CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

The Genetic Diversity and Population Genetic Structure of the Shovelnose Guitarfish (Pseudobatos productus) from Southern California to Baja California Sur
A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science in Biology
By

Alexandra Elyse Meyer

The thesis of Alexandra Elyse Meyer is approved:	
Dr. Christopher L. Chabot	Date
Dr. Jeanne M. Robertson	Date
Dr. Larry G. Allen, Chair	Date

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Dedication

To my parents, who taught me to always ask questions and never give up.

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Abstract

The Genetic Diversity and Population Genetic Structure of the Shovelnose Guitarfish

(Pseudobatos productus) from Southern California to Baja California Sur

By

Alexandra Elyse Meyer

Master of Science in Biology

The Shovelnose Guitarfish, *Pseudobatos productus* (Ayres 1854), is a shark-like batoid ray that lives in shallow soft-bottom habitats such as bays and estuaries. This species ranges from San Francisco Bay, California to the Gulf of California, Mexico, and is targeted in the artisanal elasmobranch fisheries throughout Baja California and the Gulf of California. In many cases, localized fishing pressure can lead to a loss of genetic diversity, a reduction in effective population size, and local extinction. Genetic assessments of fish stocks are seldom applied to management strategies, though there is strong evidence that such assessments will better inform policy. This study sought to assess the genetic diversity and connectivity of *P. productus* from southern California to Baja California Sur, Mexico. Genetic diversity and connectivity were assessed using the complete mitochondrial control region as well as genomic SNP data produced using the 2b-RAD library preparation and sequencing protocol. Both markers show evidence of high levels of connectivity from southern California to northern Baja California Sur, indicating admixture across international boundaries. Mitochondrial DNA evidence

supports higher levels of population structure while genomic SNP data supports admixture between mainland localities. This may be indicative of sex-specific movement patterns, with higher levels of male-mediated gene flow between localities. Based on these data, *P. productus* should be managed as a bi-national species with sex-based restrictions as males likely contribute more to genetic admixture than females.

Introduction

Genetic Diversity and Exploitation

Similar to the effect of species diversity on the long-term stability of communities, intraspecific genetic diversity is often integral to the temporal stability of a population as well as influence its system function (Agashe, 2009; Prieto *et al.*, 2015). Populations lacking genetic diversity are often at risk of collapse or localized extinction due to limited adaptive potential (Willi *et al.*, 2006) or the effects of increased inbreeding (Frankham, 1995). In the case of species harvested for human use, this risk is often related to the level of exploitation. In heavily exploited populations, the removal of individuals either by targeted or non-selective means typically results in a loss of genetic diversity. This in turn can lead to a loss of effective population size as observed in species such as the Giant Sea Bass (*Stereolepis gigas*) and Orange Roughy (*Hoplostethus atlanticus*), particularly when breeding aggregations are targeted (Smith *et al.*, 1991; Gaffney *et al.*, 2007; Chabot *et al.*, 2015b).

Despite the relationship between genetic diversity and population stability, seemingly stable fisheries stocks are commonly assessed using generalized models that frequently do not factor in specific life history characteristics and these stocks are often reassessed only when there are significant changes in stock abundance or distribution (Begg *et al.*, 1999). Numerous studies have supported the use of genetic stock identification to inform fisheries management policies, however, this is rarely implemented (Ryman, 1991; Shaklee and Bentzen, 1998; Shaklee *et al.*, 1999). Additionally, many species span political boundaries, either internationally or at the state level, complicating management policies even further. These "trans-boundary" species

are often managed by unique political entities with different policies despite possibly belonging to the same genetic stock (Chabot *et al.*, 2015b; Reiss *et al.*, 2009).

Elasmobranchs in general are particularly vulnerable to the effects of this mismatch between genetic stocks and management due to their often-similar life history characteristics. Many elasmobranch life histories are characterized by long lifespans, slow growth rates, late age-of-maturity, long gestation periods that produce large, wellformed young, low fecundity, and aggregating behaviors for the purposes of feeding or breeding (Hoenig and Gruber, 1990; Musick et al., 2000). In addition, species that reach a total maximum length over 100 cm in combination with these life-history characteristics appear to be particularly vulnerable to overfishing and population collapse (Frisk et al., 2001). The historic over-exploitation of species such as the Soupfin Shark (Galeorhinus galeus), Porbeagle (Lamna nasus), Basking Shark (Cetorhinus maximus), Spiny Dogfish (Squalus acanthias), and Barndoor Skate (Raja laevis) have led to population collapse and near extinction in some cases (Casey and Myers, 1998; Stevens et al., 2000). Of the 1,050 known species of elasmobranchs, the International Union for the Conservation of Nature classifies 204 species in one of three threatened categories (Vulnerable, Endangered, Critically Endangered) and another 115 as Near-Threatened ("IUCN Red List of Threatened Species," 2019). Due to its life-history characteristics, distribution, and fisheries status, population genetic assessment is critical for the conservation and management of the Shovelnose Guitarfish (Pseudobatos productus, Ayres 1854).

Distribution and Putative Biogeographic Barriers

Pseudobatos productus ranges from San Francisco Bay, California through the Gulf of California, Mexico, though it is most common south of Point Conception, California (Timmons and Bray, 1997; Love, 2011; Figure 1.). Shovelnose Guitarfish are a coastal benthos-associated species, commonly inhabiting shallow sandy or muddy embayments and estuaries shallower than 13 m, though they have been observed at depths as great as 91 m (Love, 2011). Pseudobatos productus mate and pup during the late spring and summer months, with females arriving earlier in the spring and remaining there until fall and males arriving in the summer as mating aggregations form (Villaviencio-Garayzar, 1993; Márquez-Farías, 2007; Love, 2011). Shovelnose Guitarfish are thought to move offshore outside of breeding season, though there is evidence of site fidelity to proposed nursery sites and other nearshore sites from year to year (Dubois, 1981; Villaviencio-Garayzar, 1993; Sandoval-Castillo et al., 2004).

Populations of *P. productus* span several predominant phylogeographic barriers along the coast of California and Baja California, including Point Conception, the San Pedro Channel, the San Quintín Upwelling-Zone, Punta Eugenia, and Cabo San Lucas, all which have been shown to have varying degrees of impact on population connectivity of coastal fishes. For example, Point Conception often provides a biogeographic barrier, but not a strong phylogeographic break, between central and southern California, and is believed to be the transition zone between the northern Oregonian Province and southern San Diegan Province (Briggs, 1974; Horn and Allen, 1978; Horn *et al.*, 2006). By contrast, Cabo San Lucas often provides both a phylogeographic and biogeographic break between the Gulf of California and the Pacific Coast of Baja California, limiting gene flow between the two regions as seen in species such as the Golden Cownose Ray

(Rhinoptera steindachneri) and the Pacific Angel Shark (Squatina californica) (Dawson et al., 2006; Ramírez-Amaro et al., 2017; Sandoval-Castillo and Rocha-Olivares, 2011). Populations of demersal species such as the Round Stingray (Urobatis halleri) show restricted gene flow across the San Pedro Channel, separating the southern California Channel Islands from mainland southern California (Plank et al., 2010), while numerous other demersal and shallow-water species show varying levels of phylogeographic structure across Punta Eugenia (Dawson, 2001).

Life History

Maximum total length estimates for *Pseudobatos productus* range from 162 cm in females to 114 cm in males (Downton-Hoffmann, 2007; Juaristi Videgaray, 2016) and the species has an estimated longevity of 11 years and a generation time of 9 years (Timmons and Bray, 1997). Location-based estimates of size at sexual maturity range from 57–111 cm in females and 63–110 cm in males, with both sexes maturing at approximately 7 years (Downton-Hoffmann, 2007; Dubois, 1981; Juaristi Videgaray, 2016; Márquez-Farías, 2007; Timmons and Bray, 1997; Villaviencio-Garayzar, 1993). Total gestation time in *P. productus* is approximately 12 months with a 5-month embryonic growth phase. The reproductive cycle of *P. productus* is annual, producing a single litter of pups per year with ovulation soon after parturition (Márquez-Farías, 2007; Romo-Curiel et al., 2016). Additionally, there is no evidence supporting sperm storage, indicating that fertilization occurs during the reproductive event (Juaristi Videgaray, 2016). Pseudobatos productus are a lecithotrophic viviparous species in which pups are nourished exclusively by the yolk sac with no additional maternal contribution (Romo-Curiel et al., 2016). Litter sizes range from 2–18 pups, however the average litter size is 5 pups with larger females producing larger litters (Downton-Hoffmann, 2007; Márquez-Farías, 2007; Villaviencio-Garayzar, 1993). Similar to size at sexual maturity, size at parturition varies by location ranging from 17–23 cm (Márquez-Farías, 2007; Villaviencio-Garayzar, 1993). As with all elasmobranchs, pups are born fully formed and independent of parental care.

Fisheries

Pseudobatos productus is classified as "Near-Threatened" with a declining population trend, likely as the result of fisheries harvest and human impact with the primary threat being targeted elasmobranch fisheries (Farrugia et al., 2016). In Californian waters there is no commercial fishery for *P. productus* and individuals are landed as the result of incidental trawling by-catch or from limited harvest by recreational anglers (Farrugia et al., 2016). In Mexican waters, both within the Gulf of California and along the Pacific coast, *P. productus* is landed as a primary target of the artisanal ray fishery as well as bycatch in demersal trawls from other fisheries (Farrugia et al., 2016). In some locations, gillnets used to catch demersal species are modified with additional nylon lines to increase the entanglement and retention rate of guitarfish (Salazar-Hermoso and Villavicencio-Garayzar, 1999; Márquez-Farías, 2011). Pseudobatos productus is the most commonly landed species in the artisanal elasmobranch fishery, comprising at least 20% of the total reported catch of any state yearly with recorded landings often numbering in the thousands (Bizzarro et al., 2009; Cartamil et al., 2011; Ramirez-Amaro et al., 2013; Smith et al., 2009a). In one extreme example, nearly 46,000 individuals were landed in 1999 as part of the artisanal fishery in Sonora (Bizzarro et al., 2009). Although the catch of P. productus has a strong seasonal

bias towards the spring and summer months, individuals are landed throughout the year at most locations (Márquez-Farías, 2007).

Unlike, the artisanal shark fishery in the Mexican Pacific which has been documented since the 1930s, the targeted ray fishery is a relatively recent development, with the first documentation of landings in 1986, developing alongside the use of bottomset gillnets in commercial teleost fisheries (Márquez-Farías, 2002). Despite being a relatively young documented fishery, catches in some locations such as Bahía Almejas, Baja California Sur have steeply declined from the late 1990s likely as a result of intense fishing pressure that frequently removes mature individuals (Farrugia et al., 2016). Gillnets are often set at the mouths of bays, channels, and estuaries frequently used by P. productus to move inshore during the breeding season, increasing the chance of catching reproductive individuals (Márquez-Farías, 2011; Salazar-Hermoso and Villavicencio-Garayzar, 1999). Large, gravid females are frequently caught during the spring and summer months due to the targeted fishing of these breeding aggregations (Bizzarro et al., 2009; Márquez-Farías, 2011; Smith et al., 2009a). Catches of P. productus often deviate from a 1:1 sex ratio, with higher numbers of females being landed when compared to males, sometimes doubling or tripling the male catch (Bizzarro et al., 2009; Cartamil et al., 2011; Márquez-Farías, 2011; Ramirez-Amaro et al., 2013; Salazar Hermoso and Villavicencio-Garayzar, 1999; Smith et al., 2009a). This deviation is often attributed to the difference in size between males and females, with larger individuals being more susceptible to entanglement, as well as the difference in the duration of time spent in shallow-water habitats vulnerable to fishing (Márquez-Farías, 2005; Márquez-Farías, 2011). This removal of mature females is of particular concern as

prior assessments of population connectivity reveal genetic isolation of populations, indicating low female migration between some sites (Sandoval-Castillo *et al.*, 2004).

Assessment of Population Genetic Structure

Over the last several decades, the use of mitochondrial DNA, particularly the non-coding mitochondrial control region (mtCR) or "D-loop", has been the marker of choice for phylogeographic studies due to its relatively rapid mutation rate with no genetic recombination between generations (Figure 2). Frequently, mtCR analyses have been combined with neutral nuclear based approaches (e.g., microsatellites) in order to provide more complete assessments of population structure, as nuclear approaches remove the female-specific bias inherent to mitochondrial markers due to bi-parental inheritance (Freeland *et al.*, 2011). More recently, with the advent of next generation sequencing technology (NGS), studies now typically incorporate the use of single nucleotide polymorphisms (SNPs) discovered through genome-wide screening for the assessment of population differentiation. These SNPs are often produced through various restriction-site associated DNA sequencing methods (RADseq) as they reliably generate large numbers of small fragments that are distributed throughout the genome and are independent of each other (Andrews and Luikart, 2014; Puritz *et al.*, 2014; Rowe *et al.*, 2011).

The population genetic structure of *Pseudobatos productus* was previously assessed using Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the mitochondrial control region (mtCR) (Sandoval-Castillo *et al.*, 2004). The study assessed the degree of differentiation between populations located on the Pacific coast of Baja California Sur and those located in the northern Gulf of California, finding indications of a deep phylogenetic separation across the Baja

Castillo *et al.*, 2004). However, there has been no assessment of the population connectivity of *P. productus* along the Pacific coast including the waters within the United States. Therefore, the aim of this study was to assess the population structure of *P. productus* from southern California to Baja California Sur using the analysis of SNPs generated via the 2b-RAD protocol (Wang *et al.*, 2012) as well as complete mitochondrial control region sequences.

Materials and Methods

Sample Collection

Tissue samples were collected from the fins and muscle tissues of *P. productus* caught from southern California to Baja California Sur. Tissues were collected using sterile techniques and stored in 95% ethanol in a -20 °C freezer for long term storage. In southern California, specimens were collected using hook and line, beach seines, and shallow water otter trawls. In Baja California Sur, muscle tissue was collected from individuals landed as part of the artisanal elasmobranch fishery. Tissue was collected from sub-adult and adult individuals of both sexes. Samples were collected from 6 localities from Los Angeles to Baja California Sur from 2012–2018, with the majority of samples collected in the spring and summer. At least 6 individuals were collected from each locality, with the exception of Dockweiler Beach, California where only 1 individual was collected (Santa Catalina Island, CA, n=13; Long Beach, CA, n=6; La Jolla, CA, n=10; Guerrero Negro, BCS, n=15; Bahía Tortugas, BCS, n=27) (Table 1; Figure 1). The sample from Dockweiler beach was grouped with individuals from Long Beach due to geographic proximity to form a general locality hereafter referred to as San Pedro that is comprised of samples from the Los Angeles/Long Beach region.

Total genomic DNA was extracted from each sample using the DNEasy Blood and Tissue Kit® (Qiagen, Valencia, CA) following the manufacturer's protocol. DNA concentration (ng/µl) and 260/280 nm absorbance ratio were quantified using a NanoDropTM 2000 Spectrophotometer (Thermo Scientific) and overall quality was assessed visually using 1% agarose gels.

Mitochondrial DNA Amplification and Sequencing

The currently unpublished mitochondrial control region (mtCR) of *P. productus* was amplified using species-specific primers designed by Sandoval-Castillo et al. (2004) for use in PCR-RFLP analysis: FWD: CbRhino1163 5'- CYT ACT TCT CAT TAT TCC TNA TCC TCC TAC C -3' and REV: 12Srev326 5'- ACT CGT ATA ACC GCG GTG GCT -3'. Oligonucleotides were ordered from Integrated DNA Technologies (San Diego, CA) and reconstituted to a concentration of 100µM using Buffer AE (10mM Tris-Cl and 0.5mM EDTA; Qiagen, Valencia CA). Amplification was performed in a 10ul volume using 5µl HotStarTaq Master Mix® (Qiagen, Valencia, CA), 1µl each of 2µM forward and reverse primers, 2.1µl nuclease free water (NFW), 0.4µl of 10µM Bovine Serum Albumin (BSA, New England Biolabs, Ipswich, MA), and 1.5µl DNA template (10nM-140nM). Polymerase Chain Reaction (PCR) was performed in an Applied Biosystems GeneAmp PCR System 9700® thermocycler with an initial denaturation step of 94 °C for 15 minutes, followed by 35 cycles of 94 °C for 15 seconds, 63 °C for 120 seconds, 72 °C for 45 seconds, and a final extension of 72 °C for 10 minutes. A subsample of each PCR product was visualized using a 1% TAE agarose gel and electrophoresed at 90-95 volts for 45 minutes to confirm the presence of the expected PCR product with a length of ~1500bp.

Successfully amplified PCR products were sequenced in two directions using the PCR primer CbRhino1163 as the forward primer and two nested sequencing primers (REV 12SrRNA: 12Srev195 5'- AGG CTA GGA CCA AAC CTG CT -3' and REV Internal-mtCR: 12sDLoop101 5'- GGG TTT TTC GAG GAT ACC GTG -3'). Both nested sequencing primers were designed using Geneious 10.2.3 (https://www.geneious.com) and Primer3 (Koressaar and Remm, 2007; Untergasser *et*

al., 2012). These two primers were designed and optimized from preliminary samples to reduce sequence coverage within the flanking conserved 12SrRNA region in order to increase read coverage within the mtCR as well as provide sequence overlap for the forward and reverse reads. PCR products were purified using ExoSap-IT Express (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) and sent to Laragen, Inc. (Culver City, CA) for Sanger Sequencing.

Mitochondrial DNA Sequence Verification

All sequences for each individual were aligned to each other and to publicly available mitochondrial sequences from 7 closely related species (Rhinopristiformes: *Rhinobatos hynnicephalus* GenBank Accession # (GB#)KF534708.1; *Rhinobatos schlegellii* GB#KJ140136.1; *Rhychobatus australiae* GB#KU746824.1; *Pristis clavata* GB#KF381507.1; *Pristis pectinata* GB#KP400584.1; *Anoxypristis cuspidata* GB#KP233202.1; *Rhina ancylostoma* GB#KU721837.1; Figure 2) using the De Novo Assemble tool in Geneious 10.2.3 under the High Sensetivity setting and validated by eye. A multiple sequence alignment (MSA) of all validated mitochondrial control region sequences from *P. productus* was generated in Geneious using the Multiple Alignment tool (Geneious 10.2.3) under the default settings. From the MSA produced by Geneious, single nucleotide polymorphisms (SNPs) were verified by eye and each variant sequence was given a haplotype identifier based on location (e.g., CAT 1–13, DW 1, LB 1–6, etc.) and were uploaded to GenBank (NCBI).

jModelTest 2 (Darriba *et al.*, 2012) was used to determine the best fit evolutionary model for the mtCR. It was found that the generalized time-reversal model (GTR+1+G) was the best fit model, however, this model is not available in many of the programs used for subsequent analyses. As a result, the Tamura-Nei model was used as it was also identified as good model and is available in all of the software used for downstream analyses.

Arlequin 3.5 (Excoffier and Lischer, 2010) was used to calculate standard molecular diversity indices including relative nucleotide composition, number of polymorphic loci, haplotype diversity (h), nucleotide diversity (π), mean pairwise nucleotide differences among populations, and the population mutation parameter θ_S (which is based on the number of segregating sites, sample size, and θ for a sample of non-recombining DNA). Female effective population size, N_{ef} , was estimated for each population using the mutation rates of 0.0067 mutations per million years (Schultz *et al.*, 2008) and 0.024 mutations per million years (Hoelzel *et al.*, 2006) based on minimum and maximum mutation rates observed in elasmobranchs. N_{ef} was calculated using the equation $\theta_S = 2N_{ef}u$ where $u = 2\mu k$, with μ being the mutation rate and k being the number of nucleotides in the sequence. Arlequin 3.5 (Excoffier and Lischer, 2010) was also used to calculate two estimators of recent population expansion, Tajima's D (Tajima, 1989) and Fu's FS statistic (Fu. 1997).

The estimation of genetic divergence between localities was calculated using multiple pairwise and hierarchical AMOVAs (Analysis of MOlecular VAriance; Excoffier *et al.*, 1992) to generate the diversity metrics Φ_{ST} and Φ_{RT} , which are analogous to Wright's F-statistics, using GenAlEx v6.503 (Peakall and Smouse, 2006, 2012). To

determine the significance of hierarchical and pairwise AMOVAs after multiple tests, the false discovery rate of Benjamini and Yekutieli (Benjamini and Yekutieli, 2001; Narum, 2006) was used to determine the significant value of alpha based on the total number of pairwise comparisons.

A Median-Joining haplotype network was generated using PopART to visualize the relatedness of haplotypes (Leigh and Bryant, 2015). The genetic population assignment of individuals was assessed using STRUCTURE 2.3.4 (Falush *et al.*, 2003, 2007; Hubisz *et al.*, 2009; Pritchard *et al.*, 2000). STRUCTURE uses posterior probabilities generated from estimated allele frequencies to assign individual membership to the most likely cluster, "K". Population assignment was tested for K values of 1–6 with 20 independent iterations of 1,000,000 Markov-chain Monte Carlo repetitions per K and a burn-in of 10% using the admixture model. CLUMPAK (Kopelman *et al.*, 2015) was used to determine the most likely value of K according to the Evanno method (Evanno *et al.*, 2005) and the natural-log probability of K (Falush *et al.*, 2003, 2007; Hubisz *et al.*, 2009; Pritchard *et al.*, 2000).

Genomic DNA Preparation

Given that high-quality genomic DNA (gDNA) is required for successful amplification using the 2b-RAD genotyping technique (Wang *et al.*, 2012), total gDNA concentration was assessed using a Qubit 3.0 Flourometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA) prior to library preparation. Since 2b-RAD genotyping works best at low volumes of high concentration gDNA, all samples were concentrated using ethanol precipitation based on methods described by Green and Sambrook (2016). gDNA was then reconstituted using Tris-HCl (pH 8.5) to a final concentration of ~100ng/μl.

Genomic DNA Library Generation and Sequencing

Genomic sequencing libraries were generated using the 2b-RAD genotyping technique (Wang *et al.*, 2012) with minor modifications based on updated protocols provided by Eli Meyer (2018). A complete list of oligonucleotides used in the generation of 2b-RAD libraries for sequencing can be found in Appendix C. The 2b-RAD technique consists of four steps: digestion of gDNA, ligation of double stranded library adaptors, PCR attachment of dual-indexing barcodes and amplification of gDNA, and fragment size selection to purify generated libraries (Figure 3). The digestion mix consisted of 1.2μl of 10X Buffer R (Thermo Scientific cat#:ER1801 2U/μl *AlfI* restriction enzyme kit, Waltham, MA) 0.8μl of 150μM SAM (cat#:ER1801), 0.5μl of *AlfI* restriction enzyme (cat#:ER1801), and 1.5μl of nuclease free water (NFW) per sample. A total of 8μl of concentrated gDNA was added to the 4μl digestion master mix, creating a total reaction volume of 12μl that was incubated overnight at 37 °C.

Due to *P. productus* having a large genome of ~4Gb (Hinegardner, 1976; Schwartz and Maddock, 1986), the feasibility of generating libraries capable of sequencing the entire genome of each sample with adequate depth was low. As a result, a 1/4 reduction scheme approach based on adapters from Meyer (2018) was used to generate libraries with adequate coverage of the genome, albeit reduced, with increased sequencing depth for each fragment of the genome. Immediately prior to ligation, two double stranded ligation adaptors were assembled. 2μL of 100μM 5ILL-NR oligo (Appendix C) were combined with 2μL of 100μM anti-ILL oligo (Appendix C) and 96μL NFW to create Adaptor 1. The process was repeated to create Adaptor 2, in which 2μL of 100μM 3ILL-NR oligo (Appendix C) were combined with 2μL of 100μM anti-ILL oligo (Appendix C) and 96μL NFW. Both adaptor mixes were incubated at room

temperature for 10 minutes. Following adapter assembly, a ligation mix consisting of 1µl of 10mM ATP (New England Biolabs, Ipswich, MA, #P0756L), 4µl of 10X T4 ligase buffer, 1µl of T4 DNA ligase (New England Biolabs, Ipswich, MA, #M0202L), 0.5µl of 20mg/ml Bovine Serum Albumin (BSA, New England Biolabs, Ipswich, MA), 5µl of Adaptor 1, 5µl of Adaptor 2, and 23.5µl of NFW was combined with 12µl of digested gDNA and incubated overnight at 16 °C.

Amplification of the gDNA/adapter complex and the attachment of sequencing barcodes was performed in a single PCR consisting of two reaction mixes. The first, a PCR mix consisting of 22µl of NFW, 2µl of 10mM dNTP (New England Biolabs, Ipswich, MA, #N0446S), 2µl of 10µM ILL-Lib1 (Appendix C), 2µl of 10µM ILL-Lib2 (Appendix C), 1µl of Q5 DNA Polymerase, 20µl of 5X Q5 Buffer (New England Biolabs, Ipswich, MA, #M0491L), and 1µl of 20mg/ml Bovine Serum Albumin (BSA, New England Biolabs, Ipswich, MA). The second, a gDNA/adapter complex-dualindexing mix consisting of 5µl of an Illumina i7 index (Appendix C) and 5µl of an Illumina i5 index (Appendix C) that were combined with 40µl of each gDNA/adapter complex in unique sample combinations (Table 2). For the final PCR mix, 50ul of the PCR master mix was combined with 50ul of the gDNA/adapter complex-dual-indexing mix for a total reaction volume of 100µl. PCR was performed in an Applied Biosystems GeneAmp PCR System 9700® thermocycler with an initial denaturation step of 98 °C for 30 seconds, followed by 25 cycles of 98 °C for 5 seconds, 60 °C for 20 seconds, 72 °C for 10 seconds, and a final extension of 72 °C for 2 minutes.

After PCR, 2% Tris-Borate-EDTA low melt agarose gels were used to visualize libraries followed by their extraction from the gels. For each library, 20µl of gel loading dye was combined with 100µl of the PCR product and electrophoresed on low voltage

(95 V) for 2 hours until bands were adequately separated. Gels were visualized on a blue-light table (Clare Chemical Research, Dark Reader DR-89X) to minimize damage to libraries and a target fragment size of ~166bp was cut from the gel. Libraries were then extracted from gels using a MinElute Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols.

Prior to the assembly of a pooled sequencing library, the quantity of DNA for each extracted library was determined by Qubit. Based on the quantity of DNA in each sample, concentrations were adjusted to 10nM and then individual libraries were pooled together at equimolar concentrations into a single sequencing library. Pooled libraries were then sequenced on an Illumina HiSeq 4000 Next Generation Sequencer using a 50-cycle single read protocol at the University of Chicago Genomics Facility. Sequences were demultiplexed and sequencing adaptors were trimmed following run completion by the University of Chicago prior to any data analysis.

Genomic DNA Read Processing

Raw sequence reads were processed using a custom Perl script pipeline from the Meyer Lab (Meyer, 2018) with minor modification to optimize the number of reads and their quality as well as reduce artifacts within the data inherent to high-throughput sequencing. A complete list of all scripts and their code can be found in Appendix D. Given that AlfI restriction endonucleases generate predictable 36bp-long fragments, the raw 50-character reads were trimmed to 36 characters to remove the additional 14 characters produced as an artifact of the sequencing protocol (TruncateFastq.pl). Low quality reads were removed using bbduk.sh (Bushnell, 2019) with parameters set to remove reads with an average Q<30. As P. productus does not have a known reference

genome, a de novo reference genome was generated by creating a cluster-derived reference (CDR) based on representative sequences from the raw reads (BuildRef.pl). Reads were aligned to the CDR using the SHRiMP software package (David et al., 2011). Poor reads that did not properly map to the CDR and ambiguous reads that mapped to multiple locations within the CDR were filtered using SAMFilter.pl. Genotypes were determined from nucleotide frequencies using CallGenotypes.pl. Monomorphic loci were filtered using PolyFilter.pl, leaving only loci at which 2 or more genotypes were observed through all samples. MDFilter.pl was used to filter lowcoverage loci and retain loci that were genotyped in all 72 individuals to minimize missing data in downstream analyses. Repetitive sequences were filtered using RepTagFilter.pl to remove bias inherent to high-throughput genotyping-by-sequencing methods due to the inability to correctly map repetitive sequences to corresponding unique loci in the *de novo* CDR genome. This filtering process removes loci that produce an unusually high number of SNPs, leaving only loci in which 2 SNPs are present. Putative linked SNPs that occur on the same AlfI fragment were filtered using OneSNPPerTag.pl to remove downstream bias generated by the analyses of linked traits.

Genomic DNA Analysis

The estimation of genetic divergence between localities was calculated using multiple pairwise and hierarchical AMOVAs (Analysis of MOlecular VAriance; Excoffier *et al.*, 1992) to generate the diversity metrics F_{ST} and F_{RT} in Arlequin 3.5 (Excoffier and Lischer, 2010). To determine the significance of hierarchical and pairwise AMOVAs after multiple tests, the false discovery rate of Benjamini and Yekutieli (Benjamini and Yekutieli, 2001; Narum, 2006) was used to determine the significant

value of alpha based on the total number of pairwise comparisons. The genetic population assignment of individuals was assessed using STRUCTURE 2.3.4 (Falush *et al.*, 2003, 2007; Hubisz *et al.*, 2009; Pritchard *et al.*, 2000). Population assignment was tested for *K* values of 1–6 with 20 independent iterations of 1,000,000 Markov-chain Monte Carlo repetitions per *K* and a burn-in of 10% using the admixture model. CLUMPAK (Kopelman *et al.*, 2015) was used to determine the most likely value of *K* populations according the Evanno method (Evanno *et al.*, 2005) and the natural-log probability of *K* (Falush *et al.*, 2003, 2007; Hubisz *et al.*, 2009; Pritchard *et al.*, 2000). Genetic clustering was visualized using a principal-components analysis (PCA) using *adegenet* in R (Jombart, 2008; Jombart and Ahmed, 2011).

Results

Mitochondrial DNA Analyses

Complete mitochondrial control regions were amplified from 70 individuals with each individual possessing a unique haplotype (Table 3; GenBank Accession #MN783979 – MN784048). Due to overall poor DNA quality, complete mitochondrial control regions were successfully amplified and sequenced from only 13 individuals from Guerrero Negro. The complete control region of *P. productus* is between 1,316bp – 1,320bp in length with an average composition of 22.51% cytosine, 32.63% thymine, 32.26% adenine, and 12.60% guanine. The complete control region of *P. productus* is roughly 200bp longer than the known complete control regions of other members of the family Rhinobatidae. Haplotypes of *P. productus* contained a total of 95 polymorphic sites, which is approximately 7.2% of the entire control region (Table 3). Of the 95 polymorphic sites, there were 40 transitions, 41 transversions, and 20 indels across all samples. The majority of polymorphic sites occurred between 700bp - 1,150bp in the second hypervariable region towards the tRNA^{Phe} and 12sRNA genes. Overall haplotype diversity was high (h = 1.00) while total nucleotide diversity was low ($\pi = 0.011 \pm 0.006$) (Table 4). Nucleotide diversity across all localities was consistently low $(\pi = 0.007 \pm 0.004 - 0.017 \pm 0.009)$ with the lowest being observed at Santa Catalina Island, San Pedro, and Bahía Tortugas and the highest at Guerrero Negro (Table 4). Tests of population expansion via Tajima's D statistic were non-significant across all populations, however both Santa Catalina Island and Bahía Tortugas had significantly negative Fu's F_S statistics (Table 4). Maximum effective female population sizes of P. productus ranged between 157,665 (based on a mutation rate of 0.024 mutations per

million years) and 564,770 (based on a mutation rate of 0.0067 mutations per million years) (Table 4).

Significant mitochondrial DNA genetic structure was observed with 23.18% (p = 0.0001) of global variation occurring among sampled localities (Table 5). Significant pairwise assessments of Φ_{ST} ranged in value between 0.1225 and 0.4859 with Santa Catalina Island and Bahía Tortugas significantly diverging from all other sampling localities (p \leq 0.017, Table 6). Hierarchical AMOVAs testing divergence across putative phylogeographic barriers revealed three putative mtDNA regions with two putative barriers to geneflow (Φ_{RT} = 0.1626, p = 0.0001, Table 7). The first barrier separates Santa Catalina Island from Long Beach and the second barrier separates Guerrero Negro from Bahía Tortugas while Long Beach, La Jolla, and Guerrero Negro experience geneflow between localities. There is no evidence for isolation by distance between any of the sampling localities (r^2 = 0.013, p = 0.25).

Haplotype network analyses, while heavily reticulated, revealed two distinct clusters with one grouping comprised of individuals sampled in the north and the other of individuals sampled in the south with mixing of haplotypes between the two (Figure 4). Population assignment via STRUCTURE revealed a minimum of K = 2, with Santa Catalina Island and Bahía Tortugas being assigned to different clusters. Increasing to K = 3 retains these same clusters but begins to segregate Guerrero Negro from other localities (Figure 5). The Evanno method estimated K = 2 as the maximum value of K while the natural-log probability of K estimated K = 3 as the most likely value of K (Figure 6).

Genomic DNA Analyses

A total of 573 SNP loci were detected across all individuals. The average genetic diversity across loci was high at 0.254 ± 0.013 . Significant genomic DNA structure was observed with 0.54% (p < 0.0001) of the variation occurring among populations (Table 8). Significant pairwise assessments of $F_{\rm ST}$ ranged in value from 0.004 to 0.0443 with Santa Catalina Island and San Pedro significantly diverging from all other sampling localities (p ≤ 0.017 , Table 6). Hierarchical AMOVAs testing divergence across putative phylogeographic barriers revealed no significant structure among regions based on location (Table 9). Population assignment via STRUCTURE revealed a minimum of K = 2 with Santa Catalina Island slightly separating from all other localities (Figure 7). Both the Evanno method and the natural-log probability of K method estimated K = 2 as the most likely number of clusters. The principal-components analysis (PCA) revealed an overall lack of distinct clustering supporting very little differentiation among sampled localities (Figure 8).

Discussion

Depending on the molecular marker used, data from the present study were able to detect either two or three genetically isolated populations of P. productus. Mitochondrial DNA evidence supports three divergent populations, as shown by both pairwise Φ_{ST} and hierarchical AMOVA analyses. This is further supported by both STRUCTURE analyses and the haplotype network that demonstrate two distinct clusters (Santa Catalina Island and Bahía Tortugas) with a high degree of mixing occurring between the two (Figure 4, 5). Congruent observations from both markers describe a single population of P. productus along the mainland from San Pedro, California to Guerrero Negro, Baja California Sur with an isolated population existing on Santa Catalina Island.

Putative Mechanisms Separating Populations of Pseudobatos productus
San Pedro Channel

Pseudobatos productus appears to have restricted gene flow across the San Pedro Channel, a span of water that separates the mainland from Santa Catalina Island. Of those studied at Santa Catalina Island, relatively few species show evidence of genetic divergence between the island and the mainland, likely due to the proximity of Santa Catalina Island to the mainland (approximately 45 km) and local current patterns that do not appear to inhibit larval dispersal (Dawson, 2001). Species that do show evidence of structure across the San Pedro Channel similar to that exhibited by P. productus include Round Stingray, Leopard Shark (Triakis semifasciata), and Black Surfperch (Embiotoca jacksoni), all viviparous species that prefer shallow coastal waters (Bernardi, 2000; Lewallen et al., 2007; Plank et al., 2010). The deepest part of the channel is the San

Pedro Basin, which is approximately 905 m and 20 km wide, that likely produces a sizeable barrier to viviparous species regularly inhabiting shallower waters with low dispersal potential for offspring due to reproductive characteristics (Dartnell and Gardner, 2009). Congruent molecular evidence that *P. productus* exhibits genetic structure between Santa Catalina Island and the mainland (although strong with mitochondrial markers and weak with nuclear markers) may suggest that movement varies between males and females across the channel with males potentially crossing more frequently than females.

Population Connectivity Across the United States-Mexican Border

The coastline of northern Baja California is characterized by discontinuous areas of seasonal upwelling events, particularly along the south-facing stretches of coasts of prominent headlands, such as Cabo San Quintín (Emerson, 1956). These upwelling zones can produce extreme variations in water temperature, with temperature fluctuations being as much as 10 °C cooler on the southern sides of headlands (Emerson, 1956). The upwelling zone at San Quintín is most active during the spring and summer months, characterized by strong northwest winds and offshore currents that have been shown to affect the abundance of marine organisms (Emerson, 1956). Given the hydrology of the area, the San Quintín Upwelling Zone appears to have a far greater impact on the population structure of summer-spawning fishes or species that use warm-water temperatures as settlement cues, such as the White Seabass (*Atractoscion nobilis*), Spotted Sand Bass (*Paralabrax maculatofasciatus*), and to a lesser degree, Barred Sand Bass (*Paralabrax nebulifer*) (Franklin *et al.*, 2016; Paterson *et al.*, 2015; Salomon, 2005). Species of teleost fishes that are not exclusively summer-spawners such as the California

Halibut (*Paralichthys californicus*), or species with a long pelagic larval phase such as the Opaleye (*Girella nigricans*), appear to be unaffected by the San Quintín Upwelling Zone as high levels of geneflow have been observed across it (Craig *et al.*, 2011; Terry *et al.*, 2000). Among broadcast spawning invertebrates, both the Tidepool Copepod (*Tigriopus californicus*) and the subtidal California Spiny Lobster (*Panulirus interruptus*) show connectivity across the upwelling zone despite having vastly different larval lifecycles and habitat usage (García-Rodríguez and Perez-Enriquez, 2006; Peterson *et al.*, 2013).

Viviparous teleost species such as the Black Surfperch appear to be unaffected by the San Quintín Upwelling Zone, likely due to their mode of reproduction that produces well-formed young that do not rely on current patterns for dispersal (Bernardi, 2000). Elasmobranchs appear to be similarly unaffected by the upwelling zone, as the reproductive biology of all elasmobranch species, both viviparous and oviparous, produces well-formed nektonic young that are capable of dispersal independent of local current patterns. Although relatively few studies have assessed the genetic structure of elasmobranch species in both Californian and Mexican waters, the majority show evidence of high levels of genetic connectivity. For example, assessment of the Brown Smoothhound Shark (*Mustelus henlei*) provided evidence of a single genetic population from southern California into the Gulf of California using both nuclear and mitochondrial DNA markers (Chabot et al., 2015). Likewise, the Round Stingray was shown to have similar degrees of population connectivity when assessed with microsatellite markers (Plank et al., 2010). Pseudobatos productus appears to exhibit the same degree of connectivity across the San Quintín Upwelling Zone, with a high degree of geneflow

observed between locations in southern California and those along the northern Baja California coast.

Punta Eugenia

Restricted geneflow across Punta Eugenia has been observed among several species including Opaleye, Black Surfperch, and Tidepool Copepod, however, this has been attributed to a lack of useable habitat in the form of rocky reefs and tidepools just north of Punta Eugenia in the shallow, sandy Laguna Ojo de Liebre where Guerrero Negro is located (Bernardi, 2000; Peterson *et al.*, 2013; Terry *et al.*, 2000). In addition, genetic structuring across Punta Eugenia is found in species that are often restricted to estuaries such as the California Killifish (*Fundulus parvipinnis*) and Longjaw Mudsucker (*Gillichthys mirabilis*) or in species with pelagic larval phases such as the Spotted Sand Bass that may be affected by both current patterns and a scarcity of habitat space used by breeding adults across Point Eugenia (Bernardi and Talley, 2000; Huang and Bernardi, 2001; Salomon, 2005).

Similar to these species, *P. productus* appears to exhibit mitochondrial structuring across Punta Eugenia, a finding that is unique among previously studied rays including the confamilial Banded Guitarfish (*Zapteryx exasperata*) (Castillo-Páez *et al.*, 2014). *Pseudobatos productus* and the Banded Guitarfish have similar life history characteristics and are known to frequently share habitat space, yet, the Banded Guitarfish exhibits genetic connectively along the Pacific coast of Baja California while *P. productus* does not (Castillo-Páez *et al.*, 2014). Relatively few studies have examined the population connectivity of elasmobranchs along the Pacific coast of southern California and Baja California and even fewer have included samples collected from northern Baja

California. Those that do include samples from northern Baja California frequently indicate a high degree of genetic connectivity along the Pacific coast of Baja California, even across Punta Eugenia (Castillo-Páez *et al.*, 2014; Ramírez-Amaro *et al.*, 2017). The observed mitochondrial structuring across Punta Eugenia may be the result of a physical barrier represented by the geography of the area which females are less likely to cross while males are apparently more vagile.

Peninsula of Baja California

Patterns of genetic divergence across Cabo San Lucas, dividing Pacific coast populations from those in the Gulf of California, have been observed in a number of marine species including elasmobranchs and teleost fishes. Structuring that has been observed in teleost species with a pelagic larval phase is largely attributed to differences in water temperature and current patterns that may affect the distributions of adults and settlement of larvae such as the White Seabass (Franklin et al., 2016). Strong signals of genetic divergence between the Pacific coast and the Gulf of California observed in teleost species such as the Longjaw Mudsucker, Opaleye, and Spotted Sand Bass have been attributed to population structuring caused by the formation of the Baja California Peninsula, glaciation events, as well as the possibility of incipient speciation resulting from vicariance (Bernardi et al., 2003). Similar patterns of strong divergence across Cabo San Lucas have been observed in numerous demersal and coastal-associated elasmobranch species such as the Banded Guitarfish, Golden Cownose Ray, Butterfly Ray (Gymnura marmorata), and the Pacific Angel Shark (Castillo-Páez et al., 2014; Ramírez-Amaro et al., 2017; Jonathan Sandoval-Castillo and Rocha-Olivares, 2011; Smith et al., 2009b). Although not directly tested in the present study, population

subdivision has been observed in *P. productus* across Cabo San Lucas resulting in an isolated population within the Gulf of California (Sandoval-Castillo *et al.*, 2004). Based on the phylogeographic breaks described above and the lack of population connectivity observed in previous studies along with those of the present study, there would appear to be at least four populations of *P. productus* within the northeastern Pacific based on mitochondrial data: 1) Santa Catalina Island, 2) southern California down to Punta Eugenia, 3) below Punta Eugenia to Cabo San Lucas, and 4) within the Gulf of California.

Putative Mechanisms Affecting Population Connectivity

The mitochondrial structuring exhibited by *P. productus* may potentially be a result of some degree of reproductive philopatry that may have produced geographically isolated reproductive units as seen in sawfishes of the genus *Pristis* (Phillips *et al.*, 2011). *Pseudobatos productus* has been documented to exhibit seasonal residency to particular nursery sites in California (Farrugia *et al.*, 2011) as well as some degree of site fidelity to inshore habitats between years (Dubois, 1981; Villaviencio-Garayzar, 1993; Sandoval-Castillo *et al.*, 2004). Taken in combination with the relative lack of structure suggested by genomic DNA evidence, this mitochondrial structuring may also be sex dependent. Similar patterns of discordance between genomic or nuclear markers and mitochondrial DNA have been reported in numerous coastal shark species including the Grey Reef Shark (*Carcharhinus amblyrhynchos*) (Momigliano *et al.*, 2017) and the Bonnethead Shark (*Sphyrna tiburo*) (Portnoy *et al.*, 2015). For both, discordance has been attributed to higher levels of male movement and some degree of female philopatry (Momigliano *et al.*, 2017; Portnoy *et al.*, 2015). In addition to female philopatry to nurseries or sites that

produce conditions favorable for reproduction, prey availability may influence where individuals are found throughout the year as well as their fine-scale movement (Carlisle and Starr, 2010; Nosal *et al.*, 2013).

Other causes of population structuring are usually attributed to long-term genetic patterns caused by historic changes in both the shape of the coastline and habitat availability either due to tectonic activity or glaciation periods (Dawson et al., 2006). Southern California and the Baja California peninsula have been sites of tectonic upheaval for the last several million years that may have influenced historic population structure in numerous species (Bernardi et al., 2003). The formation of the Gulf of California as well as the positioning of Baja California likely began during the late Miocene approximately 12 million to 3.5 million years ago (Holt et al., 2000). Studies of population structure and long-term phylogeographic separation in terrestrial species such as desert mice and side-blotched lizards as well as marine fauna including the Opaleye and Spotted Sand Bass support the opening and closure of numerous trans-peninsula seaways connecting the Pacific coast with the Gulf of California (Lindell et al., 2006; Riddle et al., 2000; Stepien et al., 2001; Terry et al., 2000; Upton and Murphy, 1997). During the late Pliocene, approximately 3 million years ago, two seaways were proposed that connected the northern and southern Gulf of California with the Pacific Ocean (Bernardi et al., 2003). Further genetic structuring in both marine and terrestrial fauna has been attributed to the opening of a mid-peninsular seaway connecting the Pacific Ocean to the Gulf of California south of Punta Eugenia approximately 1 million years ago during the mid-Pleistocene (Bernardi et al., 2003; Lindell et al., 2006; Upton and Murphy, 1997). While these Pliocene and Pleistocene seaways may have had an impact on the structure of populations of *P. productus* between the Pacific coast and the Gulf of

California, they are unlikely to have contributed to the structure observed in Baja
California Sur as the proposed Pleistocene mid-peninsular seaway crossed the peninsula
at Punta Abreojos south of Punta Eugenia.

Climate change and variation in sea levels associated with the Last Glacial Maximum (LGM) approximately 20 thousand years ago have been cited as potential causes of population structure in species such as the California Killifish and the Tidewater Goby (*Eucyclogobius newberryi*) in both southern California and the Baja peninsula (Dawson *et al.*, 2001; Dolby *et al.*, 2018). Reconstructions of coastal habitats and tidal estuaries have hypothesized that sea level recession caused by the LGM likely reduced the amount of usable habitat and decreases in water temperatures impacted the genetic structure of the California Killifish, Longjaw Mudsucker, and Shadow Goby (*Quietula y-cauda*) (Dolby *et al.*, 2018). These species were likely restricted to shallow water refugia during periods of low sea level followed by colonization into suitable habitat as sea level rose. This loss of shared habitat space could account for structuring seen at the mitochondrial marker in *P. productus*. Similarly, habitat space in southern California was likely heavily affected by the sea level decrease associated with the LGM.

Historic geological data suggests that the northern California Channel Islands may have been connected to the mainland during the LGM, allowing for expansion and contraction of numerous species based on habitat availability (Dawson, 2001; Dawson *et al.*, 2006). It is unlikely, however, that populations of *P. productus* between the mainland and the southern Channel Islands would experience increased connectivity during this period as the land bridge generated between the mainland and California Channel Islands likely connected farther north near Ventura rather than San Pedro (Bernardi, 2000; Dawson, 2001). In addition, due to the shape of the San Pedro Shelf and San Pedro Basin,

it is likely that shallow coastal habitat useable by *P. productus* would be further restricted across the deeper waters.

The low levels of mitochondrial nucleotide diversity along with the high haplotype diversity observed in the present study of *P. productus* are often attributed to a demographic expansion from a small effective population (Grant and Bowen, 1998). Evidence of rapid population expansion was detected in two sites (Santa Catalina Island and Bahía Tortugas) using the Fu's F_S statistic (Table 4), however, this test was found to be non-significant in all other sample localities. Similar to the Barred Sand Bass, the haplotype network generated in this study for *P. productus* lacks the typical starburst pattern often associated with haplotypic expansion from a bottleneck or small population indicating that such population expansion may have occurred in the distant past (Paterson et al., 2015). Estimates of effective population size for P. productus are fairly high, indicating the possibility of large, genetically stable populations throughout the range similar to patterns observed in the Brown Smoothhound Shark (Chabot et al., 2015a). Despite genetic patterns of expansion and long-term stability, populations of *P. productus* appear to be declining within Baja California based on observational studies (Farrugia et al., 2016). Due to this incongruence, future monitoring of the genetic diversity of P. productus may be essential for the successful management of the species, particularly in areas where harvesting occurs.

Cryptic Species

Within the Gulf of California, putative cryptic species have been suggested for *P. productus*, the Golden Cownose Ray, and the Butterfly Ray due to high levels of mitochondrial genetic divergence observed between/among sampled populations

(Sandoval-Castillo et al., 2004; Sandoval-Castillo and Rocha-Olivares, 2011; Smith et al., 2009b). Recently, a new species of *Pseudobatos* was described from 82 specimens obtained from the Gulf of California in the 1940s and 1950s (Rutledge, 2019). Pseudobatos buthi is described as being morphologically most similar to P. productus in most aspects including coloration but differs in the size and shape of the rostrum as well as in the scale and thorn patterns found on the head (Rutledge, 2019). Pseudobatos buthi was found to have a smaller size at maturity in both sexes (48 cm) and shorter observed total length (68.5 cm) than *P. productus* (Rutledge, 2019). This difference in size at maturity and maximum total length observed in P. buthi may explain differences in life history characteristics reported for P. productus between the Gulf of California and along the Pacific coast (Downton-Hoffmann, 2007; Dubois, 1981; Juaristi Videgaray, 2016; Márquez-Farías, 2007; Timmons and Bray, 1997; Villaviencio-Garayzar, 1993). An assessment of the genetic structure of P. buthi and phylogenetic comparisons of P. productus and P. buthi would be helpful in order to further resolve the evolutionary relationships between the two species.

In addition to *P. buthi*, there are two other described congeneric species of guitarfish found within the Gulf of California that share habitat space with *P. productus*: the Speckled Guitarfish (*Pseudobatos glaucostigmus*) and the Whitesnout Guitarfish (*Pseudobatos leucorhynchus*). All four species of guitarfish are morphologically very similar with slight variations in color and size being the immediately distinguishable characteristics. These species inhabit coastal waters in a clinal pattern that includes overlapping habitat space within the Gulf of California. While *P. productus* is found from California waters and into the Gulf of California, the Speckled Guitarfish ranges from southern Baja California Sur to Ecuador, the Whitesnout Guitarfish occurs within the

Gulf of California and south to Ecuador, and *P. buthi* has been described only within the Gulf of California (Bizzarro 2016a; Bizzarro 2016b; Farrugia *et al.* 2016; Rutledge 2019). Of the four species, population trends of only *P. productus* have been assessed by the IUCN within the last decade with the species being classified as Near-Threatened with a declining trend, while the Whitesnout Guitarfish was assessed in 2006 and classified similarly (Bizzarro, 2016b; Farrugia *et al.*, 2016). Both *P. buthi* and the Speckled Guitarfish are considered Data Deficient by the IUCN despite likely being impacted by both intentional harvest in artisanal fisheries and accidental bycatch in trawlbased fisheries (Bizzarro, 2016a; Rutledge, 2019). In order to fully understand the impact of removal of individuals on population structure and diversity of Pacific *Pseudobatos* spp., further studies and stock assessments are required as there is a scarcity of information on the Whitesnout Guitarfish, the Speckled Guitarfish, and *P. buthi*.

Fisheries Management

While *P. productus* is a heavily fished, bi-national species, relatively little management strategies have been enacted in order to protect the species from overexploitation. In California, there are no specific regulations to the recreational harvest of guitarfish, just a general catch limit of 10 individuals per day regardless of size and sex ("California ocean sport fishing regulations," 2019). In Mexico, there has been an increase in fishing regulations applied to the artisanal fishery, however, none specifically have been applied for *P. productus*. In 2007, a regulation was implemented which required the use of a logbook for accurate catch reporting as well as defined restricted fishing areas (Farrugia *et al.*, 2016). As a result of the 2007 regulations, *P. productus* as well as other guitarfish species are recorded in the fishery as "pez guitarra", however,

differentiation between species is not required (Farrugia *et al.*, 2016). In 2012, a regulation was implemented to close the fishery from May 31 to July 31, a period that coincides with the peak of breeding season for many coastal elasmobranch species (Farrugia *et al.*, 2016). Despite this ban, *P. productus* remains vulnerable to fishing pressures due to their extended use of shallow coastal areas throughout the year.

The implementation of management policies specific to the genus *Pseudobatos* along with species-specific management strategies based on observed population structuring in *P. productus* will likely promote both the health of the species as well as that of the fishery. Within the Mexican fishery, region-based catch limits and size restrictions should be set, particularly during the spring months when large, gravid female P. productus remain unprotected by the fishing closure. Under the current management system, only late-arriving individuals to aggregation sites are protected by policy while those who arrive earlier in the year are vulnerable (Márquez-Farías, 2007). Due to the observed mitochondrial genetic structure described in the present study, populations of P. productus north of Punta Eugenia should be assessed and managed independently of those to the south. Current assessments of catch by the artisanal fisheries are typically conducted within broad ranges of the states of Baja California and Baja California Sur, so an assessment of catches within regions of genetic similarity are necessary for a more accurate understanding of the impact of harvest on populations. Given the evidence of strong genetic divergence of *P. productus* across Cabo San Lucas, populations should be managed as separate entities between southern Baja California Sur and the Gulf of California. Additionally, current fisheries along the Pacific coast and within the Gulf of California should be assessed for the presence of *P. productus* and *P. buthi* as both species are very similar morphologically and incorrect identifications may result in

erroneous catch counts (Rutledge, 2019). The inclusion of accurate species identifications of *Pseudobatos* spp. within the fisheries (along with bycatch) would be an ideal addition to current management policies, particularly in the Gulf of California where species' ranges overlap.

Collaborative management between Californian and Baja Californian fisheries authorities is necessary to protect the area of genetic continuity that stretches from southern California to Guerrero Negro. Currently, management policy is divided between the two entities with different levels of harvest regulations ("California ocean sport fishing regulations," 2019; Farrugia et al., 2016). As previously mentioned, there are no specific regulations to the recreational harvest of guitarfish within Californian waters. This policy should be revised to include guitarfish with other federally regulated "groundfish" species and to have catch limits set similarly to those species with shared levels of population structuring, life history characteristics, and that inhabit similar environments as P. productus. Additionally, the implementation of regionally based policies that manage stocks at the California Channel Islands separately from those on the mainland will help maintain genetic diversity within isolated populations. Though the protection of *P. productus* was not the aim of restrictions to trawling fisheries and the implementation of harvest restrictions within marine protected areas, the species has likely benefitted from them as mortality due to bycatch has been greatly reduced (Farrugia *et al.*, 2016).

The combination of population genetic structure and accurate stock assessments are essential for the creation of management strategies used to promote the health and longevity of targeted species as well as the fisheries that harvest them. In addition, studies of genetic diversity can provide a genetic baseline for populations that can be used to

detect localized declines in genetic diversity. Trans-international assessments of population structure are also essential in species which may experience different harvest rates and policy applications throughout its range, to further prevent depletion within isolated populations. As shown with *P. productus*, observed patterns of population connectivity seldom align with international or state boundaries that are often used to delineate management units.

Conclusion

This study sought to assess the genetic diversity and population genetic structure of *P. productus* from southern California to the southern Pacific coast of Baja California. Both mitochondrial and genomic DNA evidence suggests a high degree of geneflow and connectivity between San Pedro, California and Guerrero Negro, Baja California Sur with an isolated population being detected at Santa Catalina Island. Mitochondrial evidence supports further structuring of *P. productus* with an additionally isolated population being observed at Bahía Tortugas, BCS. Based on these findings, populations of *P. productus* should be managed based on areas of genetic relatedness instead of by political boundaries. Furthermore, international cooperation may be necessary to protect areas of high geneflow, such as observed across the United States-Mexico border.

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Appendix A: Figures



Figure 1. Distribution of *Pseudobatos productus* in shaded area with marked sampling locations. 1) Santa Catalina Island, CA, 2.1) Dockweiler Beach, CA, 2.2) Long Beach, CA (2.1 and 2.2 later combined as the sampling region San Pedro), 3) La Jolla, CA, 4) Guerrero Negro, BCS, 5) Bahía Tortugas, BCS. Major known phylogeographic or biogeographic barriers indicated by red arrows.

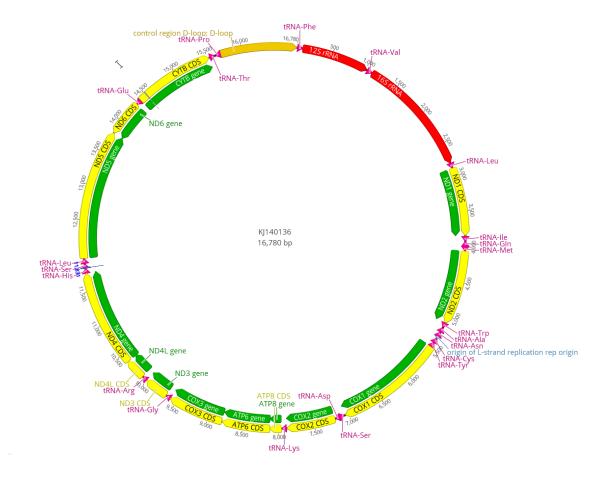


Figure 2. Complete mitochondrial genome of *Rhinobatos schlegelii* (GenBank Accession #KJ140136.1) indicating the position of the control region (D-Loop) and flanking tRNAs.

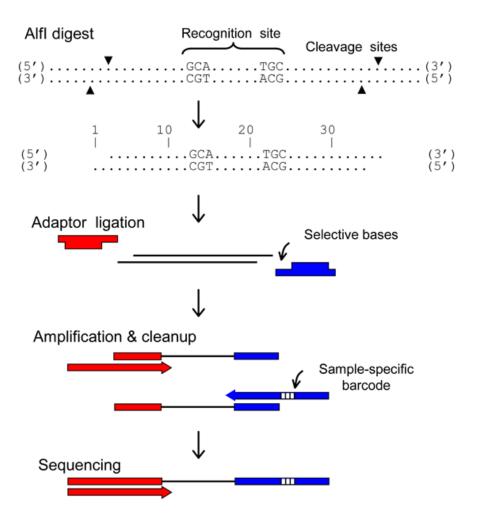


Figure 3. The steps of 2b-RAD library generation. From Eli Meyer (2018).

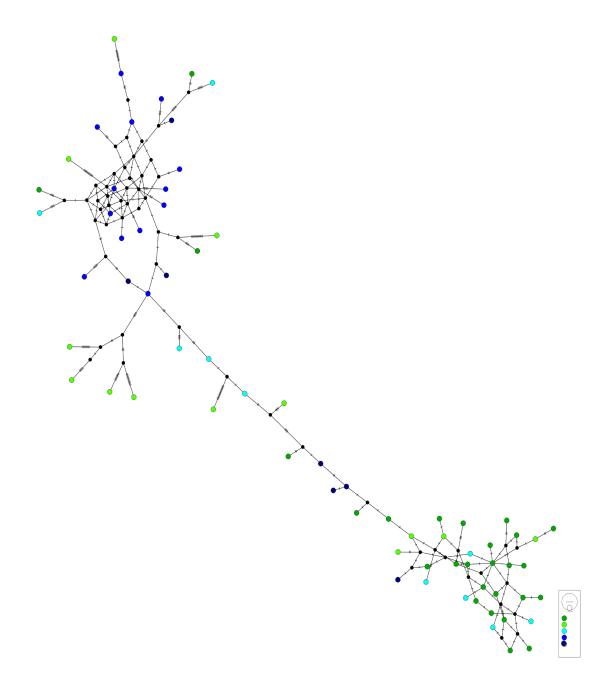


Figure 4. Haplotype network for *Pseudobatos productus*. Each colored circle represents a unique haplotype and each color corresponds to a sampling location. Bahía Tortugas (dark green), Guerrero Negro (light green), La Jolla (bright blue), Catalina (blue), and San Pedro (dark blue). Circle size corresponds to the number of individuals per haplotype. Each black circle represents a hypothetical "ancestor" or missed haplotype. The hashmarks on joining lines represent the number of mutational steps between haplotypes. A total of 70 unique haplotypes were found in 70 individuals.

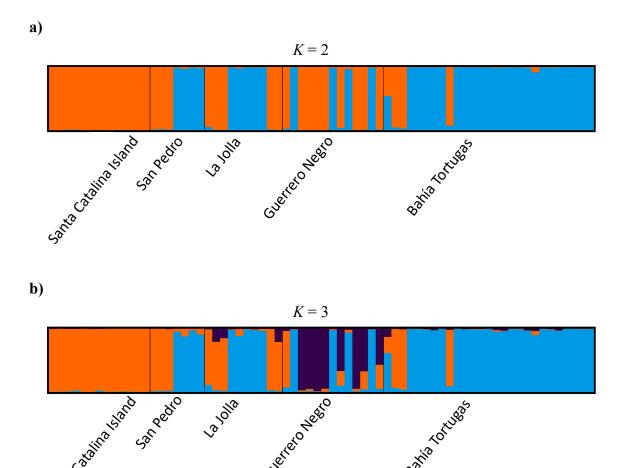


Figure 5. Population assignment of *Pseudobatos productus* estimated by STRUCTURE using mitochondrial DNA. Each bar represents a single individual by population with percentage ancestry to K populations represented vertically. Californian sampling locations: Santa Catalina Island, San Pedro, and La Jolla. Mexican sampling locations: Guerrero Negro, BCS and Bahía Tortugas, BCS. **a)** Assignment to K = 2. **b)** Assignment to K = 3.

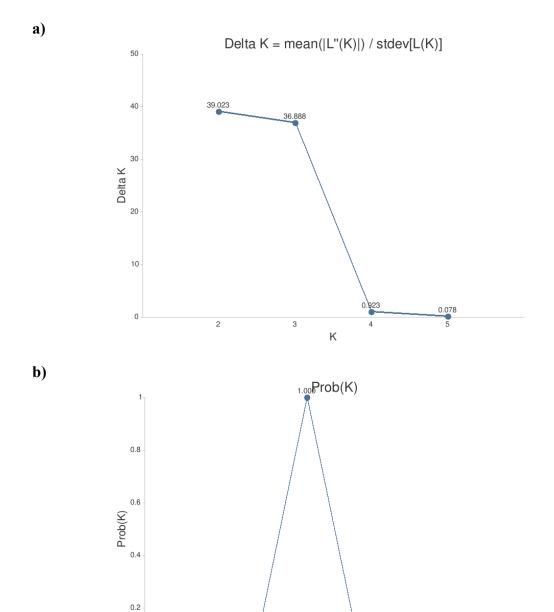


Figure 6. Determination of the most likely value of K estimated by CLUMPAK using mitochondrial DNA. **a)** Evanno DeltaK method estimates K = 2. **b)** Natural-log probability of K estimates K = 3.

0.000

0.000

0.000

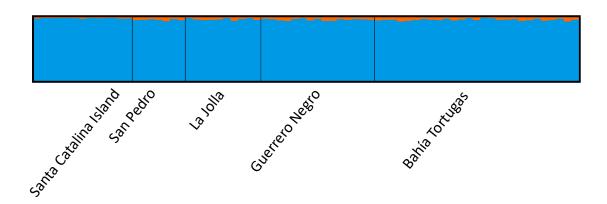


Figure 7. Population assignment of *Pseudobatos productus* estimated by STRUCTURE using genomic DNA. Each bar represents a single individual by population with percentage ancestry to *K* populations represented vertically. Californian sampling locations: Santa Catalina Island, San Pedro, and La Jolla. Mexican sampling locations: Guerrero Negro, BCS and Bahía Tortugas, BCS.

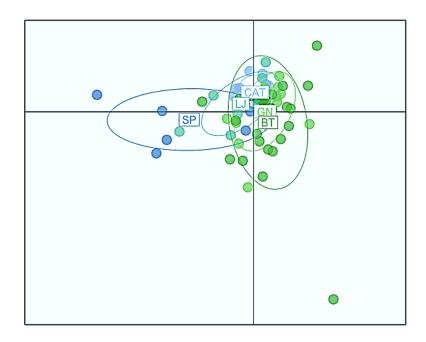


Figure 8. Genetic clustering via principal-components analysis (PCA) using *adegenet* for *Pseudobatos productus* using genomic DNA.

Appendix B: Tables

 Table 1. Samples of Pseudobatos productus.

Sample ID	Location	Sex	TL (mm)	Analysis
RPCAT1	Santa Catalina Island, CA	F	1230	mtCR/gDNA
RPCAT2	Santa Catalina Island, CA	F	1270	mtCR/gDNA
RPCAT3	Santa Catalina Island, CA	F	1320	mtCR/gDNA
RPCAT4	Santa Catalina Island, CA	F	1235	mtCR/gDNA
RPCAT5	Santa Catalina Island, CA	F	1230	mtCR/gDNA
RPCAT6	Santa Catalina Island, CA	F	1310	mtCR/gDNA
RPCAT7	Santa Catalina Island, CA	F	1120	mtCR/gDNA
RPCAT8	Santa Catalina Island, CA	F	1265	mtCR/gDNA
TS263	Santa Catalina Island, CA	F	880	mtCR/gDNA
TS264	Santa Catalina Island, CA	M	1130	mtCR/gDNA
TS270	Santa Catalina Island, CA	M	1030	mtCR/gDNA
TS272	Santa Catalina Island, CA	M	960	mtCR/gDNA
TS273	Santa Catalina Island, CA	M	1090	mtCR/gDNA
RPDW1	Dockweiler Beach, CA	M	890	mtCR/gDNA
RPLBH1	Long Beach, CA	F	583	mtCR/gDNA
RPLBH2	Long Beach, CA	М	320	mtCR/gDNA
RPLBH3	Long Beach, CA	F	760	mtCR/gDNA
RPLBH4	Long Beach, CA	М	650	mtCR/gDNA
RPLBH5	Long Beach, CA	F	620	mtCR/gDNA
RPLBH6	Long Beach, CA	F	670	mtCR/gDNA
RP001	La Jolla, CA	F	1320	mtCR/gDNA
RP002	La Jolla, CA	F	1420	mtCR/gDNA
RP003	La Jolla, CA	F	1230	mtCR/gDNA
RP116	La Jolla, CA	F	1360	mtCR/gDNA
RP216	La Jolla, CA	M	1240	mtCR/gDNA
RP316	La Jolla, CA	F	1470	mtCR/gDNA
RP416	La Jolla, CA	F	1400	mtCR/gDNA
RP516	La Jolla, CA	F	1360	mtCR/gDNA
RP616	La Jolla, CA	М	1350	mtCR/gDNA
RP716	La Jolla, CA	F	1460	mtCR/gDNA
LCRP3	Guerrero Negro, BCS	M	810	mtCR/gDNA
LCRP4	Guerrero Negro, BCS	М	770	mtCR/gDNA
LCRP5	Guerrero Negro, BCS	M	860	mtCR/gDNA
LCRP11	Guerrero Negro, BCS	M	880	mtCR/gDNA
LCRP13	Guerrero Negro, BCS	M	740	mtCR/gDNA
LCRP15	Guerrero Negro, BCS	М	850	gDNA

 Table 1. Samples of Pseudobatos productus (cont.).

Sample ID	Location	Sex	TL (mm)	Analysis
LCRP22	Guerrero Negro, BCS	M	580	mtCR/gDNA
LCRP25	Guerrero Negro, BCS	F	1000	gDNA
LCRP48	Guerrero Negro, BCS	F	_	mtCR/gDNA
LCRP49	Guerrero Negro, BCS	F	_	mtCR/gDNA
LCRP50	Guerrero Negro, BCS	M	_	mtCR/gDNA
LCRP51	Guerrero Negro, BCS	M	_	mtCR/gDNA
LCRP54	Guerrero Negro, BCS	F	_	mtCR/gDNA
LCRP56	Guerrero Negro, BCS	F	1415	mtCR/gDNA
LCRP57	Guerrero Negro, BCS	M	1030	mtCR/gDNA
BTRP1	Bahía Tortugas, BCS	F	786	mtCR/gDNA
BTRP2	Bahía Tortugas, BCS	F	922	mtCR/gDNA
BTRP3	Bahía Tortugas, BCS	M	1024	mtCR/gDNA
BTRP4	Bahía Tortugas, BCS	F	904	mtCR/gDNA
BTRP5	Bahía Tortugas, BCS	M	996	mtCR/gDNA
BTRP6	Bahía Tortugas, BCS	F	1054	mtCR/gDNA
BTRP7	Bahía Tortugas, BCS	F	1008	mtCR/gDNA
BTRP8	Bahía Tortugas, BCS	M	928	mtCR/gDNA
BTRP9	Bahía Tortugas, BCS	Н	756	mtCR/gDNA
BTRP10	Bahía Tortugas, BCS	M	1146	mtCR/gDNA
BTRP11	Bahía Tortugas, BCS	F	842	mtCR/gDNA
BTRP12	Bahía Tortugas, BCS	F	922	mtCR/gDNA
BTRP13	Bahía Tortugas, BCS	F	926	mtCR/gDNA
BTRP14	Bahía Tortugas, BCS	F	936	mtCR/gDNA
BTRP15	Bahía Tortugas, BCS	F	1064	mtCR/gDNA
BTRP16	Bahía Tortugas, BCS	F	924	mtCR/gDNA
BTRP17	Bahía Tortugas, BCS	M	842	mtCR/gDNA
BTRP18	Bahía Tortugas, BCS	F	842	mtCR/gDNA
BTRP19	Bahía Tortugas, BCS	M	1014	mtCR/gDNA
BTRP20	Bahía Tortugas, BCS	F	980	mtCR/gDNA
BTRP21	Bahía Tortugas, BCS	F	1122	mtCR/gDNA
BTRP22	Bahía Tortugas, BCS	F	910	mtCR/gDNA
BTRP23	Bahía Tortugas, BCS	F	970	mtCR/gDNA
BTRP24	Bahía Tortugas, BCS	F	1036	mtCR/gDNA
BTRP25	Bahía Tortugas, BCS	F	1160	mtCR/gDNA
BTRP26	Bahía Tortugas, BCS	F	940	mtCR/gDNA
BTRP27	Bahía Tortugas, BCS	M	762	mtCR/gDNA

Table 2. Unique dual-indices applied to samples of *Pseudobatos productus* for genomic

library sequencing.

Sample	i7 Index	i7 Index	i5 Index	i5 Index
	Name	Sequence	Name	Sequence
RPCAT1	UTBC74	ACGATT	HT28	CAGCA
RPCAT2	UTBC74	ACGATT	HT29	GGCGA
RPCAT3	UTBC74	ACGATT	HT33	TGTTG
RPCAT4	UTBC74	ACGATT	HT38	AAAAT
RPCAT5	UTBC74	ACGATT	HT5	AACCC
RPCAT6	UTBC91	TGCAAA	HT13	ATTAC
RPCAT7	UTBC91	TGCAAA	HT17	CCAAT
RPCAT8	UTBC91	TGCAAA	HT20	CACCG
TS263	UTBC91	TGCAAA	HT28	CAGCA
TS264	UTBC91	TGCAAA	HT29	GGCGA
TS270	UTBC91	TGCAAA	HT33	TGTTG
TS272	UTBC91	TGCAAA	HT38	AAAAT
TS273	TSBC06	GCCAAT	HT7	GGAAC
RPDW1	TSBC06	GCCAAT	HT9	CGTAG
RPLBH1	TSBC06	GCCAAT	HT12	TATCT
RPLBH2	TSBC06	GCCAAT	HT19	TGGTC
RPLBH3	TSBC15	ATGTCA	HT7	GGAAC
RPLBH4	TSBC15	ATGTCA	HT9	CGTAG
RPLBH5	TSBC15	ATGTCA	HT12	TATCT
RPLBH6	TSBC15	ATGTCA	HT19	TGGTC
RP001	UTBC74	ACGATT	HT13	ATTAC
RP002	UTBC74	ACGATT	HT17	CCAAT
RP003	UTBC74	ACGATT	HT20	CACCG
RP116	UTBC72	AAGTAT	HT17	CCAAT
RP216	UTBC72	AAGTAT	HT20	CACCG
RP316	UTBC72	AAGTAT	HT28	CAGCA
RP416	UTBC72	AAGTAT	HT29	GGCGA
RP516	UTBC72	AAGTAT	HT33	TGTTG
RP616	UTBC72	AAGTAT	HT38	AAAAT
RP716	UTBC91	TGCAAA	HT5	AACCC
LCRP3	TSBC42	TAATCG	HT20	CACCG
LCRP4	TSBC42	TAATCG	HT28	CAGCA
LCRP5	UTBC68	GGAACT	HT29	GGCGA
LCRP11	TSBC42	TAATCG	HT33	TGTTG
LCRP13	TSBC42	TAATCG	HT38	AAAAT
LCRP15	UTBC68	GGAACT	HT5	AACCC

Table 2. Unique dual-indices applied to samples of *Pseudobatos productus* for genomic library sequencing (cont.).

Sample	i7 Index	i7 Index	i5 Index	i5 Index
	Name	Sequence	Name	Sequence
LCRP22	UTBC68	GGAACT	HT13	ATTAC
LCRP25	UTBC68	GGAACT	HT17	CCAAT
LCRP48	UTBC68	GGAACT	HT20	CACCG
LCRP49	UTBC68	GGAACT	HT28	CAGCA
LCRP50	TSBC42	TAATCG	HT29	GGCGA
LCRP51	UTBC68	GGAACT	HT33	TGTTG
LCRP54	UTBC68	GGAACT	HT38	AAAAT
LCRP56	UTBC72	AAGTAT	HT5	AACCC
LCRP57	UTBC72	AAGTAT	HT13	ATTAC
BTRP1	TSBC12	CTTGTA	HT5	AACCC
BTRP2	TSBC12	CTTGTA	HT13	ATTAC
BTRP3	TSBC12	CTTGTA	HT17	CCAAT
BTRP4	TSBC12	CTTGTA	HT20	CACCG
BTRP5	TSBC12	CTTGTA	HT28	CAGCA
BTRP6	TSBC12	CTTGTA	HT29	GGCGA
BTRP7	TSBC12	CTTGTA	HT33	TGTTG
BTRP8	TSBC12	CTTGTA	HT38	AAAAT
BTRP9	TSBC13	AGTCAA	HT5	AACCC
BTRP10	TSBC13	AGTCAA	HT13	ATTAC
BTRP11	TSBC13	AGTCAA	HT17	CCAAT
BTRP12	TSBC13	AGTCAA	HT20	CACCG
BTRP13	TSBC13	AGTCAA	HT28	CAGCA
BTRP14	TSBC13	AGTCAA	HT29	GGCGA
BTRP15	TSBC13	AGTCAA	HT33	TGTTG
BTRP16	TSBC13	AGTCAA	HT38	AAAAT
BTRP17	TSBC34	CATGGC	HT5	AACCC
BTRP18	TSBC34	CATGGC	HT13	ATTAC
BTRP19	TSBC34	CATGGC	HT17	CCAAT
BTRP20	TSBC34	CATGGC	HT20	CACCG
BTRP21	TSBC34	CATGGC	HT28	CAGCA
BTRP22	TSBC34	CATGGC	HT29	GGCGA
BTRP23	TSBC34	CATGGC	HT33	TGTTG
BTRP24	TSBC34	CATGGC	HT38	AAAAT
BTRP25	TSBC42	TAATCG	HT5	AACCC
BTRP26	TSBC42	TAATCG	HT13	ATTAC
BTRP27	TSBC42	TAATCG	HT17	CCAAT

56

SITE 39 227 242 252 473 519 sednence.

Table 3. Nucleotide changes representative of haplotypes of Pseudobatos productus relative to the consensus

1029 1040 1044 1046 1049 1050 1057 1058 1058 1058 1058 1057 1103 1107 1102 1125 1127 1129 1143 1158 1158 1219 1219 121 1254 1318 1319 1320 1321 1323 1321 1325 Table 3. Nucleotide changes representative of haplotypes of Pseudobatos productus relative to the consensus sequence (cont.).

Table 4. Summary of mtDNA genetic diversity of *Pseudobatos productus*. Haplotype number (N), haplotype diversity (h), mean

size (N_{ef}) based on mutation rates of 0.024 and 0.0067 mutation per million years respectively, Tajima's D, and Fu's F_{S}	utation ra	ites of 0.()24 and 0.0067 mu	ıtation per million yea	ars respecti	vely, Tajima's D, a	nd Fu's Fs	
Locality	>	ų	п	MPD	θ_{S}	N_{ef} T	Tajima's <i>D</i> Fu's F _s	Fu's Fs
Santa Catalina Island	13	1.00	0.007 ± 0.004	8.551 ± 4.229	8.3784	66,117 - 236,839 -0.256	-0.256	-6.451*
San Pedro	7	1.00	0.007 ± 0.004	9.429 ± 4.934	7.7551	61,199 - 219,219 0.452	0.452	-1.668
La Jolla	10	1.00	0.012 ± 0.006	15.200 ± 7.433	12.372	97,633 - 349,729 0.428	0.428	-2.398
Guerrero Negro	13	1.00	0.017 ± 0.009	22.923 ± 10.801	19.979	157,665 - 564,770 -0.180	-0.180	-2.856
Bahía Tortugas	27	1.00	0.007 ± 0.004	8.749 ± 4.167	9.599	75,753 - 271,352 -0.841	-0.841	-22.509*

Table 5. Fixation index for Pseudobatos productus based on mitochondrial DNA.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component P	Degrees of Freedom Sum of Squares Variance Component Percentage of Variation
Among Population	4	122.108	1.853	23.18%
Within Populations	99	399.207	6.142	76.82%
Total	69	521.314	7.995	100.00%
Fixation Index (Φ_{ST}) 0.2318				
p < 0.0001				

Table 6. Pairwise Φ_{ST} and F_{ST} values for *Pseudobatos productus*. Φ_{ST} values are given below the diagonal and F_{ST} values are given above. * indicates p-values ≤ 0.017 after Benjamini and Yekutieli modified false discovery rate correction. ** indicates p-values ≤ 0.0001 .

	Santa Catalina Island	San Pedro	La Jolla	Guerrero Negro	Bahía Tortugas
Santa Catalina Island	I	0.0443**	0.0198**	0.0062*	*6900.0
San Pedro	0.2095*	I	0.0040*	0.0128*	0.0149**
La Jolla	0.2498**	0.0000	I	0.0000	0.0000
Guerrero Negro	0.1847**	0.0567	0.0673	I	0.0000
Bahía Tortugas	0.4859**	0.1993*	0.1225*	0.2506**	I

Table 7. Results of hierarchical AMOVA using mitochondrial DNA testing putative biogeographic barriers, San Pedro Channel and Punta Eugenia. Catalina was separated from San Pedro and Guerrero Negro was separated from Bahía Tortugas.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Degrees of Freedom Sum of Squares Variance Component Percentage of Variation
Among Regions	2	92.666	1.334	16.26%
Among Populations	2	26.442	0.730	8.89%
Within Populations	99	399.207	6.142	74.84%
Total	69	521.314	8.206	100.00%
Fixation Index (Φ _{RT}) 0.1626	9			
p = 0.0001				

 Table 8. Fixation index for Pseudobatos productus based on genomic DNA.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Degrees of Freedom Sum of Squares Variance Component Percentage of Variation
Among Population	4	33.267	0.03923	.54%
Within Populations	139	1007.893	7.25103	99.46%
Total	143	1041.16	7.29026	100.00%
Fixation Index (FsT)	0.0054			
p < 0.0001				

Table 9. Results of hierarchical AMOVAs using genomic DNA testing putative biogeographic barriers.

a) Separating Santa Catalina Island from all other localities

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percentage of Variation
Among Regions	1	8.223	-0.00407	0.18%
Among Populations	к	25.044	0.04082	0.44%
Within Populations	139	1007.893	7.25103	99.38%
Total	143	1041.160	7.28778	100.00%
Fixation Index (F _{RT}) 0.0000	0			
p = 0.4003				

b) Separating Santa Catalina Island and San Pedro from all other localities

D) Separating Santa Catanna Island and San I caro noin an onici rocannes	a Sail I calo Holli all Oulci 10ce	nitics		
Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percentage of Variation
Among Regions	1	8.873	0.01296	0.18%
Among Populations	3	24.394	0.03233	0.44%
Within Populations	139	1007.893	7.25103	99.38%
Total	143	1041.160	7.29633	100.00%
Fixation Index (FRT) 0.0018	8			
p = 0.2997				

c) Separating Santa Catalina Island and Bahía Tortugas from all other localities

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percentage of Variation
Among Regions	2	16.190	-0.02896	0.00%
Among Populations	2	17.077	0.06337	0.87%
Within Populations	139	1007.893	7.25103	99.53%
Total	143	1041.160	7.28544	100.00%
Fixation Index (F _{RT}) 0.0000				
p = 0.5028				

Appendix C

a) Oligonucleotides used in the generation of ligation adaptors. These oligonucleotides are attached during the ligation process.

Name	Sequence
anti-ILL	AGATCGGAAGAGC(InvdT)
5ILL-NR	CTACACGACGCTCTTCCGATCTNR
3ILL-NR	CAGACGTGTGCTCTTCCGATCTNR

b) Oligonucleotides used to bind gDNA constructs to the Illumina flow-cell for sequencing. These oligonucleotides are attached and amplified during the PCR process.

Name	Construct End	Sequence
ILL-Lib1	5'	AATGATACGGCGACCACCGA
ILL-Lib2	3'	CAAGCAGAAGACGGCATACGA

c) Complete oligonucleotides used to attach index sequences to the Adaptor 1 end of the gDNA/adaptor complex. These oligonucleotides are attached and amplified during the PCR process.

Name	Sequence
HT5	AATGATACGGCGACCACCGAGATCTACACAACCCACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT07	AATGATACGGCGACCACCGAGATCTACACGGAACACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT09	AATGATACGGCGACCACCGAGATCTACACCGTAGACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT12	AATGATACGGCGACCACCGAGATCTACACTATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT13	AATGATACGGCGACCACCGAGATCTACACATTACACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT17	AATGATACGGCGACCACCGAGATCTACACCCAATACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT19	AATGATACGGCGACCACCGAGATCTACACTGGTCACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT20	AATGATACGGCGACCACCGAGATCTACACCACCGACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT28	AATGATACGGCGACCACCGAGATCTACACCAGCAACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT29	AATGATACGGCGACCACCGAGATCTACACGGCGAACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT33	AATGATACGGCGACCACCGAGATCTACACTGTTGACACTCTTTCCCTACACGACGCTCTTCCGATCT
HT38	AATGATACGGCGACCACCGAGATCTACACAAAATACACTCTTTCCCTACACGACGCTCTTCCGATCT

d) Complete oligonucleotides used to attach index sequences to the Adaptor 2 end of the gDNA/adaptor complex. These oligonucleotides are attached and amplified during the PCR process.

Name	Sequence
UTBC68	CAAGCAGAAGACGGCATACGAGATAGTTCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
UTBC72	CAAGCAGAAGACGGCATACGAGATATACTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
UTBC74	CAAGCAGAAGACGGCATACGAGATAATCGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
UTBC91	CAAGCAGAAGACGGCATACGAGATTTTGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC06	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC12	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC13	CAAGCAGAAGACGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC15	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC34	CAAGCAGAAGACGGCATACGAGATGCCATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
TSBC42	CAAGCAGAAGACGGCATACGAGATCGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC

Appendix D

All perl scripts were written by Eli Meyer (Meyer, 2018)

Custom perl-script pipeline used to prepare genomic data for analyses:

- 1. Truncate reads using TruncateFastq.pl
- 2. Quality filter reads using bbduk.sh
- 3. Generate a cluster derived reference genome using BuildRef.pl
- 4. Align reads to reference genome using gmapper function within SHRiMP
- 5. Filter weak and ambiguous reads using SAMFilter.pl
- 6. Parse alignments and call base pairs using SAMBaseCounts.pl
- Combine all base calls into a single file prior to calling genotypes with CombineBaseCounts.pl
- 8. Determine genotypes from nucleotide frequencies using CallGenotypes.pl
- 9. Filter to include only polymorphic loci using PolyFilter.pl
- 10. Filter reads to exclude low coverage loci that were genotyped in too few samples to be informative using MDFilter.pl
- 11. Filter samples to exclude repetitive sites using RepTagFilter.pl
- 12. Filter samples to exclude samples with multiple SNPs per tag to remove linkage using OneSNPPerTag.pl

TruncateFastq.pl

```
#!/usr/bin/perl
# written by E Meyer, <a href="mailto:eli.meyer@science.oregonstate.edu">eli.meyer@science.oregonstate.edu</a>
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///q:
$usage = <<USAGE;</pre>
Truncates a set of short reads in FASTQ format to keep the region specified
Usage: $scriptname -i input -s start -e end -o output
Required arguments:
                       file of short reads to be filtered, fastq format
       -i input
                       beginning of the region to keep, nucleotide position
       -s start
       -e end
                       end of the region to keep, nucleotide position
       -o output
                       a name for the output file (fastq format)
USAGE
if ($#ARGV < 3 || $ARGV[0] eq "-h") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n": exit;}
use Getopt::Std;
# get variables from input
getopts('i:s:e:o:h');# in this example a is required, b is optional, h is help
if (!$opt_i ||!$opt_s ||!$opt_e ||!$opt_o || $opt_h) {print "\n", "-"x60, "\n",
$scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
my $seqfile = $opt i;
                              # raw reads, fastq format
my $startloc = $opt_s-1;
                              # beginning of region to keep, base 1
my sendloc = sopt_e-1;
                                     # end of region to keep, base 1
                              # name for output file, fastg format
my $outfile = $opt o;
# loop through fastq file and truncate sequences and quality scores
open (IN, $seqfile);
open (OUT, ">$outfile");
my $switch = 0;
while(<IN>)
       {
       chomp;
       $count++;
       if ($count==1) {$ss = substr($_, 0, 4);}
       if ($_ =~ /^$ss/)
               print OUT $_, "\n";
               next:
               }
       if ($_ =~ /^\+$/)
               print OUT "$_", "\n";
               next:
               }
       else
               $ssi = $_;
               $slen = length($ssi);
               $subi = substr ($ssi, $startloc, $endloc-$startloc+1);
               print OUT $subi, "\n";
close(IN);
```

BuildRef.pl

```
#!/usr/bin/env perl
# written by E Meyer, <a href="mailto:eli.meyer@science.oregonstate.edu">eli.meyer@science.oregonstate.edu</a>
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///g:
$usage = <<USAGE;</pre>
Builds a reference for de novo analysis of 2bRAD sequences from samples
without a sequenced genome. The script filters, clusters, and compares
similar sequences to infer the set of loci present in the species of
interest, using a subset of reads from the samples themselves.
Usage: $scriptname -i input -o output <OPTIONS>
Required arguments:
       -i input
                     The set of processed (truncated, HQ) reads (FASTQ) to be used
for reference
                     development. Ideally this should include 10-20 million reads
spanning the range
                     of known diversity (e.g. from all populations in your study).
Prepare this
                     ahead of time by concatenating together a subset of reads from
your samples.
                     a name for the output file, to be used as a reference in
       -o output
mapping and genotyping
Options:
       -v overwrite 0=do not overwrite existing files; use them for analysis.
(default)
                     1=do not use existing files; overwrite them with new files.
       -n mincov
                     Minimum depth to qualify as a valid allele. (default=2)
       -q threshold Quality scores below this threshold are low quality
(default=30)
       -x number
                     Maximum number of low quality bases allowed for reference
construction (default=0)
       -m mismatches Maximum number of mismatches allowed in clustering of related
alleles (default=2)
                     Minimum number of bases required to resolve sub-clusters
       -d distance
(default=1)
       -a haplotypes For very large clusters containing more than this number of
unique sequences,
                      do not attempt to resolve sub clusters. These indicate
repetitive sequences
                     which are not useful for genotyping anyway, and resolving these
large clusters
                      is computationally intensive. (default=32)
IISAGE
if ($#ARGV < 2 || $ARGV[0] eq "-h") {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
# -- module and executable dependencies
# use this block if checking for executable dependencies
# copy the block and edit to check for additional Perl modules required by the
script
$mod1="File::Which";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use File::Which;
$mod2="Bio::TreeI0";
unless(eval("require $mod2")) {print "$mod2 not found. Exiting\n"; exit;}
use Bio::TreeIO;
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
$mod1="Bio::SeqIO";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Bio::SeqIO;
```

```
$mod2="Bio::Seq::Ouality":
unless(eval("require $mod2")) {print "$mod2 not found. Exiting\n"; exit;}
use Bio::Seq::Quality;
# use this block and edit to check for executables required by the script
$dep1 = "raxmlHPC":
unless (defined(which($dep1))) {print $dep1, " not found. Exiting.\n"; exit;}
$dep2 = "cd-hit-est";
unless (defined(which($dep2))) {print $dep2, " not found. Exiting.\n"; exit;}
# get variables from input
getopts('i:o:v:n:g:x:m:d:a:h');
if (!$opt_i || !$opt_o || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if ($opt_v) {$ow_opt=$opt_v;} else {$ow_opt = 0;}# overwrite options: 1=overwrite
if ($ow opt ne 0 and and $ow opt ne 1) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if (sopt_n) {smincov=sopt_n;} else {smincov = 2;}# min coverage for valid alleles
if ($opt_q) {$qthd=$opt_q;} else {$qthd = 30;}
                                                             # quality score threshold
if ($opt_x) {$maxlq=$opt_x;} else {$maxlq = 0;}
if ($ont_a) {$maxlq=$opt_x;} else {$maxlq = 0;}
                                                             # max LO positions threshold
if ($opt_a) {$maxmems=$opt_a;} else {$maxmems = 32;}
                                                           # maximum cluster size for
sub cluster analysis
$infile = $opt_i;
                               # name of the input sequence file (fasta format)
$outfile = $opt_o;
                              # a name for the final output file (reference to be used
for mapping)
if ($opt_d) {$depthi = $opt_d;} else {$depthi = 1;}
$mindepth = 0.028 * $depthi;# min branch length and depth to select clades. 0.028
corresponds to 1 bp distance
if ($opt_m) {$maxmm = $opt_m;} else {$maxmm = 2;}
\sin tc = (36 - \frac{36}{max})/36; # 0.944 = 2 bp distance for initial clustering of
alleles and paralogs
# define additional variables. edit these if you understand the consequences.
$site = "\\w{12}GCA\\w{6}TGC\\w{12}"; # recognition site in Perl regexp
$minmems = 4; # minimum number of sequences in a clade to consider tree building.
Don't set below 4.
# convert fastq input to fasta
my \ \$wrap = 1000;
                               # only needs to be longer than maximum read length
my $fastqfile = $infile;
$nameroot = $fastqfile;
nerver = s/\..+//;
my $osfile = $nameroot.".fasta";
my $ogfile = $nameroot.".qual";
if ($ow_opt eq 0)
        if (-e $osfile andand -e $ogfile)
               print "Found $osfile and $oqfile . Using these ...\n";
               goto CONV;
print "Converting FASTQ input into FASTA...\n";
my $inseqs = new Bio::SeqIO(-file=>$fastqfile, -format=>"fastq");
my $outseqs = new Bio::SeqIO(-file=>">$osfile", -format=>"fasta", -width=>$wrap);
my $outquals = new Bio::SeqIO(-file=>">$oqfile", -format=>"qual", -width=>$wrap);
my %sh; my $scount = 0;
while ($seq = $inseqs->next seq)
        {$sh{$seq->display_id} = $seq->seq;
        $outseqs->write_seq($seq);
        $outquals->write_seq($seq);
        $scount++;
print "Converted ", $scount, " reads from FASTQ to FASTA and QUAL.\n";
```

```
CONV:
                              # end of file conversion section
$seqfile = $osfile;
$qualfile = $oqfile;
# fix sequence name characters because raxmlHP is picky
print "Checking input sequence names for \":\" characters...\n";
$checkseq = `head -n 1 $seqfile | grep \"\:\" -m 1`;
$checkqual = `head -n 1 $qualfile | grep \"\:\" -m 1`;
print "Finished checking input files.\n";
if (length($checkseq)>0)
       print "Found \":\" in $seqfile. Correcting...\n";
       system("perl -pi -e \"s/\:/-/g\" $seqfile");
       print "Finished.\n";
if (length($checkqual)>0)
       print "Found \":\" in $qualfile. Correcting...\n";
       system("perl -pi -e \"s/\:/-/g\" $qualfile");
       print "Finished.\n";
# -- counting input
$inseq = `grep \">\" -c $seqfile`;
$inqual = `grep \">\" -c $qualfile`;
chomp($inseq); chomp($inqual);
# -- screen for Ns in input reads
print "Checking for Ns in input...\n";
open(SF, $seqfile);
while(<SF>)
       {chomp;
       if (\$\_ = \ />/) {\$name = \$\_; \$name = \ s/>//; \$name = \ s/\ *//; next;
       else
               if (\$\_ = \sim /N/i)
                       $nhash{$name}++;
                       $withn++;
               }
       }
print "Finished.\n";
# -- designate positions to exclude from quality filtering
# low diversity at these positions leads to falsely low quality scores,
# and we'll be checking for accuracy at those positions more directly anyway
Qexcvec = qw{13 14 15 16 22 23 24};
foreach $e (@excvec) {$exch{$e}++;}
# -- stringently filter and truncate raw reads and scores
if ($ow_opt eq 0)
       if (-e "VHQ.qual" and -e "VHQ.fasta")
               print "VHQ.fasta and VHQ.qual found. Using existing files...\n";
               $outqual = `grep -c \">\" VHQ.qual`;
$outseq = `grep -c \">\" VHQ.fasta`;
               chomp($outseq); chomp($outqual);
               $nlq = $inseq - $outseq - $withn;
               goto VHQ;
print "Conducting stringent quality filtering...\n";
my $ngual = $lqn = 0;
open(QF, $qualfile);
open(QQ, ">VHQ.qual");
```

```
while(<QF>)
        {chomp;
        if (\$_ =~ />/) {\$name = \$_; \$name =~ \$//; \$name =~ \$/\s+.*//; next;}
                {@qa = split(" ", $_);
        else
                foreach $q (@qa)
                        $poscount++;
                        if (exists($exch{$poscount})) {next;}
                        if (q < qthd) {qthd}
                        }
                if (exists($nhash{$name})) {next;}
                if ($lqn>$maxlq) {$nlq++; next;}
                elsif ($lqn <= $maxlq)</pre>
                        {print 0Q ">", $name, "\n";
print 0Q join(" ", @qa), "\n";
$gh{$name}++;
                        $outqual++;
                }
close(QF);
open(SF, $seqfile);
open(OS, ">VHQ.fasta");
while(<SF>)
        {chomp;
        if ($_ =~ />/) {$name = $_; $name =~ s/>//; $name =~ s/\s+.*//; next;}
                if (exists($gh{$name}))
                        {print OS ">", $name, "\n"; print OS $_, "\n";
                        $outseq++;
                }
close(SF);
close(0S);
print "Finished quality filtering.\n";
                                         # end of quality filtering section.
print $inseq, " sequences in.\n";
print $inqual, " scores in.\n";
print $withn, " sequences with Ns excluded.\n";
print $nlq, " low quality sequences excluded.\n";
print $outseq, " sequences out.\n";
print $outqual, " scores out.\n";
# -- Filter for perfect match to restriction site
print "Filtering for perfect matches to restriction site...\n";
if ($ow_opt eq 0)
        if (-e "matches.fasta")
                print "matches.fasta found. Using existing file...\n";
                goto MATCHES;
system("grep -P \"$site\" VHQ.fasta -B 1 > matches.fasta");
system("perl -pi -e \"s/^--\n//g\" matches.fasta");
MATCHES:
                                         # end of restriction site filtering section
$nmatches = `grep \">\" matches.fasta -c`;
chomp($nmatches);
print "Done filtering for site matches. $nmatches matches found.\n";
```

```
# -- if VHQ sites coverage is high (> 10 million) enforce a minimum coverage setting
of at least 1/5M reads
newmincov = int(nmatches/5000000+0.5);
if ($newmincov > $mincov) {$mincov = $newmincov;}
# -- this step identifies sequences observed repeatedly in the VHQ dataset
# -- we call these "valid alleles"
# -- cluster reads at 1.0 and extract valid alleles based on depth
print "Beginning initial clustering step...\n";
if ($ow_opt eq 0)
      if (-e "va.clstr")
             {
             print "va.clstr found. Using existing file...\n";
             goto CLSTR1;
system("date");
system("cd-hit-est -i matches.fasta -M 0 -d 0 -c 1 -o va >va.log");
print "Finished with initial clustering.\n";
                                  # end of initial clustering section
print "Parsing initial clusters...\n";
open(IN, "va.clstr");
while(<IN>)
      chomp;
      if ($_ =~ /^>/)
             $cname = $_;
             $cname =~ s/ /_/;
             $cnum++;
      else
             $ch{$cname}++;
             $allreads++;
close(IN);
# -- apply coverage filter to identify sequences identified more than $mincov times
in the VHQ data
foreach $c (sort(keys(%ch)))
      {
      if ($ch{$c}<$mincov) {next;}</pre>
       $pass++;
       $passh{$c}++;
# -- identify representative sequences from valid clusters
open(IN, "va.clstr");
$status = 0;
while(<IN>)
       chomp;
       if ($_ =~ /^>/)
             $cname = $_;
              if (exists($passh{$cname})) {$status = 1;}
       elsif (\$status > 0 and \$_= \sim /\*/)
```

```
$repname = $;
              $repname =~ s/.*>//;
              representation = ~ s/\..+//;
              $reph{$repname}++;
              status = 0;
close(IN);
# -- extract representative sequences from valid clusters
open(IN, "va");
open(OUT, ">filtered_va.fasta");
$status = 0;
while(<IN>)
       chomp;
       if ($_ =~ />/)
              $rname = $_;
              rname = ~ s/>//;
              rname = ~ s/ .+//;
              if(exists($reph{$rname})) {print OUT $_, "\n"; $status++;}
       elsif ($status > 0)
              print OUT $_, "\n";
              $status = 0;
close(IN);
close(OUT);
print "Finished parsing initial clusters. \n";
print "Identified $pass putative alleles (sequences observed at least $mincov
times).\n";
system("date");
# cluster valid alleles to identify groups of related alleles that may represent
paralogs or alleles
print "Beginning second clustering step...\n";
if ($ow_opt eq 0)
       if (-e "gra.clstr")
              print "gra.clstr found. Using existing file...\n";
              goto CLSTR2;
system("cd-hit-est -i filtered va.fasta -M 0 -d 0 -c $initc -o gra >gra.log");
print "Finished second clustering step.\n";
CLSTR2:
                                    # end of second clustering step
print "Parsing cluster output...\n";
# parse clusters to record cluster membership of each sequence
open(IN, "gra.clstr");
$status = 0; $gra_c = 0;
while(<IN>)
       chomp;
       if ($_ =~ /^>/)
              section 5 = 5;
              $cname =~ s/ /_/;
              $cname =~ s/>//;
              $gra c++;
       else
```

```
memname = \$_;
              memname =  s/.* //;
              $memname =~ s/\..+//;
              if ($_ =~ /\*/)
                      $grareps{$cname} = $memname;
              $grah{$cname}{$memname}++;
print "Finished parsing.\n";
print $gra_c, " clusters (groups of related alleles) identified.\n";
system("date");
# -- build trees for each cluster in an effort to distinguish between paralogs and
print "Testing for clusters containing multiple loci...\n";
$ctestno = 0;
@allcs = keys(%grah);
$nallcs = @allcs;
foreach $c (sort(keys(%grah)))
                             # count members in each cluster
       %cih = %{$grah{$c}};
       @cmems = sort(keys(%cih));
       $ncmems = @cmems;
       $ctestno++;
# If fewer than 4 alleles were observed, it's not possible to use sequence
relationships
# among alleles to infer the number of loci in the cluster.
       if ($ncmems < $minmems)</pre>
# pick representative seq from this cluster and add it to reference as a single
locus
              $outh{$grareps{$c}}++;
              if ($ncmems < 2) {$mono loc++;}</pre>
              else
                      {$poly_one++;}
              next;
# If more than the maximum number of sequences were observed, this indicates a
highly
# repetitive cluster that we'd like to exclude because such loci will be error
prone.
       if ($ncmems > $maxmems)
# pick representative seq from this cluster and add it to reference as a single
locus
              $outh{$grareps{$c}}++;
              $poly_max++;
              next;
# for clusters of the right size for tree building
       print STDERR "Resolving cluster number $ctestno of $nallcs, with $ncmems
members ... ";
# extract sequences of all cluster members
       $c4tree++;
       found = 0;
       open(SEQ, "filtered_va.fasta");
open(TMP, ">tmp.fasta");
       while(<SEQ>)
              chomp;
              if ($_ =~ />/)
                      status = 0;
                      = < s/>//;
```

```
$_ =~ s/\s.*//;
if (exists($cih{$_}))
                             $outname = $_;
                             $outname =~ s/\:/-/g;
                             print TMP ">", $outname, "\n";
                             $status++;
                             $found++;
                             next;
                             }
                     }
              elsif ($status > 0)
                     {
                     print TMP $_, "\n";
                     status = 0;
              if ($found >= $ncmems) {last;}
       close(SEQ); close(TMP);
# tree building code begins here
# prepare for tree building
       if(glob("*.tree"))
                            {system("rm *.tree");}
       %cladeh = %deph = %blenh = ();
# build tree describing relationships among members of the cluster
       $nseqs = `grep -c \">\" tmp.fasta`;
       chomp($nseqs);
       if ($nseqs ne 0)
       system("raxmlHPC -s tmp.fasta -p 123 -m GTRCAT -n out.tree > ml.log");
# parse tree to identify clades that differ by at least the critical
# minimum difference ($mindepth variable)
       if (-e "RAxML_bestTree.out.tree")
              $treeobj = new Bio::TreeIO(-file=>"RAxML_bestTree.out.tree", -
format=>"newick");
              $tree = $treeobj->next_tree;
              $rootnode = $tree->get_root_node;
# store id, depth, and branch length for each node
       foreach $node ($rootnode->get all Descendents)
              if (defined($node->id))
                     {
                     @lineage = $tree->get_lineage_nodes($node);
                     $cladeh{$lineage[-1]->internal_id}{$node->id}++;
                     $deph{$lineage[-1]->internal_id} = $lineage[-1]->depth;
                     $blenh{$lineage[-1]->internal_id} = $lineage[-1]-
>branch_length;
                     }
              }
# identify number of loci in each cluster and representative sequence for each locus
       %tmpfateh = ();
       foreach $tclade (sort(keys(%cladeh)))
              {
              $cladetest++; $cci = 0;
              %th = %{$cladeh{$tclade}};
              @tha = sort(keys(%th));
              $ntha = @tha;
# store a representative of clade at root
              if ($deph{$tclade}==0)
                     {
```

```
$outh{$tha[0]}++;
                         $tmpfateh{$tclade} = $tha[0];
# store a representative of each clade judged to be sufficiently different to
# represent a seperate locus
                 elsif ($deph{$tclade}>=$mindepth and and $blenh{$tclade}>=$mindepth)
                         $outh{$tha[0]}++;
                         $tmpfateh{$tclade} = $tha[0];
                 }
# end tree building code
        @tmpkeys = keys(%tmpfateh);
        $nkeys = @tmpkeys;
        if ($nkeys > 1)
                 $splitclust++;
                 $newclust += $nkeys;
                 print STDERR " split into $nkeys loci.\n";
        else
                 $remainsingle++;
                 print STDERR " left as a single locus.\n";
        }
        }
print "Finished tree analysis.\n";
system("date");
print $mono_loc, " singleton clusters\n";
print $poly_one, " clusters made of too few sequences to build useful trees\n";
print $c4tree, " cluster with sufficient diversity to build trees\n";
print $remainsingle, " clusters lacked sub-clusters and remained together\n";
print $splitclust, " clusters were split into two or more sub-clusters\n";
print "Those clusters were split into $newclust sub-clusters.\n";
@outlocs = sort(keys(%outh));
$nout = @outlocs;
print $nout, " loci identified altogether.\n";
# write out valid sites to a de novo reference file
open(IN, "filtered_va.fasta");
open(OUT, ">$outfile");
status = 0; stound = 0;
while(<IN>)
        {
        chomp;
        if ($_ =~ /^>/)
                 sid = _;
                 $sid =~ s/>//;
                 $sid =~ s/ .*//g;
                 $altsid = $sid;
                 $altsid =~ s/\:/-/g;
                 if (exists($outh{$sid}) || exists($outh{$altsid}))
                         status = 1;
                         $found++;
                         print OUT ">denovoLocus", $found, "\n";
                         next;
                         }
                 else
                         {
                         next;
```

SAMFilter.pl

```
#!/usr/bin/env perl
# written by E Meyer, eli.meyer@science.oregonstate.edu
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///q:
$usage = <<USAGE;</pre>
Filters the alignments produced by mapping short reads against a reference,
excluding ambiguous, short, and weak matches.
NOTE: make sure that when a read matches multiple reference sequences (ambigous)
your mapper reports at least two alignments in the output. This is NOT the default
behavior for some mappers, but is required to exclude ambiguous matches before
genotyping.
Usage: $scriptname -i input -m matches -o output <options>
Required arguments:
              -i input
                                           Output from any short read mapper, in SAM format.
              -m matches
                                          Minimum number of matching bases required to consider an
alignment valid.
                                          A name for the filtered output (SAM format).
              -o output
Options:
              -c option
                                          1: Report the number of reads matching each reference sequence
                                           in a separate output files "counts.tab". 0: Don't produce this
file (default).
              -l length
                                          Minimum length of aligned region (match, mismatch, + gaps)
required to consider
                                           an alignment valid. Only relevant if your mapper uses local
alignment. For global
                                          alignments, this is set equal to -m.
USAGE
if (\$ARGV < 3 \parallel \$ARGV[0] = "-h") {print "\n", "-"x60, "\n", $scriptname, "\n", "-"x60, "\n", $scriptname, "\n", "-"x60, "\n", $scriptname, "\n", "-"x60, "\n", $scriptname, "\n", "-"x60, "\n", "\
$usage, "-"x60, "\n\n"; exit;}
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
getopts('i:m:o:c:l:h');
                                                        # in this example a is required, b is optional, h is
help
if (!$opt_i || !$opt_m || !$opt_o || $opt_h) {print "\n", "-"x60, "\n", $scriptname,
"\n", $usage, "-"x60, "\n\n"; exit;}
if ($opt_c) {$cprint = 1;} else {$cprint = 0;}
if ($opt_l) {$athd = $opt_l;} else {$a_thd = $opt_m;}
my $infile = $opt i;
my $mthd = $opt_m;
my $outfile = $opt_o;
$ambig = $tooshort = 0;
# read in sam file output and build a hash, counting raw mappings
open(IN, $infile);
my %maph;
while(<IN>)
              {
              if ($_ =~ /^@/) {next;}
              chomp;
              rowi = _;
              $rowi =~ s/^>//;
              @cols = split("\t", $rowi);
              $ncols = @cols;
              if ($cols[2] eq "*") {next;}
```

```
$rawmap++;
# -- extract alignment length and apply alignment threshold
        $numstr = $cols[5];
        @chars = split("M", $numstr);
       $aligni = $mismatchi = 0;
       foreach $c (@chars)
               c = \sim s/.+D//g;
               if ($c > 0) {$aligni+=$c;}
       if ($aligni < $athd) {$tooshort++; next;}</pre>
# -- extract mismatches and apply mismatch threshold
       foreach $flag (@cols[11..$ncols])
               if ($flag =~ /NM\:i/)
                      flag = \sim s/NM:i://;
                      $mismatchi = $flag;
               }
       $matchi = $aligni - $mismatchi;
       if ($matchi < $mthd) {$tooweak++; next;}</pre>
# -- add extracted data to hash
       $maph{$cols[0]}{$cols[2]}{"count"}++;
       $maph{$cols[0]}{$cols[2]}{"string"} = $_;
       $maph{$cols[0]}{$cols[2]}{"align"} = $aligni;
       $maph{$cols[0]}{$cols[2]}{"match"} = $matchi;
# count number of reads with one or more matches passing thresholds
@mra = keys(%maph);
nmr = 0mra;
# select final set of unique mappings
open(OUT, ">$outfile");
open(IN, $infile);
while(<IN>)
       chomp;
       if ($_ =~ /^@/) {print OUT $_, "\n";}
       else {last;}
my %refch;
foreach $r (@mra)
       %rh = %{$maph{$r}};
       @ma = keys(%rh);
       nma = @ma;
       if ($nma>1)
               @moa = sort{$rh{$b}{"match"}<=>$rh{$a}{"match"}}(keys(%rh));
               if ($rh{$moa[0]}{"match"}==$rh{$moa[1]}{"match"})
                      $ambig++;
                      next;
                      }
               }
       if ($nma == 1) {@moa = @ma;}
       $unimap++;
       $refch{$moa[0]}++;
       print OUT $rh{$moa[0]}{"string"}, "\n";
```

SAMBaseCounts.pl

```
#!/usr/bin/perl
# written by E Meyer, eli.meyer@science.oregonstate.edu
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$usage = <<USAGE;</pre>
Counts nucleotide frequencies at each locus in a 2bRAD sequence data set.
Usage: $scriptname -i input -r reference -o <OPTIONS>
Required arguments:
                             input alignments, SAM format
       -i input
       -r reference
                             reference used to generate the input alignments, FASTA
format
       -o output
                             a name for the output file (tab delimited text)
Options:
                             loci with lower coverage are discarded (default: 3)
       -c coverage
if ($#ARGV < 2 || $ARGV[0] eq "-h") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
getopts('i:r:o:c:h');# in this example a is required, b is optional, h is help
if (!$opt_i || !$opt_r || !$opt_o || $opt_h) {print "\n", "-"x60, "\n", $scriptname,
"\n", $usage, "-"x60, "\n\n"; exit;}
if ($opt_c) {$thd = $opt_c;} else {$thd = 3;}
$infile = $opt_i;
$reffile = $opt r;
$outfile = $opt_o;
# load reference into memory
system("date");
print "beginning analysis of nucleotide frequencies in $infile...\n";
print "Reading reference...\n";
open(REF,$reffile);
while(<REF>)
       {
       chomp;
       if ($_ =~ /\>/)
              $refi = $ ;
       elsif ($_ =~ /[ACGT]/)
              @bits = split("", $_);
               bcount = 0;
              foreach $b (@bits)
                      $bcount++;
                      $rrh{$refi}{$bcount} = $b;
              }
close(REF);
print "Finished loading reference. into memory.\n";
# define positions to be ignored (recognition sites, which are invariant)
foreach $p (13, 14, 15, 22, 23, 24) {\$exch{\$p}++;}
                                                                  # for AlfI
```

```
# read in sam file output line by line
print "Reading alignments and counting nucleotides at each position...\n";
open(IN, $infile);
my %nfh;
while(<IN>)
       {
       if ($_ =~ /^@/) {next;}
       chomp;
       rowi = _;
       $rowi =~ s/^>//;
       @cols = split("\t", $rowi);
       $ncols = @cols;
       if ($cols[2] eq "*") {next;}
       $rawmap++;
# -- build a key for each read matching each position to a reference position
       scig = scols[5];
       @ciga = split(/(?<=\D)/, $cig);</pre>
       @codea = (); $refloc = $cols[3];
       foreach $c (@ciga)
               {
               if ($c = \sim /M/)
                      c = \sim s/M//;
                      for ($d=1; $d<=$c; $d++) {push @codea, $refloc; $refloc++;}</pre>
               elsif ($c = \sim /D/)
                      {
                      c = \sim s/D//;
                      for ($d=1; $d<=$c; $d++) {$refloc++;}</pre>
               elsif ($c = \sim /I/)
                      {
                      c = \sim s/I//;
                      for ($d=1; $d<=$c; $d++) {push @codea, 0;}</pre>
                      }
# -- count each base toward nucleotide frequencies at the corresponding reference
position
       @reada = split("",$cols[9]);
       if (@reada ne @codea) {print "Something is wrong.\n @reada \n @codea\n";
exit;}
       readpos = -1;
       foreach $b (@reada)
               {
               $readpos++;
               if ($codea[$readpos] eq 0) {next;}
               if ($codea[$readpos] eq "") {next;}
               $nfh{$cols[2]}{$codea[$readpos]}{$b}++;
print "Finished reading and counting alignments.\n";
# -- write output, applying coverage threshold per locus
print "Writing output...\n";
open(OUT, ">$outfile");
print OUT "Tag\tLocus\tRef\tA\tC\tG\tT\n";
@nbins = qw\{A C G T\};
rownum = 0;
foreach $ref (sort(keys(%nfh)))
       {
       %refh = %{$nfh{$ref}};
       foreach $loc (sort{$a<=>$b}(keys(%refh)))
```

CombineBaseCounts.pl

```
#!/usr/bin/perl
# written by E Meyer, <a href="mailto:eli.meyer@science.oregonstate.edu">eli.meyer@science.oregonstate.edu</a>
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$usage = <<USAGE;</pre>
Counts the number of times each allele was observed, for each locus, in a collection
of
2bRAD data describing nucleotide frequencies for each locus and sample (the output
from
SAMBaseCounts.pl).
Output format: columns 1=tag, 2=position, 3=reference allele,
5=allele counts for sample 1 (A/C/G/T), 6=for sample 2, etc..
Missing data are shown as "NA" for all alleles.
Usage: $scriptname file_1 file_2 ... file_n > output_file
Where:
       files 1-n:
                      nucleotide frequencies (output from SAMBasecaller.pl) for each
sample
       output_file: a name for the output; tab-delimited text
USAGE
if (\#ARGV < 1 \parallel \#ARGV[0] = q "-h") {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
my %bigh;
my $maxthird = 2;
print "Tag\tLocus\tRef\t";
scountrow = 0;
foreach $argi (0..$#ARGV)
       $countrow++;
       if ($countround==1) {next;}
       $tmpname = $ARGV[$argi];
       #$tmpname = < s/(.+).tab/;
       ($tmpname, $foo) = split (/\.tab/, $tmpname);
       \#$tmpname =~ s/.+\///g;
       print $tmpname, "\t";
       print STDERR "reading ", $ARGV[$argi], "\n";
       open(TAB, $ARGV[$argi]);
       rownum = 0;
       while(<TAB>)
               {
               chomp:
               $rownum++; if ($rownum==1) {next;}
               @cols = split("\t", $_);
               $bigh{$cols[0]}{$cols[1]}{"ref"} = $cols[2];
               $bigh{$cols[0]}{$cols[1]}{$argi}{"A"} = $cols[3];
               $bigh{$cols[0]}{$cols[1]}{$argi}{"C"} = $cols[4];
$bigh{$cols[0]}{$cols[1]}{$argi}{"G"} = $cols[5];
               $bigh{$cols[0]}{$cols[1]}{$argi}{"T"} = $cols[6];
print "\n";
foreach $r (sort(keys(%bigh)))
       ch = { \frac{s}{shigh{sr}}};
       foreach $l (sort{$a<=>$b}(keys(%ch)))
               print $r, "\t", $l, "\t", $bigh{$r}{$l}{"ref"}, "\t";
```

CallGenotypes.pl

```
#!/usr/bin/perl
# written by E Meyer, <a href="mailto:eli.meyer@oregonstate.edu">eli.meyer@oregonstate.edu</a>
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///q:
$usage = <<USAGE;</pre>
Determines SNP genotypes from nucleotide frequencies. Input file contains nucleotide
from multiple samples. Output file lists the genotypes called from those
frequencies.
Usage: $scriptname -i input -o output <OPTIONS>
Required arguments:
                     Input file, tab delimited text file of nucleotide frequencies
       -i input
(output from NtFrequences.pl)
                      column = tag, column 2 = locus, column 3 = reference genotype
                      subsequent columns = nucleotide frequences in each sample, as
A/C/G/T (e.g. 0/0/10/12)
                     A name for the output file (tab delimited text)
       -o output
Options:
       -c coverage
                     Minimum coverage required to determine genotypes. Lower
coverage loci wil be discarded.
                     Default: 10
                     y: exclude terminal positions in alignments where errors may
       -e ends
arise during ligation. (default)
                      n: do not exclude terminal positions.
                      "nf": nucleotide frequencies (classic method; the default).
       -m method
                        This method determines genotypes directly from nucleotide
frequencies, using thresholds
                        defined by the user. If minor allele frequency (MAF) <= x at</pre>
a locus, the genotype is called
                        homozygous for the major allele at that locus. If MAF >= n.
genotype is called heterozygous.
                        Genotypes are not called at intermediate MAF (if x > MAF > n)
where errors are likely.
                       pgf": NF informed by population genotype frequencies. (an
update on the classic method)
                        This method first identifies valid alleles at each locus
based on their frequency in the
                        population (the two most common alleles with frequencies >=y
times in >= q individuals),
                        then applies relaxed nucleotide frequency thresholds for
those alleles (using y instead
                        of n for valid alleles).
                      "bgc" = Bayesian Genotype Caller
                        This method calls the BGC software, which implements a
maximum-likelihood (ML) method for
                        calling genotypes that incorporates prior population-level
information on genotype
                        frequencies and error rates from a genotype-frequency
estimator. For more details see
                        (Maruki and Lynch, [doi: 10.1534/g3.117.039008], and cite
that paper if using this option.
       Options for method "nf" or "pgf":
                     Maximum frequency of the minor allele you're willing to ignore
       -x max_MAF
and call the position
                      homozygous for the major allele (0-1). Default: 0.01
                     Minimum frequency of the minor allele you're willing to accept
       -n min MAF
as evidence of
                      heterozygosity, and call the locus heterozygous (0-1). Default:
0.25
```

```
-r min reads Because low frequencies translate into 1 or fewer reads at low
coverage, the script
                                          also imposes a minimum read number for detection of
heterozygotes. (default: 2)
              Options for method "pgf":
              -y frequency Minimum frequency a second allele must be detected to be
considered valid.
                                          (default: 0.05)
                                          Each allele must present in at least q samples to be considered
              -q samples
valid.
                                          (default: 2)
              Options for method "bgc":
                                          Critical p-value for the chi-square polymorphism test (BGC)
              -p p-value
                                          (default: 0.05)
                                          Coverage at which the pipeline switches from BGC (for low
              -v maxcov
coverage data) to
                                         HGC (for high coverage data). Default=80 (i.e. HGC above 80).
Examples:
    CallGenotypes.pl -i allele_counts.tab -o genotypes.tab
                                                                                                                             # basic usage
    CallGenotypes.pl -i allele_counts.tab -o genotypes.tab -c 20
coverage threshold
    CallGenotypes.pl -i allele_counts.tab -o genotypes.tab -m bgc
                                                                                                                                       # use Bayesian
Genotype Caller
   CallGenotypes.pl -i allele_counts.tab -o genotypes.tab -m pgf -y 0.05
population method
USAGE
if (\#ARGV < 1 \parallel \#ARGV[0] = q "-h") {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel \#ARGV[0] = q \parallel -h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel +h \parallel) {print "\n", "-"x60, "\n", \#ARGV = 1 \parallel +h \parallel) {print "\n", \#ARGV = 1 \parallel +h \parallel}}
$usage, "-"x60, "\n\n"; exit;}
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
$mod1="Bio::SeqI0";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Bio::SeqIO;
$mod1="File::Which";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use File::Which;
# get variables from input
getopts('i:o:e:c:m:x:n:y:q:p:r:v:h');  # in this example a is required, b is
optional, h is help
if (!$opt_i || !$opt_o || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
$ftabfile = $opt_i; # input filename
$outfile = $opt_o; # output filename
$outfile = $opt_o;
                                                                                  {\$method = "nf";}
if ($opt m)
                          {$method = $opt m;} else
if ($method ne "bgc" andand $method ne "nf" andand $method ne "pgf") {print "\n", "-
"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
if ($method eq "bgc")
              $dep1 = "GFE";
              unless (defined(which($dep1))) {print $dep1, " not found. Exiting.\n"; exit;}
              $dep1 = "BGC";
              unless (defined(which($dep1))) {print $dep1, "not found. Exiting.\n"; exit;}
              $dep1 = "HGC";
              unless (defined(which($dep1))) {print $dep1, " not found. Exiting.\n"; exit;}
if ($opt_c) {$mincov = $opt_c;} else {$mincov = 10;}
                                                                                                                             # default min
coverage to be genotyped
if ($opt_x) {$maxhom = $opt_x;} else {$maxhom = 0.01;}
                                                                                                                           # default maximum MAF
ignored and called homozygous for major allele
if ($opt n) {$minhet = $opt n;} else {$minhet = 0.25;}
                                                                                                                           # default minimum MAF
considered heterozygous
```

```
if ($opt r) {$minreads = $opt r;} else {$minreads = 2;}
                                                                 # default minimum MA
read depth for heterozygous calls
if ($opt_y) {$minall = $opt_y;} else {$minall = 0.05;}
                                                                 # default minimum
coverage per allele to define valid alleles
if ($opt_q) {$minsamp = $opt_q;} else {$minsamp = 2;}
                                                                 # default minimum
number of samples to define valid alleles
if ($opt_p) {$bgcp = $opt_p;} else {$bgcp = 0.05;}
                                                                 # default critical p
value for polymorphism test (BGC)
if ($opt_e) {$eopt = $opt_e;} else {$eopt = "y";}
                                                          # exclude terminal positions
from alignments
if ($opt v) {$maxcov = $opt v;} else {$maxcov = 80;}
                                                                 # max coverage for
system("date");
# Nucleotide Frequency Method - the classic approach
if ($method eq "nf")
{
# make a big hash of all base calls
print "Reading $ftabfile ...\n";
my %bh;
@boa = qw{A C G T};
open(FTAB, $ftabfile);
rowno = 0;
while(<FTAB>)
       {chomp;
       $rowno++;
       @cols = split("\t", $_);
       if ($rowno == 1)
              @header = @cols;
              next;
       for ($i=3; $i<@cols; $i++)</pre>
              @bases = split("/", $cols[$i]);
              $jno = 0;
              foreach $j (@bases)
                     $bh{$cols[0]}{$cols[1]}{$header[$i]}{$boa[$jno]} = $j;
                     $jno++;
              }
print "Finished. \n";
print $rowno-1, " loci with nucleotide frequency data. \n";
system("date");
close(FTAB);
@samlabs = @header[3..@cols-1];
# loop through all loci and samples, applying genotyping rules using threshold
defined above
# in this method, genotyping is based directly on nucleotide frequency thresholds
print "Determining genotypes from nucleotide frequencies ...\n";
open(OUT,">$outfile");
print OUT "scaffold", "\t", "position", "\t", join ("\t", @samlabs), "\n";
@sites = sort(keys(%bh));
$countgt = $gtlocno = $sampno = 0;
foreach $s (@sites)
       %siteh = %{$bh{$s}};
       @loca = sort{$a<=>$b}(keys(%siteh));
       foreach $1 (@loca)
              $sgt = 0;
```

```
if ($l == 1 | $l == 36)
                      if($eopt eq "y")
                             next:
                             }
                     }
              %loch = %{$siteh{$l}};
              @gta = ();
              foreach $s (@samlabs)
                      {
                      %samh = %{$loch{$s}};
                     %tmph = ();
foreach $b (@boa) {$tmph{$b} = $samh{$b};}
                      @saa = sort{$tmph{$b}<=>$tmph{$a}}(keys(%tmph));
                      $majmin = $tmph{$saa[0]}+$tmph{$saa[1]};
                      $minreject = $majmin * $maxhom;
                      $gti = ""; $rati = "";
                      print $saa[0], ":", $tmph{$saa[0]}, ",", $saa[1], ":",
$tmph{$saa[1]}, "[", $majmin, "]";
# first conditions where we arent interested in or cannot compare nucleotide
frequencies
# exclude loci with >2 alleles observed, and loci with low coverage
                      if (($tmph{$saa[2]}>=$minreject)||($majmin<$mincov))</pre>
                             \{\$gti = 0;\}
# then conditions where we can compare nucleotide frequencies
                      else
                             $rati = $tmph{$saa[1]}/($majmin);
                             print "(", $rati, ")";
#
                             if ($rati<$maxhom) {$gti = $saa[0];}</pre>
                             elsif ($rati>=$minhet) {@rsa = sort(@saa[0..1]); $gti =
"@rsa";}
                             elsif (($rati >= $maxhom and and $rati <</pre>
$minhet)||($tmph{$saa[1]} < $minreads)) {$gti = 0;}</pre>
                      print "\t";
#
                      push @gta, $gti;
                      if ($gti =~ /[ACGT]/) {$sgt++;}
              print join("\t", @gta), "\n";
#
              $countgt += $sgt;
              if ($sgt > 0)
                      {
                      $gtlocno++;
                      print OUT $s, "\t", $l, "\t", join("\t", @gta), "\n";
              }
print "Finished. \n";
$sampno = @samlabs;
print "Called genotypes at $gtlocno loci in $sampno samples ($countgt genotypes
altogether)\n";
system("date");
# Nucleotide Frequencies Informed by Population Genotype Frequencies
# in this method, valid alleles are defined at each locus by counting the number of
times each
# allele occurs at high enough coverage in enough samples. Each sample is then
genotyped only for those
# alleles based on observed numbers and user defined nucleotide frequency
thresholds. The increased
# confidence in the valid alleles provided by population analysis makes it possible
to relax MAF
```

```
# threshold for heterozygous calls, determining genotypes at loci with intermediate
MAFs that could
# otherwise not be determined directly from nucleotide frequencies.
if ($method eq "pgf")
# make a big hash of all base calls
print "Reading $ftabfile ...\n";
my %bh;
@boa = qw{A C G T};
open(FTAB, $ftabfile);
rowno = 0:
while(<FTAB>)
       {chomp;
       $rowno++;
       @cols = split("\t", $_);
       if (srowno == 1)
               @header = @cols;
               next;
       for ($i=3; $i<@cols; $i++)
               @bases = split("/", $cols[$i]);
               $jno = 0;
               foreach $j (@bases)
                      $bh{$cols[0]}{$cols[1]}{$header[$i]}{$boa[$jno]} = $j;
                      $jno++;
               }
       }
print "Finished. \n";
print $rowno-1, " loci with nucleotide frequency data. \n";
system("date");
close(FTAB);
@samlabs = @header[3..@cols-1];
# loop through all loci and samples, applying genotyping rules using thresholds
defined above.
print "Determining genotypes from population genotype frequencies and sample
nucleotide frequencies ...\n";
open(OUT,">$outfile");
print OUT "scaffold", "\t", "position", "\t", join ("\t", @samlabs), "\n";
@sites = sort(keys(%bh));
foreach $s (@sites)
       {
       %siteh = %{$bh{$s}};
       @loca = sort{$a<=>$b}(keys(%siteh));
# filter out terminal bases
       foreach $1 (@loca)
               $sgt = 0;
               if ($l == 1 | $l == 36)
                      if($eopt eq "y")
                             {
                             next;
                              }
                      }
               %loch = %{$siteh{$l}};
               @gta = (); %vah = ();
# first identify valid alleles
               foreach $s (@samlabs)
                      %samh = %{$loch{$s}};
```

```
%tmph = (); $covi = 0;
                      foreach $b (@boa) {$tmph{$b} = $samh{$b}; $covi += $samh{$b};}
                      @saa = sort{$tmph{$b}<=>$tmph{$a}}(keys(%tmph));
                             if ((($tmph{$saa[0]}/$covi) >=
$minall)andand($tmph{$saa[0]} >= $minreads)) {$vah{$saa[0]}++;}
                              if ((($tmph{$saa[1]}/$covi) >=
minall) and and (\text{mph}\{\text{saa}[1]\} >= \text{minreads})) {\text{svah}\{\text{saa}[1]\}++;}
                             }
              @tmp = sort{$vah{$b}<=>$vah{$a}}(keys(%vah));
              %sva = (); $nva = 0;
              foreach $t (@tmp)
                      if ($vah{$t} >= $minsamp) {$sva{$t}++; $nva++;}
              print $s, "\t", $l, "\t", $nva, " valid alleles\n";
# filter out positions with more than two alleles
# then genotype samples based on frequency of those alleles in each sample
# taking into account the population genotype frequencies determined above
              foreach $s (@samlabs)
                      %samh = %{$loch{$s}};
                      %tmph = ();
                      foreach $b (@boa) {$tmph{$b} = $samh{$b};}
                      @saa = sort{$tmph{$b}<=>$tmph{$a}}(keys(%tmph));
                      $majmin = $tmph{$saa[0]}+$tmph{$saa[1]};
                      $minreject = $majmin * $maxhom;
                      $gti = ""; $rati = "";
                      print $saa[0], ":", $tmph{$saa[0]}, ",", $saa[1], ":",
$tmph{$saa[1]}, "[", $majmin, "]";
# first conditions where we arent interested in or cannot compare nucleotide
frequencies
# exclude loci with >2 alleles observed, and loci with low coverage
                      if (($tmph{$saa[2]}>=$minreject)||($majmin<$mincov))</pre>
                             $gti = 0;
                             print "r";
# then conditions where we can compare nucleotide frequencies
                      else
                             $rati = $tmph{$saa[1]}/($majmin);
                             print "(", $rati, ")";
#
                             if ($rati<$maxhom) {$gti = $saa[0];}</pre>
                             elsif ($rati>=$minhet) {@rsa = sort(@saa[0..1]); $gti =
"@rsa";}
                             elsif (($rati >= $maxhom and and $rati <</pre>
$minhet)||($tmph{$saa[1]} < $minreads))</pre>
                                     if(exists($sva{$saa[1]}))
                                                                  # if minor allele in
this sample is one
                                            {
                                                                   # of top 2 in pgf,
handle this way
                                            if (($rati >= $minall) andand
($tmph{$saa[1]} >= $minreads))
                                                    {@rsa = sort(@saa[0..1]); $gti =
"@rsa":}
                                            else
                                                    \{ \text{sgti} = 0; \}
                                            }
                                                                  # if minor allele is
                                     else
not one of the top
                                            {
                                                                  # 2 in pgf handle it
this way
```

```
$qti = 0;
                                    }
                     print "\t";
#
                     push @gta, $gti;
                     if ($gti ne 0) {$sgt++;}
              print join("\t", @gta), "\t", $sgt, "\n";
#
              $countgt += $sgt;
              if ($sgt > 0)
                     {
                     $gtlocno++;
                     print OUT $s, "\t", $l, "\t", join("\t", @gta), "\n";
              }
print "Finished. \n";
$sampno = @samlabs;
print "Called genotypes at $gtlocno loci in $sampno samples ($countgt genotypes
altogether)\n":
system("date");
# Bayesian Genotyper Caller Method
if ($method eq "bgc")
{
# estimate genotype frequencies in population
$gfe_out = $ftabfile.".gfe.out";
print "Running GFE ...\n";
system "GFE -cv $bgcp -mode c -as 1 -min_cov $mincov -max_cov $maxcov -in $ftabfile
-out $gfe_out > gfe_log.txt";
print "Finished.\n";
system("date");
# call genotypes at low coverage sites using ML approach informed by prior
population estimates
$bgc_out = $ftabfile.".bgc.out";
print "Running BGC ...\n";
system "BGC -as 1 -min_cov $mincov -max_cov $maxcov -in $gfe_out -out $bgc_out >
bgc_log.txt";
print "Finished.\n";
system("date");
# call genotypes at high coverage sites using ML approach informed by prior
population estimates
$hgc_out = $ftabfile.".hgc.out";
print "Running HGC ...\n";
system "HGC -in $ftabfile -out $hgc_out -min_cov $maxcov > hgc_log.txt";
$numrows=`wc -l $hgc_out`;
numrows = ~ s/\s.+//;
chomp($numrows);
if ($numrows eq 1) {goto HGC;}
                                           # because HGC sometimes exits without
throwing an error. This catches it.
print "Finished.\n";
system("date");
# integrate output from BGC and HGC
$rownum=0;
open(IN, $hgc_out);
while(<IN>)
       {
       chomp;
       $rownum++;
       @cols = split("\t", $_);
```

```
if ($rownum==1) {@header=@cols; next;}
       $len=@cols; $end=$len-8;
       for ($a=11; $a<$end; $a++)
              unless ($cols[$a] eq "NA")
                      $fh{$cols[0]}{$cols[1]}{$header[$a]} = $cols[$a];
              }
       }
close(IN);
open(IN, $bgc_out);
open(OUT, ">integrated_bgc.tab");
$rownum=0;
while(<IN>)
       {
       chomp;
       $rownum++;
       @cols = split("\t", $_);
       if ($rownum==1) {print OUT $_, "\n"; @header=@cols; next;}
       $end=@cols-1;
       for ($a=5; $a<$end; $a++)
              if(exists($fh{$cols[0]}{$cols[1]}{$header[$a]}))
                      $cols[$a] = $fh{$cols[0]}{$cols[1]}{$header[$a]};
       print OUT join("\t", @cols), "\n";
close(IN);
# convert output to same format as the other methods
print "Converting output file...\n";
open(OUT, ">$outfile");
foreach $a (qw{0 1 2 3 4})
       {$exc_col{$a}++;}
foreach a (qw{2 3 4})
       {$exa_col{$a}++;}
open(IN, "integrated_bgc.tab");
$rownum = 0;
while(<IN>)
       {
       $rownum++;
       chomp;
       @cols = split("\t", $_);
       $cno = 0;
       if ($rownum==1)
              foreach $c (@cols)
                      unless (exists($exa_col{$cno}))
                             print OUT $c, "\t";
                      $cno++;
              print OUT "\n";
              next:
              }
       nonmd = 0; scno = 0;
       foreach $c (@cols)
              unless (exists($exc_col{$cno}))
```

```
{
    if ($c ne "NA")
                                                  {$nonmd++;}
                $cno++;
        if ($nonmd < 1) {next;}</pre>
        if ($eopt eq "y")
                if ($cols[1] == 1 || $cols[1] == 36)
                        {
                         next;
                         }
                }
        $cno = 0; $gtino = 0;
        foreach $c (@cols)
                if ($cno < 2)
                         {
                         print OUT $c, "\t";
                unless (exists($exc_col{$cno}))
                         @alls = split("", $c);
if ($c eq "NA")
                                 print OUT "0\t";
                         elsif ($alls[0] eq $alls[1])
                                 $countgt++; $gtino++;
print OUT $alls[0], "\t";
                         else
                                 $countgt++; $gtino++;
print OUT $alls[0], " " , $alls[1], "\t";
                $cno++;
        if ($gtino>0) {$gtloc++;}
        print OUT "\n";
print "Finished.\n";
$sampno = @cols - 5;
print "Genotyped $gtloc loci in $sampno samples ($countgt genotypes altogether)\n";
system("date");
```

PolyFilter.pl

```
#!/usr/bin/env perl
# written by E Meyer, eli.meyer@science.oregonstate.edu
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///g:
$usage = <<USAGE;</pre>
Excludes loci containing too few classes of genotypes or numbers of alleles (keeps
polymorphic loci).
Usage: $scriptname -i input <OPTIONS>
Required arguments:
       -i input
                      name of the input file, a matrix of genotypes or allele counts
                      (see -m for format)
Options:
       -n genotypes minimum number of unique genotypes (for -m g) or alleles (for -
ma)
                      required to consider a locus polymorphic (default=2)
                      g=genotypes (default).
       -m mode
                        Input file contains genotypes from individuals.
                        Input format: rows=loci and columns=samples.
                           row 1 = \text{column label, column } 1 = \text{tag, column } 2 = \text{position}
                           subsequent columns contain genotypes for each sample
                      a=allele counts.
                        Input file contains allele counts from pooled samples.
                        Input format: rows=loci and columns=samples.
                           row 1 = \text{column label}, \text{column } 1 = \text{tag}, \text{column } 2 = \text{position}
                           column 3 = major allele, column 4 = minor allele
                           subsequent pairs of columns contain allele counts
                        (major then minor) for each sample
       -v min cov
                      (for -m a) minimum coverage required to consider an allele
present
                      (default=2)
       -s min_samp
                      minimum number of samples in which an allele must be present to
be counted.
                      (default=1)
                      y=print filtered loci and summary; n=only print summary
       -p option
                      (default=n)
                      a name for the output file (loci passing this filter) (required
       -o output
if -p y)
USAGE
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
qetopts('i:o:n:p:s:v:m:h'); # in this example a is required, b is optional, h is
if (!$opt_i || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60,
"\n\n"; exit;}
if ($opt_n) {$minall = $opt_n;} else {$minall = 2;}
                                                          # min genotypes or alleles
to consider polymorphic
if ($opt p) {$pvar = $opt p;} else {$pvar = "n";}# whether to print loci passing
filter
if (pvar eq "y" and and !print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
if ($opt_o) {$ovar = $opt_o;} else {$ovar = "null";}
                                                           # name for output file
if ($opt_m) {$mode = $opt_m;} else {$mode = "g";}# mode
if ($mode ne "g" and and $mode ne "a") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
if ($opt_v) {$mincov = $opt_v;} else {$mincov = 2;} # min cov, for -m a
```

```
if ($opt s) {$minfreq = $opt s;} else {$minfreq = 1;} # min samps, for -m a
my $opt = $pvar;
# mode q
if ($mode eq "g")
open(IN, $opt_i);
open(OUT, ">$ovar");
while(<IN>)
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
       @cols = split("\t", $_);
       $ncols = @cols;
       ahi = ();
       for ($a=2; $a<$ncols; $a++)</pre>
               if ($cols[$a] eq "0") {next;}
               $ahi{$cols[$a]}++;
              }
       @alla = sort(keys(%ahi));
       passno = 0;
       foreach $z (@alla)
               if ($ahi{$z} >= $minfreq) {$passno++;}
       $nalla = @alla;
       if ($passno < $minall) {$nopoly++; next;}</pre>
       if ($opt eq "y") {print OUT $_, "\n";}
close(IN); close(OUT);
print STDERR $rowcount-1, " loci in input file\n";
print STDERR $nopoly, " loci with fewer than ", $minall, " genotypes\n";
print STDERR $rowcount-1-$nopoly, " polymorphic loci (at least ", $minall, "
genotypes in $minfreq or more samples)\n";
# mode a
elsif ($mode eq "a")
open(IN, $opt_i);
open(OUT, ">$ovar");
while(<IN>)
       {
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
       @cols = split("\t", $_);
       $ncols = @cols;
       $gt_no = $alt_no = $alt_freq = 0;
       for ($a=4; $a<$ncols; $a+=2)</pre>
               {
               if ($cols[$a] eq "NA") {next;}
               $gt_no++;
               if ($cols[$a+1] >= $mincov)
                       $alt_no++;
       $alt_freq = $alt_no / $gt_no;
       if ($alt_freq eq 0) {$mono++; next;}
       if ($alt_freq < $minfreq) {$toolow++; next;}
if ($opt eq "y") {print OUT $_, "\n";}</pre>
```

MDFilter.pl

```
#!/usr/bin/env perl
# written by E Meyer, eli.meyer@science.oregonstate.edu
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///q:
$usage = <<USAGE;</pre>
Excludes loci containing too many missing data (genotyped in too few samples)
Usage: $scriptname -i input -n min data <OPTIONS>
Required arguments:
       −i input
                      name of the input file, a matrix of genotypes or allele counts
                      (see -m for format)
       -n min data
                      loci that were genotyped in fewer samples than this will be
excluded
Options:
       -m mode
                      q=qenotypes (default).
                        Input file contains genotypes from individuals.
                        Input format: rows=loci and columns=samples.
                           row 1 = \text{column label, column } 1 = \text{tag, column } 2 = \text{position}
                           subsequent columns contain genotypes for each sample
                      a=allele counts.
                        Input file contains allele counts from pooled samples.
                        Input format: rows=loci and columns=samples.
                           row 1 = \text{column label, column } 1 = \text{tag, column } 2 = \text{position}
                           column 3 = major allele, column 4 = minor allele
                           subsequent pairs of columns contain allele counts
                        (major then minor) for each sample
       -p option
                      y=print filtered loci and summary; n=only print summary
                      (default=n)
       -o output
                      a name for the output file (loci passing this filter) (required
if -p y)
USAGE
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
getopts('i:o:n:p:m:h');
                             # in this example a is required, b is optional, h is
help
if (!$opt_i || !$opt_n || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if ($opt_p) {$pvar = $opt_p;} else {$pvar = "n";}# whether to print loci passing
filter
if ($pvar eq "y" andand !$opt_o) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if ($opt o) {$ovar = $opt o;} else {$ovar = "null";}
                                                          # name for output file
if ($opt_m) {$mode = $opt_m;} else {$mode = "g";} # name for output file
if ($mode ne "g" andand $mode ne "a") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
my $mindat = $opt n;
my $opt = $pvar;
# mode g
if ($mode eq "q")
{
open(IN, $opt_i);
open(OUT, ">$ovar");
$toofew = 0;
```

```
while(<IN>)
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
       @cols = split("\t", $_);
       $ncols = @cols;
       if ($rowcount==2)
              {\rm maxmiss} = {\rm sncols} - 2 - {\rm smindat};
       $missi = 0;
       for ($a=2; $a<$ncols; $a++)</pre>
              if ($cols[$a]eq "0") {$missi++;}
       if ($missi>$maxmiss) {$toofew++; next;}
       if ($opt eq "y") {print OUT $_, "\n";}
close(IN); close(OUT);
print STDERR $rowcount-1, " loci\n";
print STDERR $toofew, " loci with more than ", $maxmiss, " missing data (i.e.
genotyped in fewer than $mindat samples)\n";
print STDERR $rowcount-1-$toofew, "loci with no more than more than ", $maxmiss, "
missing data\n";
}
# mode a
elsif ($mode eq "a")
open(IN, $opt_i);
open(OUT, ">$ovar");
$toofew = 0;
while(<IN>)
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
       @cols = split("\t", $_);
       $ncols = @cols;
       if ($rowcount==2)
              \max = (\text{sncols} - 4)/2 - \min ;
       missi = 0;
       for ($a=4; $a<$ncols; $a+=2)</pre>
              if (($cols[$a] eq 0 and $cols[$a+1] eq 0)||($cols[$a] eq "NA"
andand $cols[$a+1] eq "NA")) {$missi++;}
       if ($missi>$maxmiss) {$toofew++; next;}
       if ($opt eq "y") {print OUT $_, "\n";}
       ļ
close(IN); close(OUT);
print STDERR $rowcount-1, " loci\n";
print STDERR $toofew, " loci with more than ", $maxmiss, " missing data (i.e.
genotyped in fewer than $mindat samples)\n";
print STDERR $rowcount-1-$toofew, " loci with no more than more than ", $maxmiss, "
missing data\n";
}
if (-e "null")
        system("rm null");
```

RepTagFilter.pl

```
#!/usr/bin/env perl
# written by E Meyer, <a href="mailto:eli.meyer@science.oregonstate.edu">eli.meyer@science.oregonstate.edu</a>
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///g:
$usage = <<USAGE;</pre>
Excludes tags containing too many SNPs, suggesting repetive regions of the genome
Usage: $scriptname -i input -n max snps <OPTIONS>
Required arguments:
       −i input
                        name of the SNP input file, a matrix of genotypes or allele
counts
                        (see -m for format)
                        note: the script assumes the input only includes polymorphic
loci
                        all SNPs from tags containing more than this number of SNPs
       -n max_snps
will be excluded
Options:
        -m mode
                        g=genotypes (default).
                          Input file contains genotypes from individuals.
                          Input format: rows=loci and columns=samples.
                             row 1 = \text{column label}, \text{column } 1 = \text{tag}, \text{column } 2 = \text{position}
                             subsequent columns contain genotypes for each sample
                        a=allele counts.
                          Input file contains allele counts from pooled samples.
                          Input format: rows=loci and columns=samples.
                             row 1 = \text{column label}, \text{column } 1 = \text{tag}, \text{column } 2 = \text{position}
                             column 3 = major allele, column 4 = minor allele
                             subsequent pairs of columns contain allele counts
                          (major then minor) for each sample
                        y=print filtered loci and summary; n=only print summary
        -p option
                        (default=n)
                        a name for the output file (loci passing this filter) (required
        -o output
if -p y
USAGE
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
                              # in this example a is required, b is optional, h is
getopts('i:o:n:p:m:h');
if (!$opt_i || !$opt_n || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if ($opt_p) {$pvar = $opt_p;} else {$pvar = "n";}# whether to print loci passing
filter
if ($pvar eq "y" andand !$opt_o) {print "\n", "-"x60, "\n", $scriptname, "\n",
$usage, "-"x60, "\n\n"; exit;}
if ($opt_o) {$ovar = $opt_o;} else {$ovar = "null";}
                                                               # name for output file
if ($opt_m) {$mode = $opt_m;} else {$mode = "g";}# name for output file if ($mode ne "g" and and $mode ne "a") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
# note that choice of mode does nothing in this script
# its only included for consistency with other filtering scripts
my $maxsnps = $opt n;
my $opt = $pvar;
open(IN, $opt_i);
```

```
open(OUT, ">$ovar");
while(<IN>)
         chomp;
         $rowcount++;
         if($rowcount==1){next;}
        @cols = split("\t", $_);
$ncols = @cols;
         $ch{$cols[0]}++;
close(IN);
$rowcount = 0;
open(IN, $opt_i);
while(<IN>)
         {
        chomp;
        $rowcount++;
         if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
        @cols = split("\t", $_);
         $ncols = @cols;
         if ($ch{$cols[0]}>$maxsnps) {$toomany++; next;}
         if ($opt eq "y") {print OUT $_, "\n";}
close(IN); close(OUT);
if (-e "null")
         system("rm null");
print STDERR $rowcount-1, " loci\n";
print STDERR $toomany, " loci with more than ", $maxsnps, " SNPs per tag\n";
print STDERR $rowcount-1-$toomany, " loci with no more than more than ", $maxsnps, "
SNPS per tag\n";
```

OneSNPPerTag.pl

```
#!/usr/bin/env perl
# written by E Meyer, eli.meyer@science.oregonstate.edu
# distributed without any guarantees or restrictions
# -- check arguments and print usage statement
$scriptname=$0: $scriptname =~ s/.+\///g:
$usage = <<USAGE;</pre>
Selects a single SNP from each tag in a matrix or genotypes or allele counts.
Chooses the locus with the least missing data.
Usage: $scriptname -i input <OPTIONS>
Required arguments:
       -i input
                       name of the input file, a matrix of genotypes or allele counts
                        (see -m for format)
Options:
        -m mode
                        g=genotypes (default).
                          Input file contains genotypes from individuals.
                          Input format: rows=loci and columns=samples.
                             row 1 = \text{column label}, \text{column } 1 = \text{tag}, \text{column } 2 = \text{position}
                             subsequent columns contain genotypes for each sample
                        a=allele counts.
                          Input file contains allele counts from pooled samples.
                          Input format: rows=loci and columns=samples.
                             row 1 = \text{column label}, \text{column } 1 = \text{tag}, \text{column } 2 = \text{position}
                             column 3 = major allele, column 4 = minor allele
                             subsequent pairs of columns contain allele counts
                          (major then minor) for each sample
                       y=print filtered loci and summary; n=only print summary
       -p option
                        (default=n)
                       a name for the output file (loci passing this filter) (required
       -o output
if -p y
USAGE
# -- module and executable dependencies
$mod1="Getopt::Std";
unless(eval("require $mod1")) {print "$mod1 not found. Exiting\n"; exit;}
use Getopt::Std;
# get variables from input
getopts('i:o:p:m:h');# in this example a is required, b is optional, h is help
if (!$opt_i || $opt_h) {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60,
"\n\n"; exit;}
if ($opt_p) {$pvar = $opt_p;} else {$pvar = "n";}# whether to print loci passing
filter
if (pvar eq "y" and and !print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
if ($opt_o) {$ovar = $opt_o;} else {$ovar = "null";}
                                                              # name for output file
if ($opt_m) {$mode = $opt_m;} else {$mode = "g";}# name for output file
if ($mode ne "g" and and $mode ne "a") {print "\n", "-"x60, "\n", $scriptname, "\n", $usage, "-"x60, "\n\n"; exit;}
my $opt = $pvar;
if ($mode eq "q")
{
open(IN, $opt_i);
open(OUT, ">$ovar");
while(<IN>)
        {
        chomp;
        $rowcount++;
        if($rowcount==1){next;}
```

```
@cols = split("\t", $_);
       $ncols = @cols;
       $mdcount = 0;
       foreach $a (@cols[2..$ncols]) {if ($a eq "0") {$mdcount++;}}
       $ch{$cols[0]}{$cols[1]} = $mdcount;
close(IN);
foreach $tag (sort(keys(%ch)))
       %tagh = %{$ch{$tag}};
       @sorted = sort{$tagh{$a}<=>$tagh{$b}}(keys(%tagh));
       $useh{$tag} = $sorted[0];
open(IN, $opt_i);
$rowcount = 0;
while(<IN>)
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
       @cols = split("\t", $_);
       if($useh{$cols[0]} ne $cols[1]) {next;}
       if ($opt eq "y") {print OUT $_, "\n";}
       $kept++;
close(IN); close(OUT);
print STDERR $rowcount-1, " loci\n";
print STDERR $kept, " SNPs selected.\n";
elsif ($mode eq "a")
{
open(IN, $opt_i);
open(OUT, ">$ovar");
while(<IN>)
       chomp;
       $rowcount++;
       if($rowcount==1){next;}
       @cols = split("\t", $_);
       $ncols = @cols;
       mdcount = 0;
       for ($a=4; $a<$ncols; $a+=2)</pre>
              {
              if (($cols[$a] eq "0" and and $cols[$a+1] eq "0")||($cols[$a] eq "NA"
andand $cols[$a+1] eq "NA"))
                      {$mdcount++;}
       $ch{$cols[0]}{$cols[1]} = $mdcount;
close(IN);
foreach $tag (sort(keys(%ch)))
       %tagh = %{$ch{$tag}};
       @sorted = sort{$tagh{$a}<=>$tagh{$b}}(keys(%tagh));
       $useh{$tag} = $sorted[0];
$rowcount = 0;
open(IN, $opt_i);
while(<IN>)
       {
       chomp;
       $rowcount++;
       if($rowcount==1){if($opt eq "y") {print OUT $_, "\n";} next;}
```

```
@cols = split("\t", $_);
    if($useh{$cols[0]} ne $cols[1]) {next;}
    if ($opt eq "y") {print OUT $_, "\n";}
        $kept++;
    }
close(IN);
print STDERR $rowcount-1, " loci\n";
print STDERR $kept, " SNPs selected.\n";
}
if (-e "null")
    {
        system("rm null");
    }
}
```

Appendix E

>Haplotype: CAT1 GB#MN784029 (Sample: RPCAT1)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGGTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATTGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: CAT2 GB#MN784030 (Sample: RPCAT2)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAAATATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTGTCACCACTATGGCAT

>Haplotype: CAT3 GB#MN784031 (Sample: RPCAT3)

>Haplotype: CAT4 GB#MN784032 (Sample: RPCAT4)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGGTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAA GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAAATATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: CAT5 GB#MN784033 (Sample: RPCAT5)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAAATATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: CAT6 GB#MN784034 (Sample: RPCAT6)

>Haplotype: CAT7 GB#MN784035 (Sample: RPCAT7)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATTGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGGGGGTAAA GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: CAT8 GB#MN784036 (Sample: RPCAT8)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATACTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGGGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: CAT9 GB#MN784044 (Sample: TS263)

>Haplotype: CAT10 GB#MN784045 (Sample: TS264)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTTGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: CAT11 GB#MN784046 (Sample: TS270)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: CAT12 GB#MN784047 (Sample: TS272)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT

CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: CAT13 GB#MN784048 (Sample: TS273)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: DW1 GB#MN784037 (Sample: RPDW1)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTTCTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: LB1 GB#MN784038 (Sample: RPLBH1)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: LB2 GB#MN784039 (Sample: RPLBH2)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGGTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATTGTTACGGGAGATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: LB3 GB#MN784040 (Sample: RPLBH3)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TCTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT

>Haplotype: LB4 GB#MN784041 (Sample: RPLBH4)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: LB5 GB#MN784042 (Sample: RPLBH5)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: LB6 GB#MN784043 (Sample: RPLBH6)

>Haplotype: LJ1 GB#MN784019 (Sample: RP001)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGATAATTGGGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: LJ2 GB#MN784020 (Sample: RP002)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TCATTAATCCTCATTACTAGAAAATCCTCTACTCCATTACAATCATTCACTCCCCCTCATTAATTTACTTC TAATACTGCTTATGCGGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTG GTACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTTT TTTAATTGGGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGAT TTTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGCTACCCTTGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGGGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATT TATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGA ATTAATTATCATCATTATATATAGTGTCACACGTATGGCAT

>Haplotype: LJ3 GB#MN784021 (Sample: RP003)

>Haplotype: LJ4 GB#MN784022 (Sample: RP116)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTGTCACAATGGCAT

>Haplotype: LJ5 GB#MN784023 (Sample: RP216)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: LJ6 GB#MN784024 (Sample: RP316)

>Haplotype: LJ7 GB#MN784025 (Sample: RP416)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTCTTAATCGTCTCAAGAT TTCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATT TATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGA ATTAATTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: LJ8 GB#MN784026 (Sample: RP516)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: LJ9 GB#MN784027 (Sample: RP616)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTTGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: LJ10 GB#MN784028 (Sample: RP716)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTACCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGGGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTTGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAAATACTTTAACATTCATAGGACATTT TTTGTCAAGATTGAGGTAATTAATTGTTACGGGGGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTATCACACGTATGGCAT

>Haplotype: GN1 GB#MN784006 (Sample: LCRP3)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAAAGAAGCAATCGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATTGTTACGGGAGAATAATTGGGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT

>Haplotype: GN2 GB#MN784007 (Sample: LCRP4)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: GN3 GB#MN784008 (Sample: LCRP5)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGGTGGGGAAATAACCAATACTTTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATTGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATTGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGGTACCCTCGACATAATATTTGTATGACCTAAATACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATTGTTACGGGAGATAATTGGGAAGTCATAATTGACCCAGTGGGTAAA GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTGTTCATTATATGGCAT

>Haplotype: GN4 GB#MN784009 (Sample: LCRP11)

>Haplotype: GN5 GB#MN784010 (Sample: LCRP13)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGGGGGGTGGGGAGAAATAACCAATACTTTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATTGGGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAAAGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGGGCTACCCTCGACATAATATTTGTATGGCCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGGCCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATATAGTGTCCCAATATGGCAT

>Haplotype: GN6 GB#MN784011 (Sample: LCRP22)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGGGGGGGGGGGAAATAACCAATACTTTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTTT TTAATTGGGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATTGTTACGGGAGAATAATTGGGAAGTCATAATTGGCCCAGTGGGTAAA GAATAATCAGATATACATTTAAGAAAGGCATTTCATGGACTTTGACCCTTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCTCACTATGGCATGCTAGTGTAGCTT

>Haplotype: GN7 GB#MN784012 (Sample: LCRP48)

>Haplotype: GN8 GB#MN784013 (Sample: LCRP49)

CTTAATGGCCGAAGTCCATGAAATGTCTTTCTTAAATGTATATCTGATTATTCATTACCCCCTGGGTCAAT TATGACTTCCCAATTATTCTCCCGTAACGATTAATTACCTCAATCTTGACAAATAATGTCCTATGAATGTT AAAGTAGTTAGGTCATACAAATATTATGTCAAGGGTAGCACAGGTTTAAATGATAAAGCCGTATCCAACCC TGGGGGGTTAACGATTGCTTCATTCCCCTAAAAAAAGACTGGGAGGACTGAAAAAATCTTGAAACGATTAAA AGTCGAAATGACAATTAAGGGAACTAGAGGAAAAGGAGTTCTGACGCGATTAAAAAAACGTATACCTTTTTA AGGGTGCCAAATGAGGGATTTGAACAATTGATGGGGATTATTCGATTACAGTACCACAAACCGTGCAAAGG GGTCTTATAAGAGAGTATTGGTTATTTCTCGCCAGCCCGCATAAGCAGTATTAGAAGTAAATTAATGAGGG TGAGTGAATGATTGTAATGGAGTAGAGGATTTTCTAGTAATGAGGATTAATGATACGTAAGATGTAATGAA ATAGGGGAATGTCTATTAATGGGGATTAAGCATAGTATGTTAATGAGGATTAAGCATAGTATGTACTATGG GGACAGTCTATTAATGAGGATTAAGCATAGCATGTAATGGAGTAGGGGAGTATGGATTAATGGGGATTAAG TAAATAGTTTATATGTCAGACTGACCAACCTGCATAATTTTTGCAATTGCATTTGTGTTTTTCACAAATGCAT TTGCGTTCACGCAAGTACATGCGTTTATGCAGGTTGGT

>Haplotype: GN9 GB#MN784014 (Sample: LCRP50)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TCTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: GN10 GB#MN784015 (Sample: LCRP51)

>Haplotype: GN11 GB#MN784016 (Sample: LCRP54)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTTTTTTATAAGACCCCTTTGCACGGTTTGTGG GTACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTTT TTTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTTCAAGAT TTTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGGTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGCTAATTAATTGTTACGGGAGAATAATTGTGAAGTCATAATTGACCCAGGGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGGCCATTAAGTGTAGTGAACAACTATT TATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACAGTGTAG AATTAATTTATCATCATTATATATATAGTGTCACGCTATGGCAT

>Haplotype: GN12 GB#MN784018 (Sample: LCRP56)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATATATATCACACTATGGCAT

>Haplotype: GN13 GB#MN784017 (Sample: LCRP57)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGGGAGAAATAACCAATACTCTTTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTCGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATTGTTTCGGGAGAATAATTGTGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGGCTTTGACCATTAAGTGTAGTGAACAAATATTT AATTAATGTAAGGAGCCTTGAACTACATGGTACATATATCGATATTTTGGCATAGACTTTTTTATATGGGT TTAATTATCATCATTATATATATGTGTCACCCTATGGCAT

>Haplotype: BT1 GB# MN783979 (Sample: BTRP1)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA CTAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTG GTACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTTT TTTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGAT TTTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATT TATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGA ATTAATTTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT2 GB# MN783980 (Sample: BTRP2)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTTTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTTGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTTGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGGTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT

>Haplotype: BT3 GB# MN783981 (Sample: BTRP3)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTTTAGTTCCCTTAATTGTCATTTTGACTTTTAATTGTTTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTT TTTGTCAAGATTGAGGTAATTAATTGTTACGGGGGAATAATTGGGAAGTCATAATTGACCCAGGGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: BT4 GB# MN783982 (Sample: BTRP4)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATATAGTATCACACTATGGCAT

>Haplotype: BT5 GB# MN783983 (Sample: BTRP5)

>Haplotype: BT6 GB#MN783984 (Sample: BTRP6)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTTCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: BT7 GB#MN783985 (Sample: BTRP7)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC ATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCCA TACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGCT TAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTAT AATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGGT ACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTCT TAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATTT CTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTAT CATTTAAACCTGTGCTACCCTCGACATAATATTTTGTATGACCTAACTACTTTAACATTCATAGGACATTAT TTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAATG AATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTTA TAATTATCATCATTATATATAGTGTGCACACTATGGCAT

>Haplotype: BT8 GB#MN783986 (Sample: BTRP8)

>Haplotype: BT9 GB#MN783987 (Sample: BTRP9)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCCACCCTTAAAAAGGTATACGTCT TTTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTTGACTTTTAATCGTTTTCAAGAT TTTTCAGTCCTCCCAGTTTTTTTTTAGGGGAAAGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGCTACCCTTGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGGGAAGTCATAATTGACACAGTGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTTGACCATTAAGTGTAGTGAACAACTATT TACCCCTGGGTTGAAAAGAAAAAAAAAAAAAATATACATAAATTTTTTGGAAAAACCCCCCCTCCCCCAAA ATTAATTATCATCATTATATATAGTGTCACACTTATGGCAT

>Haplotype: BT10 GB#MN783988 (Sample: BTRP10)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT11 GB#MN783989 (Sample: BTRP11)

>Haplotype: BT12 GB#MN783990 (Sample: BTRP12)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTT ATCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATT ATTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACCCAGTGGGTAA TGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATT TATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGA ATTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT13 GB#MN783991 (Sample: BTRP13)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACCCAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: BT14 GB#MN783992 (Sample: BTRP14)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: BT15 GB#MN783993 (Sample: BTRP15)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTT TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCCCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT16 GB#MN783994 (Sample: BTRP16)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT

>Haplotype: BT17 GB#MN783995 (Sample: BTRP17)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT18 GB#MN783996 (Sample: BTRP18)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATACTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: BT19 GB#MN783997 (Sample: BTRP19)

>Haplotype: BT20 GB#MN783998 (Sample: BTRP20)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATTGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT TTAATTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: BT21 GB#MN783999 (Sample: BTRP21)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT22 GB#MN784000 (Sample: BTRP22)

>Haplotype: BT23 GB#MN784001 (Sample: BTRP23)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA CTAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTG GTACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCT CTTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGAT TTTCTCAGTCCTCCCAGTTTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTT TATCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACAT TATTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTA ATGAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTAT ATATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAG AATTAATTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT24 GB#MN784002 (Sample: BTRP24)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTCTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGCCGATTATATATTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATAGTGTCACACTATGGCAT

>Haplotype: BT25 GB#MN784003 (Sample: BTRP25)

AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA AAAGATCAACACATGACCAACCTGCATAAACGCATGTATTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTCTTAATCGTCTCAAGATT TCTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTTATCATCATTATATATATAGTGTCACACTATGGCAT

>Haplotype: BT26 GB#MN784004 (Sample: BTRP26)

AAAGATCAACACATGACCAACCTGCATAAACGCATGTACTTGCGTGAACGCAAATGCATTTGTGAAAACAC AAATGCAATTGCAAAATTATGCAGGTTGGTCAGTCTGACATATAAACTATTTAGCCCCATACTATGTATAT CCTCATTAAATATACATACTATGCTTAATCCTCATTAATAGACTGTCCCCATATACATATTATGCTTAATC AATCCACATCATACATACGTCTATCTCACTCCCCATACACACATATATGTGTGCTTAATCCCCATTAATCC ATACTCCCCTACTCCATTACATGCTATGCTTAATCCTCATTAATAGACTGTCCCCATAGTACATACTATGC TTAATCCTCATTAACATACTATGCTTAATCCCCATTAATAGACATTCCCCTATTTCATTACATCTTACGTA TAATACTGCTTATGCGGGCTGGCGAGAAATAACCAATACTCTCTTATAAGACCCCTTTGCACGGTTTGTGG TACTGTAATCGAATAATCCCCATCAATTGTTCAAATCCCTCATTTGGCACCCTTAAAAAAGGTATACGTCTC TTAATCGCGTCAGAACTCCTTTTCCTCTAGTTCCCTTAATTGTCATTTCGACTTTTAATCGTTTCAAGATT TTTCAGTCCTCCCAGTCTTTTTTTAGGGGAATGAAGCAATCGTTAACCCGCCAGGGTTGGATACGGCTTTA TCATTTAAACCTGTGCTACCCTCGACATAATATTTGTATGACCTAACTACTTTAACATTCATAGGACATTA TTTGTCAAGATTGAGCTAATTAATCGTTACGGGAGAATAATTGTGAAGTCATAATTGACACAGTGGGTAAT GAATAATCAGATATACATTTAAGAAAGACATTTCATGGACTTCGACCATTAAGTGTAGTGAACAACTATTT ATACACGGTATCCTCGAAAAACCCCTAAAACGAGGGCCGATTATATATTTTTTCTTTTAATGACATGTAGAA TTAATTATCATCATTATATATATGTGTCACACTATGGCAT

>Haplotype: BT27 GB#MN784005 (Sample: BTRP27)