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Chapter 1

Introduction

It defines the objectives and the importance of the research. It focus on the the application of Next Generation Sequencing to molecular biology, wheat genetics and ultimately to breeding programs. It also mentions the current status of the wheat reference genome and other resources (genetic maps, markers) the need of tools to query them effectively.

Chapter 2

Literature review

It describes the current status of the wheat genome, genetics and other resources.

2.1 Wheat Breeding

An overview of how breeding is carried on currently, the different sources of genetic diversity and the relevance of fixing agriculturally important traits.

2.2 Wheat Genetics

The section describes alleles and the concept of gene, both as a locus in the genome (Quantitative Trait Locus, QTL) and an specific transcript (central dogma of molecular biology). Finally, it discusses traditional Mendelian inheritance and the effect of polyploidy.

2.3 Wheat Genomics

A description of the current status of the wheat genome (Mayer et al. (2014), Chapman et al. (2015)), the different available assemblies and approaches to sort the scaffolds (Genome Zipper, the various genetic maps).

2.4 Sequencing

The importance of the selection of the library preparation and the sequencing platforms available. A brief summary of RNA-Seq, Exome capture, Whole Genome Shotgun, etc. and on which cases are more suitable for different experiments. Mention the new technologies developed during the years of the PhD (Ren-Seq, PacBio?).

2.5 Sequence analysis

This section discusses the criteria to decide analysis done after sequencing, when to do re-alignments or *de novo* assemblies, how to do SNP calling in diploid and polyploid organisms and the bulk frequency ratios.

2.6 Wheat online resources

A compilation of the currently available resource for wheat genetics and genomics. MAS wheat, CerealsDB, Ensembl, etc.

Chapter 3

Genetic mapping of *Yr15*

This section describes in detail than the paper of Ramirez-Gonzalez et al. (2014)

3.1 (Introduction) *Yr15*

Breeding importance of *Yr15* and original source (an introgression of *T. diccocooides*).

3.2 Segregating population and resistance essays

A description of the starting material and how the population was generated.

3.3 Sequencing and mapping

RNA-Seq and the decision to call SNPs on gene models rather than the whole reference. Details of the mapping against the Wheat UniGenes Pontius et al. (2002) and the UCW. Krasileva et al. (2013) gene models.

3.4 SNP Calling

. Ruby implementation of the methodology described by Trick et al. (2012).

3.5 Bulk Frequency Ratios

Results of the simple SNP calls from the progenitors and how the score of the Bulk Frequency Ratios(BFR) improve the location of the SNPs.

3.6 *In silico* mapping

Mapping of the gene models to the IWGSC CSS Mayer et al. (2014) reference and the location of the SNPs using the genetic map from Wang et al. (2014).

3.7 Assay selection

. The selection criteria to decide which SNPs were selected to produce the genetic map: BFR>6, in the short arm of chromosome group 1 and from the *Yr15* progenitor.

3.8 Genetic map

The three versions of the genetic map: With a subset of the F₂ population

3.9 Assembly of the transcriptome

A comparison between the known unigenes and the transcript from the progenitors. Since *Yr15* comes from an introgression with *T. diccoides*, some novel transcripts can be extracted. Analysis of the gels from Mitaly?

3.10 Conclusions

Remarks on how this technique can be used to do fine-mapping and that if I were to start the project now I would use exome capture or Ren-Seq.

Chapter 4

PolyMarker: A fast polyploid primer design pipeline

One of the main challenges of working with polyploid species is the design of genome specific molecular markers. This is particularly true when targeting conserved homoeologue regions, where a primer could bind to a pair, or triplet, of identical sequences. For that reason, designing primers for polyploids require to include bases that are specific to the target, in addition to the physicochemical properties of the primer. The traditional methodology to find primer candidates include a blast search and a local alignment, select the primer candidates manually, and finally, validate the primers with a tool, like **Primer3** (Rozen and Skaletsky, 2000). To reduce the time invested in designed primers I have developed PolyMarker (Ramirez-Gonzalez et al., 2015), a pipeline to automate the primer design for polyploid organisms.

4.1 Pipeline

PolyMarker is an automated pipeline that takes as input a list of SNPs and a reference file and produces a list of primer triplets for SNP genotyping. The list of SNPs is first converted to a **FASTA** file with ambiguity codes (Cornish-Bowden, 1985). The sequences are searched on the genomic reference using **exonerate** (Slater and Birney, 2005) to find the homoeologue regions to the target sequence. Then, the alignment between homoeologues is refined using **MAFFT** (Katoh and Standley, 2013). A list of candidate variations is produced and used as input for **Primer3** (Rozen and Skaletsky, 2000). Finally, the output of **Primer3** is parsed to find the best primer pair that contains a the targeted SNP and a base that is specific to the target genome (Figure 4.1). The pipeline is written as a Ruby script, using parsers and wrappers from BioRuby (Goto et al., 2010) and bio-samtools (Etherington et al., 2015; Ramirez-Gonzalez et al., 2012). The software is open source and released as a biogem (Bonnal et al., 2012), **bio-polyploid-tools**, the source code is available in github: <https://github.com/TGAC/bioruby-polyploid-tools>.

The PolyMarker input consist on SNP list with: unique name for the marker, the target chromosome and the sequence for the marker. The alternative alleles are surrounded by square brackets within the sequence. PolyMarker can take a list of several markers and design them in batch (Figure 4.2a). A **FASTA** file is produced with all the template sequences, with the alternative alleles substituted by the IUAPC ambiguity codes (Cornish-Bowden, 1985). The flanking sequence surrounding the SNP is limited by default to 100bp to reduce the search time and avoid missing regions that diverge near the SNP, as when the variation is near an

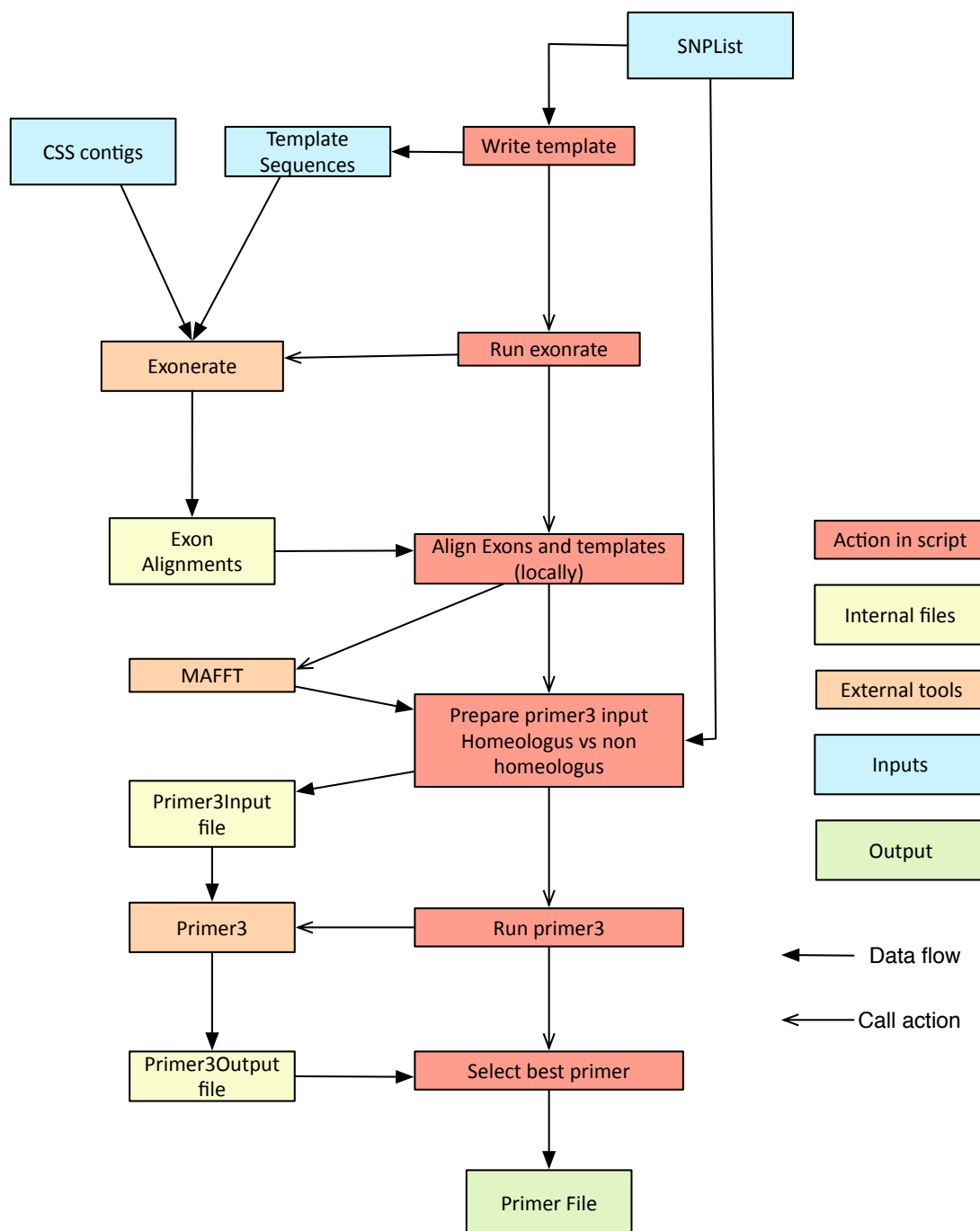


Figure 4.1: Steps and tools called by PolyMarker. The colour of the boxes represent: the step is an action inside the script (red); actions of the script (orange); temporary files (yellow); inputs (blue) and; output (green)

intron-exon junction.

The template sequences are searched in the reference sequence using `exonerate` (Slater and Birney, 2005), figure 4.2b. The alignment is run with the `--model est2genome` option, to allow the search of sequences coming from transcripts, a common source of SNPs (Allen et al., 2011). The `exonerate` output is formatted with the `--ryo` (roll your own format) to get an output easy to parse. All the hits that contain the SNP are extracted from the reference with a flanking sequence that extend out of the hit, by default, to 100bp on each side of the SNP (Figure 4.2c). The size of the flanking sequence can be set to different sizes to allow the design of different types of primers. Different homoeologues may contain small indels (Figure 4.2d). To enable a comparison base-per-base, a local alignment with `MAFFT` (Katoh and Standley, 2013) is produced (Figure 4.2e).

PolyMarker searches across each base in the local alignment to identify the variations across homoeologues and the target marker. A mask is produced to highlight the bases with a variations (Figure 4.2f) on the following categories:

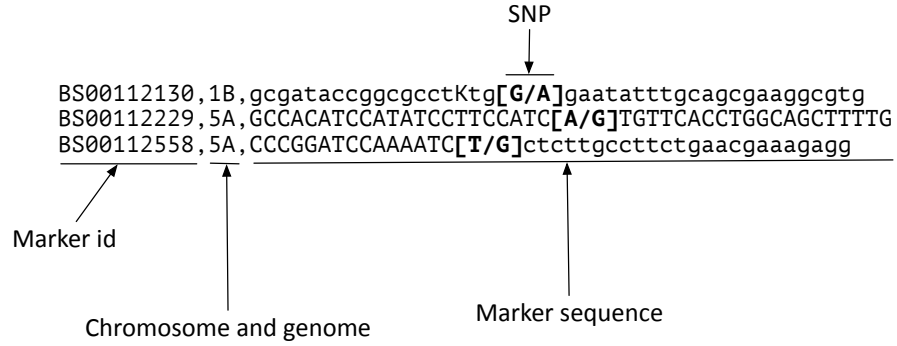
Specific	Homoeologous polymorphism which is only present in the target genome (upper case).
Semi-specific	Homoeologous polymorphism which is found in 2 of the 3 genomes, hence it discriminates against one of the off-target genomes or when not all the homoeologous sequences were found (lower case).
Non-specific	No variation is found across homoeologues (-).
Homoeologous	The target SNP is present across different chromosomes, so candidate SNP markers on this category are not expected to be reliably identify the allele (:).
Non-homoeologous	The target SNP is not present across chromosomes, so it can be used to identify an allele (&).

PolyMarker was designed to produce SNP assays for KASP genotyping (LGC Genomics, 2013), which requires a common primer and two allele-specific primers. The common primer is selected to start on a position from a: Specific; Semi-specific or; Non-specific, on that priority. This means that the common primer will be as specific as possible in the region. For the allele-specific primers, the starting position of the primer is on the base with the SNP. To ensure that the stability of the candidate primers will be met, the putative starting positions are tested with `Primer3` (Rozen and Skaletsky, 2000).

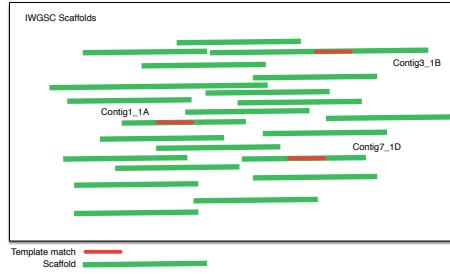
PolyMarker was designed and validated with the markers described in section 3.8. For wheat, PolyMarker uses the contigs from Mayer et al. (2014), as deposited in Ensembl. As new releases of the wheat genome are made available, different parsers to assign the chromosome to each sequence can be added with little effort to PolyMarker.

4.2 PolyMarker public web service

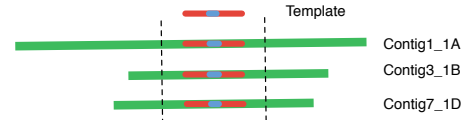
To make PolyMarker accessible to the community, a web server that allow the submission of SNPs was developed. The web interface consists on two virtual machines,



(a) PolyMarker input. The alternative alleles are surrounded by brackets.



(b) Global search of templates in the reference contigs.



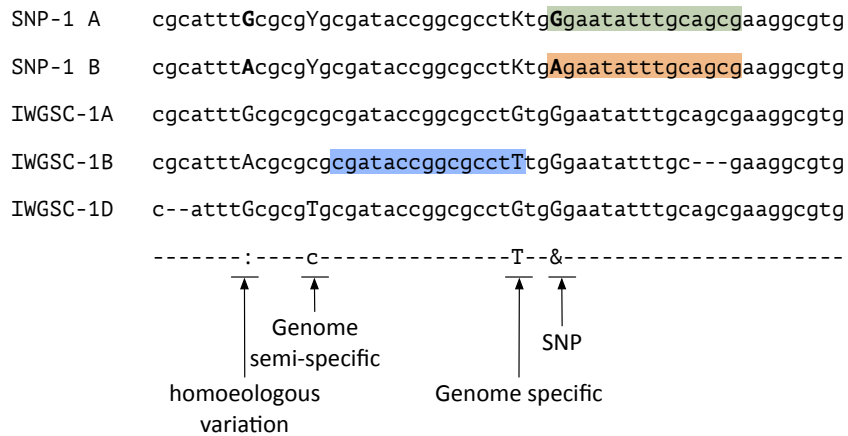
(c) Selected regions around the SNP on every chromosome.

SNP-1 A cgcatttGcgcgYgcgataccggcgccctKtgGgaatatttgcagcgaaggcgctg
 SNP-1 B cgcatttAcgcgYgcgataccggcgccctKtgAgaatatttgcagcgaaggcgctg
 IWGSC-1A cgcatttGcgcgcgcgataccggcgccctGtgGgaatatttgcagcgaaggcgctg
 IWGSC-1B cgcatttAcgcgcgcgataccggcgccctTtgGgaatatttgc---gaaggcgctg
 IWGSC-1D c--attdGcgcgTgcgataccggcgccctGtgGgaatatttgcagcgaaggcgctg

(d) Sequence of found regions around the SNP.

SNP-1 A cgcatttGcgcgYgcgataccggcgccctKtgGgaatatttgcagcgaaggcgctg
 SNP-1 B cgcatttAcgcgYgcgataccggcgccctKtgAgaatatttgcagcgaaggcgctg
 IWGSC-1A cgcatttGcgcgcgcgataccggcgccctGtgGgaatatttgcagcgaaggcgctg
 IWGSC-1B cgcatttAcgcgcgcgataccggcgccctTtgGgaatatttgc---gaaggcgctg
 IWGSC-1D c--attdGcgcgTgcgataccggcgccctGtgGgaatatttgcagcgaaggcgctg

(e) Local alignment on regions around the SNP detects indels.



(f) Alignment with mask and primer candidates.

Figure 4.2: Alignments done by PolyMarker.

Table 4.1: Count of KASP assays designed for the 40,267 SNP markers located in the genetic map from Wang et al. (2014). 4,228 assays did not align to the target chromosome. Not designed: Primer3 could not find viable primers flanking the SNP.

	Homoeologous variant	Varietal SNP	Percentage
Non-specific	1,765	5,857	21.15%
Semi-specific	7,942	6,907	41.20%
Specific	6,813	5,957	35.43%
Not designed	242	556	2.21%
Total	16,762	19,277	36,039

one with a web facing interface that stores the queries, and a dedicated node to submit jobs to an HPC cluster. The on-line interface further simplifies the design of KASP assays, a process that used to take a couple of weeks now is done in a couple of hours. Since the release of the public service in July 2014 until August 2016, 1,739 requests to PolyMarker have been done.

4.3 Applications of PolyMarker

PolyMarker is not restricted to wheat or to KASP assays, the source code is flexible and can be extended for other types of analysis. On each of the following projects, PolyMarker has been adapted to design primers in species where KASP hasn't been used before, the primers are used for regular PCR amplification, or the use of KASP is not the conventional SNP calling.

4.3.1 KASP assays for public sets of SNPs

PolyMarker was used to design KASP assays for the 81,587 markers from (Wang et al., 2014), available on the PolyMarker website and in CerealsDB (Wilkinson et al., 2012). Of those markers, 40,267 were designed using the target chromosome using the genetic map published by the genetic map. Genes without a genetic position were aligned to scaffolds sorted by chromosome from the International Wheat Genome Sequencing Consortium (Mayer et al., 2014) with BLAT (Kent, 2002) and the best hit was selected as putative location. 97.5% of the assays were designed and 76% of them are semi-specific or specific, thereby improving their expected performance with respect to randomly designed primers (Table 4.1). A set of the designed assay was used to genotype a mapping population to find resistance to Fusarium head blight (Burt et al., 2015).

4.3.2 Genotyping of *Puccinia striiformis* f. sp. *tritici* isolates.

In Hubbard et al. (2015), *Puccinia striiformis* f. sp. *tritici* (PST) isolates were sequenced and assigned to clusters, according to their genotype. The clusters are useful to monitor the changes in the pathogen population, which can be used to predict if certain wheat lines will be resistant to the isolates in the field. PolyMarker was used to design primers for PST, using the assembly PST-130 Cantu et al. (2011).

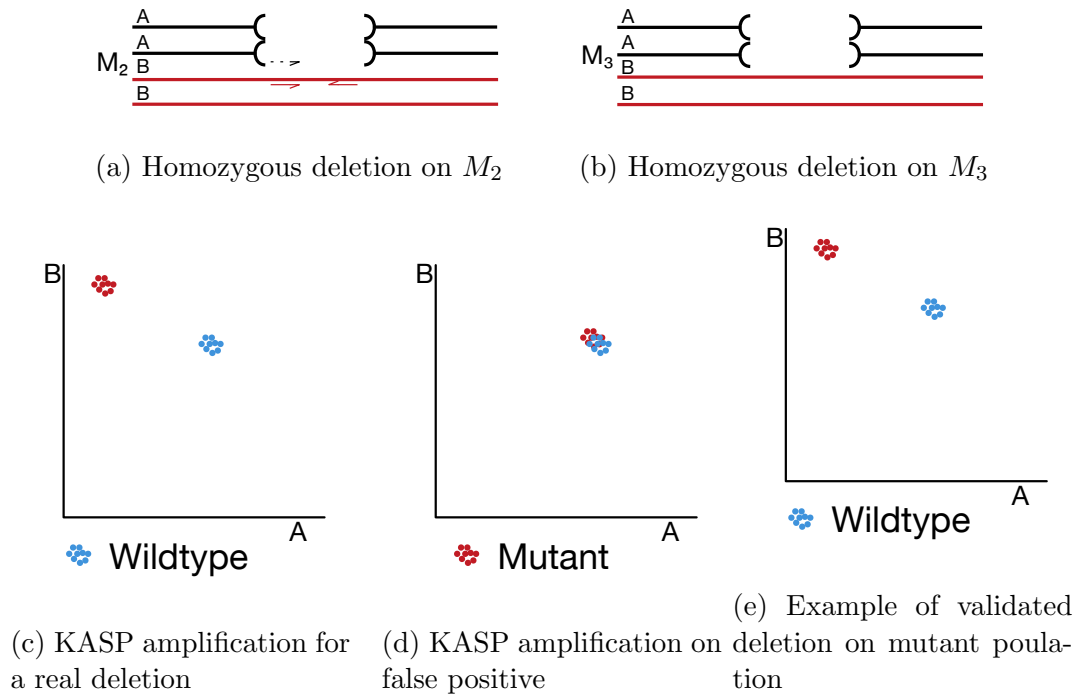


Figure 4.3: PolyMarker used to find primers to detect long deletions in tetraploid wheat.

Out of 15 assays 11 can be used to identify to which cluster of isolates a sample is likely to belong (Supplemental Table A.1).

4.3.3 Validation of SNPs in a mutant population

(Krasileva et al., submitted 2016)

4.3.4 Validation of deletions on a mutant population

Primers

4.4 Conclusions

Remarks on the importance of getting the primers right, and the time saved by automating the primer selection. Also mention other primer design tools that have been inspired by polymarker: Ma et al. (2015), Wang et al. (2016)

PolyMarker has been used successfully to design genome-specific primers in several projects.

Chapter 5

Gene expression (expVIP)

5.1 Expression experiments (Introduction)

Describe the list of previously published expression experiments and how they can potentially be used as a framework for new experiments.

5.2 Database design

Description of how the database was designed and the flexibility given by having the factors and units as variables

5.3 Analysis pipeline

Implementation of the pipeline, from running kallisto to load the data in the database

5.4 Graphical interface

How the expression can be displayed filtered, and sorted

5.5 Conclusions

The use of previously published studies is a valuable resource. Also, mention that despite the fact that there are several expression/gene browsers, none of them allow comparisons between species and don't consider polyploids.

Chapter 6

Conclusions and final remarks

This section wraps up by showing the relationship and importance of a comprehensive approach to data analysis, from the field, genetics, molecular biology and genomics. I will also remark how the technology and the resources have changed in the last 4 years. As at the references used at beginning where superseded during the PhD.

Appendix A

Supplemental tables.

Table A.1: PolyMarker used to genotype PST

Assay	Contig	Position	X	Y	Cluster I isolates		Cluster II isolates		Cluster III isolates			Cluster IV isolates	
					13/26	13/123	CL1	T-13/3	13/09	13/23	13/182	13/36	13/40
1	PST130_14470	268	C	T	X:Y	X:Y	X:X	X:X	X:X	X:X	X:X	X:X	X:X
2	PST130_8160	11876	C	T	Y:Y	Y:Y	X:Y	X:Y	X:Y	X:Y	X:Y	X:Y	X:Y
3	PST130_14628	1712	A	C	X:Y	-	X:X	X:X	X:X	X:X	X:X	X:X	X:X
4	PST130_14898	503	G	A	X:X	X:X	X:Y	X:Y	X:Y	X:Y	-	X:Y	X:Y
5	PST130_28344	2372	A	G	Y:Y	Y:Y	X:Y	X:Y	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y
6	PST130_7634	3463	A	C	Y:Y	Y:Y	X:Y	X:Y	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y
7	PST130_7629	11699	G	A	Y:Y	Y:Y	X:Y	X:Y	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y
8	PST130_10943	2979	C	T	X:Y	X:Y	X:Y	X:Y	X:X	X:X	X:X	X:Y	X:Y
9	PST130_10126	6216	G	T	Y:Y	Y:Y	X:X	X:X	X:X	X:X	-	Y:Y	Y:Y
10	PST130_22010	172	C	T	Y:Y	Y:Y	Y:Y	Y:Y	X:Y	X:Y	-	X:Y	X:Y
11	PST130_16961	1098	C	T	X:X	X:X	X:Y	X:Y	Y:Y	Y:Y	Y:Y	X:Y	X:Y
12	PST130_6915	2710	A	T	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y	X:Y	X:Y	Y:Y	Y:Y
13	PST130_12479	1428	C	T	X:X	X:X	Y:Y	Y:Y	X:X	X:X	X:X	Y:Y	X:X
14	PST130_7634	3883	C	G	X:X	X:X	X:Y	X:Y	X:X	X:X	X:Y	X:Y	X:X
15	PST130_14470	456	T	C	Y:Y	Y:Y	X:Y	X:Y	Y:Y	Y:Y	X:Y	Y:Y	Y:Y

A.1 Validation of mutations on M_4 on Kronos

IWGS contig	Line	Pos	WT	Mut	Predicted	Called on M_4	Primer 1 (Kronos)	Primer 2 (mutant)	Common Primer
IWGS.CSS.1AS.scf.3284790	Kronos3085	7449	G	A	Het	Het	ccacacttgagctcgc	ccacacttgagctcgcT	gtgattggcaggagagA
IWGS.CSS.1BL.scf.3897513	Kronos3085	1515	C	T	Het	Het	gcttcaactggctcgc	gcttcaactGggtcgcT	acAaggaactgctcgaGac
IWGS.CSS.2AL.scf.6434745	Kronos3085	3424	C	T	Het	Het	ctcGgtttgcaattctcgc	ctcGgtttgcaattctatgT	gGCaaTggcatacaacagatA
IWGS.CSS.3AS.scf.3408995	Kronos3085	732	C	T	Het	Het	agggcatttgaattcgc	agggcatttgaattcgcT	ggTgttTcaagAacctgagTG
IWGS.CSS.3B.scf.10708748	Kronos3085	2675	G	A	Het	Het	gttgatgcttcaaccagG	gttgatgcttcaaccagA	gtacaactctgagttcgtcgaC
IWGS.CSS.4AL.scf.7132733	Kronos3085	1799	C	T	Het	Het	caaccgtagtgacctcC	caaccgtagtgacctcT	aCcCctaGaaagaacctcC
IWGS.CSS.5AS.scf.1534693	Kronos3085	4605	C	T	Het	Het	caacttcctggcctcAtC	caacttcctggcctcAtT	gtaCctcagAgctcaTggagAG
IWGS.CSS.6AS.scf.4361911	Kronos3085	8857	C	A	Het	Het	tcacgaagaagcacttcaacctcC	tcacgaagaagcacttcaacctcT	catgaggtctgcactcctcA
IWGS.CSS.6BS.scf.3008326	Kronos3085	1528	G	A	Het	Het	ccatgttgtagcttggtcG	ccatgttgtagcttggtcT	ggaagcattgcCaagtgcA
IWGS.CSS.7AS.scf.4214385	Kronos3085	27835	C	T	Het	Het	cgctacgtcttggaagG	cgctacgtcttggaagA	ctcttgctcagctgataaagT
IWGS.CSS.1AL.scf.3929064	Kronos3191	1336	C	T	Het	Het	tftggcacaactgcacC	tftggcacaactgcacT	attgctcagcttctcgaC
IWGS.CSS.1BL.scf.3899789	Kronos3191	7925	C	T	Het	Het	actctcacTggcagcagC	actctcacTggcagcagT	caactgggtgccctcGtaA
IWGS.CSS.2AL.scf.6426728	Kronos3191	1481	G	A	Het	Het	gaaAcTgcgcagctcGcT	gaaAcTgcgcagctcGcT	ccaGcaGctcgtgagaaA
IWGS.CSS.2BL.scf.7960273	Kronos3191	690	C	T	Het	Het	gcaattcactcttaggcG	gcaattcactcttaggcT	acaTgaactgtcgtgagctG
IWGS.CSS.3AS.scf.3286603	Kronos3191	2975	G	A	Het*	Het	ccgtgtggtttgttggG	ccgtgtggtttgttggA	gaaaggaactgtTcaTgcaG
IWGS.CSS.5AL.scf.2694249	Kronos3191	2399	C	T	Het	Het	gctctcagatagaccGc	gctctcagatagaccGcT	gcgcacacgacatcctG
IWGS.CSS.5BL.scf.10923577	Kronos3191	3713	C	T	Het	Het	gtgattgctcgtgcttgc	gtgattgctcgtgcttgcT	tgttgccctcttggagC
IWGS.CSS.6AL.scf.5823017	Kronos3191	13225	C	T	Het	Het	cccttgcagccttggagG	cccttgcagccttggagA	tgcgagaagccctcgaA
IWGS.CSS.6BS.scf.2955394	Kronos3191	1622	C	T	Het*	Het	gtgagatgaagctctgcaagG	gtgagatgaagctctgcaagA	gatactcgtTgcaatgggtT
IWGS.CSS.7BL.scf.6739382	Kronos3191	12261	G	A	Het	Het	gagacaagcttgaattgctcC	gagacaagcttgaattgctcT	CgagtgacctTcaattccG
IWGS.CSS.1AS.scf.577873	Kronos3288	9720	C	T	Het	Het	aCcaGcaggaacAatgctcT	aCcaGcaggaacAatgctcT	atgctgcaactctcagcA
IWGS.CSS.2AL.scf.6367515	Kronos3288	6976	G	A	Het	Het	caggtcagTgtctcgcG	caggtcagTgtctcgcA	ggggatgCtggagaggC
IWGS.CSS.2AL.scf.6422019	Kronos3288	4523	G	A	Het	Het	cgttagtctcctgacagG	cgttagtctcctgacagA	atgcAagctaaacgtcgaC
IWGS.CSS.3AL.scf.4284850	Kronos3288	7901	C	T	Het	Het	tggcttggcacaactcG	tggcttggcacaactcG	tgtcAgcatcagcagcgaG
IWGS.CSS.4AS.scf.5962359	Kronos3288	13049	G	A	Het	Het	ccatcaagaagtaacagttcgaT	ccatcaagaagtaacagttcgaT	accatgccagcttgcA
IWGS.CSS.6AL.scf.577873	Kronos3288	6853	G	A	Het	Het	gagtgaccttccgtcttT	gagtgaccttccgtcttT	ggagaacagctcgcT
IWGS.CSS.6AS.scf.4392100	Kronos3288	3434	C	T	Het	Het	atggaagcacaggtgaacA	atggaagcacaggtgaacA	ggAagcgaagtgaacaaA
IWGS.CSS.7BL.scf.6744240	Kronos3288	9772	G	A	Het	Het	agctgttctctactcaag	agctgttctctactcaaa	caggtcgttctgagctcC
IWGS.CSS.1AL.scf.3887185	Kronos3413	9708	C	T	Het	Het	gcacgctttatcaggtaaaG	gcacgctttatcaggtaaaA	AGaaacacagagcgcA
IWGS.CSS.2BS.scf.3381362	Kronos3413	5160	C	T	Het	Het	caactctggctgtgtgT	caactctggctgtgtgT	tgAgaattctcgaGaaagaC
IWGS.CSS.3AS.scf.3296605	Kronos3413	6154	G	A	Het*	Het	ctgttcacgctcgtcagC	ctgttcacgctcgtcagT	cagcactgagacagatggC
IWGS.CSS.3B.scf.10693516	Kronos3413	12632	C	T	Het	Het	ctgtgtggacaacagcC	ctgtgtggacaacagcT	agcttgcattctgggcatT
IWGS.CSS.5AS.scf.1547699	Kronos3413	2686	G	A	Het	Het	gCtaacacttcaacatcG	gCtaacacttcaacatcG	gacgcttgaagtgtcatC
IWGS.CSS.5BL.scf.10856077	Kronos3413	5853	G	A	Het	Het	agagcttccaccactcG	agagcttccaccactcT	acgCacatttAatagcgaagC
IWGS.CSS.6AL.scf.5750718	Kronos3413	11046	G	A	Het	Het	cagcTtccgactcttataG	cagcTtccgactcttataA	AGagatgtgatacagatcaG
IWGS.CSS.7AL.scf.4433177	Kronos3413	3511	C	T	Het	Het	CaTgtccGtccggtcgcG	CaTgtccGtccggtcgcT	actactgacacagcttgcG
IWGS.CSS.7BL.scf.6742567	Kronos3413	667	C	T	Het	Het	gtgtcgttgcgtgagcA	gtgtcgttgcgtgagcT	catttgcacogtgcT
IWGS.CSS.1AL.scf.3976389	Kronos3935	10941	C	T	Het	Het	gttgagagatcgcGcatG	gttgagagatcgcGcatA	cagtcactacatgagagtcA
IWGS.CSS.1BL.scf.3873362	Kronos3935	1392	G	A	Het	Het	cagatcgaagctcGacatG	cagatcgaagctcGacatA	actacagatcagcacaacA
IWGS.CSS.2BL.scf.7882382	Kronos3935	2721	C	T	Het	Het	gcaagctaagatgtacgtagC	gcaagctaagatgtacgtagT	gccacagtaggagaagactT
IWGS.CSS.3AL.scf.4242376	Kronos3935	2410	C	T	Het	Het	agaacccaacccGtacttaG	agaacccaacccGtacttaA	gtagGtGctcCaaCaaagctTG
IWGS.CSS.3B.scf.10480607	Kronos3935	3349	C	T	Het	Het	gcttgagcaactcactcaacG	gcttgagcaactcactcaacT	gcaattctcttAaccgagT
IWGS.CSS.4AS.scf.5984153	Kronos3935	6006	G	A	Het	Het	agCaggtctgcgaagtTg	agCaggtctgcgaagtT	cgaatGtatgaGtaggcT
IWGS.CSS.4BL.scf.7019402	Kronos3935	9081	C	T	Het	Het	tgcatactgtgtgagctG	tgcatactgtgtgagctA	agcagatcctcagaaCcaatC
IWGS.CSS.5BL.scf.10842786	Kronos3935	3304	G	A	Het	Het	tgttcccGagcttgaaC	tgttcccGagcttgaaT	cgcatacttgaacATgagcAC
IWGS.CSS.6BS.scf.3045205	Kronos3935	2293	C	A	Het	Het	aaggacaaagccaaactcG	aaggacaaagccaaactcA	agtgatcaagcccaatgtgcA
IWGS.CSS.7AL.scf.4555249	Kronos3935	4487	C	T	Het	Het	cAgTgctcgaatgggcC	cAgTgctcgaatgggcT	cTtgcaacctctcgtatT
IWGS.CSS.1BL.scf.3918498	Kronos4240	6096	G	A	Het	Het	tfgcatgcccaagaagA	tfgcatgcccaagaagA	tggcgcaactgtgtaatgTgG
IWGS.CSS.2BS.scf.5131713	Kronos4240	5900	G	A	Het	Het	ctttatcgaagaagagacacC	ctttatcgaagaagagacacT	ccattgtgaggttcttctTttC
IWGS.CSS.5AL.scf.10460714	Kronos4240	9626	C	T	Het	Het	tgCagtggtggaacaggaG	tgCagtggtggaacaggaA	catgagtGagattctcgcT
IWGS.CSS.5BL.scf.10871091	Kronos4240	7062	G	A	Het	Het	gccaaggAaccataactgT	gccaaggAaccataactgT	GgactctggcAaccgA
IWGS.CSS.6AL.scf.5800333	Kronos4240	2360	C	A	Het	Het	cgacagatgtgagCgC	cgacagatgtgagCgT	tcagatgctgaagattcctC
IWGS.CSS.7BL.scf.6716931	Kronos4240	2613	C	A	Het	Het	gGtgGgtattTgcttgggA	gGtgGgtattTgcttgggA	tgGtgagctcgcgaGtGtaA
IWGS.CSS.2BL.scf.8029221	Kronos4346	2860	G	A	Het	Het	tgtctcgtctgtgctcC	tgtctcgtctgtgctcT	atTgcatTcgAtcgggcC
IWGS.CSS.3B.scf.10460714	Kronos4346	14359	C	T	Het	Het	ctactctggcctgcacatG	ctactctggcctgcacatA	agccccagcttcttgcG
IWGS.CSS.4AS.scf.5989735	Kronos4346	6404	C	A	Het	Het	acagatctaactcagcC	acagatctaactcagcT	actcaagataccaCcgcaC
IWGS.CSS.5BL.scf.7648030	Kronos4346	6893	C	T	Het	Het	taccttctactcagcG	taccttctactcagcA	titticagagaacacagatcA
IWGS.CSS.6AL.scf.5755840	Kronos4346	778	C	T	Het	Het	atcgatgaagctgtcacGc	atcgatgaagctgtcacGt	acctgatGcaCaccaC
IWGS.CSS.6BS.scf.2972151	Kronos4346	7876	G	A	Het	Het	gcagaaatgctActgttgG	gcagaaatgctActgttgA	gcttggaactgggcatatG
IWGS.CSS.7AL.scf.4542983	Kronos4346	18700	G	A	Het	Het	gcaaggctAcggatacC	gcaaggctAcggatacT	catctccGgttaaacatC
IWGS.CSS.7BS.scf.3098098	Kronos4346	5183	C	T	Het	Het	gCgatagtacttgcnaatgA	gCgatagtacttgcnaatgA	ttacattgttgaagcttctTttC
IWGS.CSS.1AS.scf.3259804	Kronos4485	219	C	T	Het	Het	gtcgcgaacaccttgc	gtcgcgaacaccttgcT	gcttcttaaggagcgcA
IWGS.CSS.2AL.scf.6315418	Kronos4485	10490	G	A	Het	Het	ggccctctaaCcttctcagC	ggccctctaaCcttctcagT	tticagacctCgaggaattccC
IWGS.CSS.2BS.scf.5181092	Kronos4485	3742	C	A	Het	Het	TggccagacacactgcaA	TggccagacacactgcaA	tggagatgagTgaggtAaaT
IWGS.CSS.3B.scf.10425015	Kronos4485	2372	C	T	Het	Het	gctactgaagtTgcCtcaG	gctactgaagtTgcCtcaG	cttcacacttgggggTtC
IWGS.CSS.3B.scf.10775915	Kronos4485	4701	C	T	Het	Het	ccaaggctgcagagagG	ccaaggctgcagagagA	agacctcaagGtctcC
IWGS.CSS.5AL.scf.2754304	Kronos4485	2301	G	A	Het	Het	taaccTtgcatccccc	taaccTtgcatcccccA	catgtGcagcaTgacT
IWGS.CSS.5BL.scf.10919959	Kronos4485	1867	C	T	Het	Het	gaTgccttcttggagaagA	gaTgccttcttggagaagA	tcttgttcccgaacatgtaC
IWGS.CSS.7AS.scf.4245431	Kronos4485	3402	G	A	Het	Het	aaggcccttggttctC	aaggcccttggttctT	agtaagtggaAcagtaagatcaT
IWGS.CSS.7BL.scf.6667357	Kronos4485	641	C	T	Het	Het	gaicAGtgcctatcagG	gaicAGtgcctatcagA	ttcccttgcaatgatgccC

A.2 Validation of mutations on M_4 on Cadenza

IWGSC contig	Line	Pos	WT	Mut	Predicted	Called on M_4	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGSC_CSS_3B_scaff.10445294	Cadenza1772	6019	C	T	het	het	caggatAgtGggagctgtcaaaG	caggatAgtGggagctgtcaaaA	ggagacGGGtGtggacatT
IWGSC_CSS_3DL_scaff.6955403	Cadenza1772	2418	C	T	het*	hom	tcagCggatgtggagatG	tcagCggatgtggagatG	tgctCataagTctgtccacG
IWGSC_CSS_4AL_scaff.7106846	Cadenza1772	11277	G	A	hom	hom	tgagatccatgctctacactG	tgagatccatgctctacactA	gatagtGgatttgcgctaA
IWGSC_CSS_4AS_scaff.5991335	Cadenza1772	15710	G	A	hom	hom	ctggccctgcgctgctaA	ctggccctgcgctgctaT	gtggaaGtccagaagacacG
IWGSC_CSS_4BS_scaff.4956646	Cadenza1772	252	G	A	het*	hom	gcaggttgacttcccgaaG	gcaggttgacttcccgaaA	tGaggtacgaGcTaaagAaagC
IWGSC_CSS_4DS_scaff.1715962	Cadenza1772	1225	G	A	hom	hom	cagctgtggTatctcaactgG	cagctgtggTatctcaactgA	CcCtGaaACACcGttrggatT
IWGSC_CSS_5AL_scaff.2763047	Cadenza1772	2119	G	A	hom	hom	ggacGaacctcgagatctG	ggacGaacctcgagatctA	tGggcaAtcgtCgtgcA
IWGSC_CSS_5BS_scaff.1548786	Cadenza1772	12625	C	T	het	het	AtaggcaatgttagactgaG	AtaggcaatgttagactgaA	ggattgggtgttgcacG
IWGSC_CSS_5DL_scaff.10849226	Cadenza1772	2289	C	T	het*	hom	cttgacatcatgtttcacatG	cttgacatcatgtttcacatG	caatccgggtgttgcacG
IWGSC_CSS_5BS_scaff.2270737	Cadenza1772	2262	G	A	hom	—	attcCTgtgtgttggCaatatgaG	attcCTgtgtgttggCaatatgaA	taaGcaaaAccctcagctG
IWGSC_CSS_1AL_scaff.3022915	Cadenza1661	891	C	T	hom	hom	ccacagtgagactctattigaCG	ccacagtgagactctattigaCA	atgtctgattGtGtGagtcC
IWGSC_CSS_1AS_scaff.3297240	Cadenza1661	1970	C	T	het	het	catccgccGttrctctC	catccgccGttrctctC	gctcgcgcatgaagagcT
IWGSC_CSS_1DL_scaff.3828996	Cadenza1661	1340	G	A	hom	hom	agccgagatgttagtgaacC	agccgagatgttagtgaacT	agcagcttgTgcgttaacC
IWGSC_CSS_1DS_scaff.1884529	Cadenza1661	10575	G	A	hom	hom	aCagatacaAttgtcatcgaggT	aCagatacaAttgtcatcgaggT	accctgggTgtccaatctC
IWGSC_CSS_2AL_scaff.6318370	Cadenza1661	19142	C	T	het	—	ctgtgcCGaatCtcGacG	ctgtgcCGaatCtcGacA	ttcttgggagccggcC
IWGSC_CSS_2AS_scaff.5213460	Cadenza1661	1358	G	A	hom	hom	gtcacgaaCccgtcagG	gtcacgaaCccgtcagA	aggaagagaggaaagaaGcG
IWGSC_CSS_2BS_scaff.5179331	Cadenza1661	5604	C	A	het	het	actctgtcaagactgatacaG	actctgtcaagactgatacaA	gcaGagaatgtcttgcacT
IWGSC_CSS_2DS_scaff.5341235	Cadenza1661	4673	G	A	het	het	gggaggatctcgagctG	gggaggatctcgagctA	ggcggctgtacaggtG
IWGSC_CSS_3AL_scaff.4250995	Cadenza1661	7046	G	A	hom	hom	cCaagaaacgggtgtgtccaaA	cCaagaaacgggtgtgtccaaA	ctgcagctgtcccatcagT
IWGSC_CSS_3B_scaff.10404421	Cadenza1661	4303	G	A	het	het	ctctgtcgaCaggacctG	ctctgtcgaCaggacctA	GCcagactCacAgtctcC
IWGSC_CSS_5DL_scaff.2390496	Cadenza1538	2125	C	T	hom	het	gcagttttatctcagtagcttgG	gcagttttatctcagtagcttgA	ttctgagaaTgttaatgtcGatG
IWGSC_CSS_6AL_scaff.5753680	Cadenza1538	3920	C	T	hom	hom	tgctccaattgtgacacaaTaaC	tgctccaattgtgacacaaTaaT	aaatgcaagggttaagtgttG
IWGSC_CSS_6AS_scaff.4425792	Cadenza1538	4307	C	A	hom	het	agatgcttgtCggGcaG	agatgcttgtCggGcaA	gctGaagcaacggatcaaaT
IWGSC_CSS_6BS_scaff.3003630	Cadenza1538	6933	C	T	het	het	ggcagtaagtgtggTgctgagT	ggcagtaagtgtggTgctgagT	tTgaCttrcgggttggcA
IWGSC_CSS_6DL_scaff.3246988	Cadenza1538	9186	G	A	het	het	gctaaagagagcttgagagaatC	gctaaagagagcttgagagaatT	aattctgaagagaggtgtgtatG
IWGSC_CSS_7AL_scaff.4480114	Cadenza1538	3446	C	T	het	—	gatatcccaacggcG	gatatcccaacggcG	tgagcactcttcgagttT
IWGSC_CSS_7AS_scaff.4193541	Cadenza1538	8359	C	T	het	het	agcaatttttgctatcaatagT	agcaatttttgctatcaatagT	tcactGtcttaactctactG
IWGSC_CSS_7BL_scaff.6721572	Cadenza1538	9223	C	T	het	het	gctCaggaggaagacaaagaaA	gctCaggaggaagacaaagaaA	tgctaaagaatctccagctC
IWGSC_CSS_7BS_scaff.3152945	Cadenza1538	3960	G	A	hom	hom	tcagcaaatccctgcGgC	tcagcaaatccctgcGgT	gGgcacctcatcgtttaT
IWGSC_CSS_7DS_scaff.3963838	Cadenza1538	2913	G	A	het	het	tCgttgcaagCttTtgrgT	tCgttgcaagCttTtgrgT	agaGttaTcaagGtactgtcaA
IWGSC_CSS_1AL_scaff.3903380	Cadenza1469	6193	G	A	hom	hom	ctcttcAGagatgaacggG	ctcttcAGagatgaacggA	tGtGagatGtgggttGtTtA
IWGSC_CSS_1AS_scaff.3287728	Cadenza1469	3817	C	T	het*	hom	gcacaaAAttactaaacG	gcacaaAAttactaaacG	accctcttccAGacatG
IWGSC_CSS_1BL_scaff.3815304	Cadenza1469	513	G	A	hom	hom	aacatttgcTtACaaaacGC	aacatttgcTtACaaaacGT	acacagcaagttaatagtCAAGC
IWGSC_CSS_1DL_scaff.2266648	Cadenza1469	5926	C	T	het	het	caacatgagacacacactC	caacatgagacacacactT	gtcaacgctgagagctG
IWGSC_CSS_1DS_scaff.1906671	Cadenza1469	3697	C	T	hom	hom	tggTGTgacacttggcgaA	tggTGTgacacttggcgaA	catggcgaccacACctG
IWGSC_CSS_2AL_scaff.6337088	Cadenza1469	7334	G	A	het*	hom	acaatgccAAttgtagcagttA	acaatgccAAttgtagcagttA	gggaggttgggtCagaacaT
IWGSC_CSS_2BL_scaff.7972799	Cadenza1469	8995	C	T	het	het	gTgCtctcGgactcttT	gTgCtctcGgactcttT	gatccGcgaacactagTG
IWGSC_CSS_2DL_scaff.9832343	Cadenza1469	3262	G	A	het	het	TtgcttcaACagcacCGagG	TtgcttcaACagcacCGagA	agaatctggctcagcttctT
IWGSC_CSS_2DS_scaff.5327939	Cadenza1469	3889	G	A	het	het	ttttTgccttatgtgacttagtaC	ttttTgccttatgtgacttagtaT	gagggcactcagatagcG
IWGSC_CSS_3B_scaff.10395219	Cadenza1469	1292	G	A	hom	—	aggtgctgtgtgtgtgC	aggtgctgtgtgtgtgA	ccctctctgssggctttatcG
IWGSC_CSS_3B_scaff.10592217	Cadenza0580	2994	C	T	het	—	acagcagatcaagccctC	acagcagatcaagccctT	tGatactgtgTggCggagG
IWGSC_CSS_3DS_scaff.2596771	Cadenza0580	1037	G	A	het	het	tggttatGCAcaggataatCagA	tggttatGCAcaggataatCagA	tgccaatgtgtgctataggT
IWGSC_CSS_4AL_scaff.7093953	Cadenza0580	9881	C	T	hom	hom	CacaggaagccgggttaacC	CacaggaagccgggttaacT	cttccAGaggcagtggaT
IWGSC_CSS_4BL_scaff.7037448	Cadenza0580	1837	C	T	hom	hom	CgttgaaaaGctgcaagaacttaaC	CgttgaaaaGctgcaagaacttaaT	cagttcttccTtCaCagcagataT
IWGSC_CSS_4BS_scaff.4929479	Cadenza0580	10668	G	A	hom	—	tggtatttccgcactgtC	tggtatttccgcactgtT	gtfaaacaggaatttcaagatcA
IWGSC_CSS_4DL_scaff.14359838	Cadenza0580	1408	G	A	hom	—	gCtCAttcaggatTGTcCtaTatG	gCtCAttcaggatTGTcCtaTatA	tgaCagaacagttgtgtacatC
IWGSC_CSS_4DS_scaff.2276484	Cadenza0580	8034	G	A	hom	hom	ggcggttgtagagAgaG	ggcggttgtagagAgaA	cgctcagattactgactgcaA
IWGSC_CSS_5AL_scaff.2756579	Cadenza0580	5278	G	A	het	het	tgaaatggattttgtcccggtT	tgaaatggattttgtcccggtT	ggAACTCTATgCAGAgaAaaCTG
IWGSC_CSS_5BL_scaff.10787208	Cadenza0580	10627	G	A	het	—	gcctctcaatcgaggagaC	gcctctcaatcgaggagT	acgatgtcAGgttgcGgT
IWGSC_CSS_5BS_scaff.2282179	Cadenza0580	5267	G	A	het	—	tgatgggctacgagctgC	tgatgggctacgagctG	tcggccctctgaaATcC
IWGSC_CSS_5DL_scaff.4498073	Cadenza0423	4937	C	T	hom	hom	gcacctgtgtgtgtcatC	gcacctgtgtgtgtcatT	tgagcagcaAaagcagcG
IWGSC_CSS_5DS_scaff.2738970	Cadenza0423	2319	C	T	het	—	cgtgaggtgggtgattgC	cgtgaggtgggtgattgT	tggaactagttaactgcagTTC
IWGSC_CSS_6AL_scaff.5757109	Cadenza0423	2788	G	A	hom	hom	caggaGctggcgaataaaGG	caggaGctggcgaataaaGA	ctttcGagttctcttagtttC
IWGSC_CSS_6AS_scaff.4387871	Cadenza0423	2543	G	A	hom	hom	gcagtctaacaggcgaaagA	gcagtctaacaggcgaaagA	ctcatgctctctgacttaaggtT
IWGSC_CSS_6BL_scaff.4271391	Cadenza0423	4660	C	T	hom	hom	tacgtgtcagatgtgtgtgtgtaT	tacgtgtcagatgtgtgtgtgtaT	gtttggaagtgcactagTtaccA
IWGSC_CSS_6DS_scaff.1880206	Cadenza0423	9159	G	A	het	het	ctgCGaaggctccacaaG	ctgCGaaggctccacaaA	ggatgaagaatttgcattgctC
IWGSC_CSS_7AS_scaff.4227506	Cadenza0423	952	G	A	het	—	ccatgtgttccatgtttagagC	ccatgtgttccatgtttagagT	tgccctacgttggtaagT
IWGSC_CSS_7BS_scaff.6681782	Cadenza0423	1486	C	T	hom	hom	agtaagCGtgacacgaatggG	agtaagCGtgacacgaatggA	AtgtctTtgGtgggaagtacA
IWGSC_CSS_7DS_scaff.3160328	Cadenza0423	7801	C	T	het	het	tgttaaatGataacGctcgagC	tgttaaatGataacGctcgagT	tggaatgggGgtgtgtttT
IWGSC_CSS_7DS_scaff.407428	Cadenza0423	2051	G	A	het	het	gtcCGCgcactcagacA	gtcCGCgcactcagacA	actcatcAGgtcagcccaA
IWGSC_CSS_3AL_scaff.4442479	Cadenza0364	3198	C	T	het	het	gagttcaTtaagttggtaagttggG	gagttcaTtaagttggtaagttggA	GCAcTaaCaacagatcacG
IWGSC_CSS_3AL_scaff.4447942	Cadenza0364	11917	G	A	het	het	gtcataaagattgtcctgtgaagC	gtcataaagattgtcctgtgaagA	ctcGgagtggggaagA
IWGSC_CSS_3AS_scaff.1557483	Cadenza0364	2547	C	T	het	het	aaagtcaacatgcttaccataaG	aaagtcaacatgcttaccataaA	cgaatacagcctcaccA
IWGSC_CSS_3AS_scaff.2648747	Cadenza0364	2688	C	A	het	het	tggAagCAaaggggccT	tggAagCAaaggggccT	GccgcgattggagctcG
IWGSC_CSS_3AS_scaff.3304956	Cadenza0364	1017	G	A	het	het	gtcccttgacacgaatttG	gtcccttgacacgaatttA	ccctcTggacttcaacttcaA
IWGSC_CSS_3AS_scaff.3321091	Cadenza0364	4585	C	T	het	het	caagaatATgctgagtgtggaG	caagaatATgctgagtgtggaA	acaagtgaatcgcgaatC
IWGSC_CSS_3AS_scaff.3371333	Cadenza0364	538	G	A	het	het	gggaaCGAgAcgagcgG	gggaaCGAgAcgagcgG	ccgtgtctctcaccT
IWGSC_CSS_3AS_scaff.3371815	Cadenza0364	1061	C	T	het	het	atccccagccacagA	atccccagccacagA	aAttgcccttgggtgacC
IWGSC_CSS_3AS_scaff.3440912	Cadenza0364	4498	G	A	het	het	ccgtaaactttctgtgtgT	ccgtaaactttctgtgtgT	aAtctgacaacatcatgtcG
IWGSC_CSS_3B_scaff.10343586	Cadenza0364	2242	G	A	het	—	ggttcTgTctcttctccactA	ggttcTgTctcttctccactA	tgtgttgaaccgcgaagA

IWGSC contig	Line	Pos	WT	Mut	Predicted	Called on M_4	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGSC_CSS.3AL_scaff.442479	Cadenza0364	3198	C	T	het	het	gagtaCTAaagttgtaagattgT	gagtaCTAaagttgtaagattgT	GCaGaTaaCaacagatcacG
IWGSC_CSS.3AL_scaff.4447942	Cadenza0364	11917	G	A	het	het	gtataaagattgctctctgtaaA	gtataaagattgctctctgtaaA	ctcGagtggaggaagA
IWGSC_CSS.3AS_scaff.1557483	Cadenza0364	2547	C	A	het	het	aaagtcacacatgcttaccataaG	aaagtcacacatgcttaccataaA	cgaaatccaagccatcA
IWGSC_CSS.3AS_scaff.2648747	Cadenza0364	2688	G	A	het	het	tggAagcAcaagggccT	tggAagcAcaagggccT	GcgcagatggagatcG
IWGSC_CSS.3AS_scaff.3304956	Cadenza0364	10117	G	A	het	het	gtcccttgacacacatttG	gtcccttgacacacatttG	ctctgtgactacacttcaA
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IWGSC_CSS.3AS_scaff.3440912	Cadenza0364	4498	G	A	het	het	ccgtaaactttctgctgT	ccgtaaactttctgctgT	aAtActgaactatcatgTgC
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IWGSC_CSS.5DL_scaff.4538827	Cadenza0281	1208	G	A	hom	—	acgtcagaacaacgttgaC	acgtcagaacaacgttgaC	taaatgtggttggccacC
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IWGSC_CSS.1DL_scaff.2266648	Cadenza0110	6156	G	A	het	het	actggtgggttatggagcT	actggtgggttatggagcT	ccccactcagcaacaacA
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IWGSC_CSS.2DS_scaff.5373379	Cadenza0110	2166	C	T	hom	hom	agacaanaactagtGatggcT	agacaanaactagtGatggcT	gcgtgagaattgtTgtatttG
IWGSC_CSS.3AL_scaff.4384278	Cadenza0110	1276	C	T	het	het	agcTgaactccccTgtaA	agcTgaactccccTgtaA	agggaacctGgTggatgaA
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IWGSC_CSS.7AL_scaff.4552322	Cadenza2103	1412	C	T	het	het	gcaaaagcTgatactcaacaA	gcaaaagcTgatactcaacaA	ggcAAcCAgtataaagaagC
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IWGSC_CSS.3B_scaff.10562262	Cadenza0097	7819	C	T	het	het	agaggggtgctatccatAttgA	agaggggtgctatccatAttgA	agcgtatgccaaagcttccC
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IWGSC_CSS.5AL_scaff.2767581	Cadenza0097	3737	G	A	het	het	gagaggtcctcactAtcgG	gagaggtcctcactAtcgG	cgTaatcaaatattgctggG
IWGSC_CSS.5BL_scaff.10784643	Cadenza0097	1568	C	T	hom	hom	agaaaTAcattgattgaggaCG	agaaaTAcattgattgaggaCA	catctcCCttccaCgGaaagG

IWGS contig	Line	Pos	WT	Mut	Predicted	Called on M_4	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGS.CSS.1AL_scaff.3952258	Cadenza2092	8107	C	T	het	—	ttagtagaanaatgacagtgtgG	ttagtagaanaatgacagtgtgA	tgcacattgacatgagaG
IWGS.CSS.1BL_scaff.3858008	Cadenza2092	10278	G	A	hom	hom	tttagcaggcaggatcgC	tttagcaggcaggatcgT	actcagcgtatatacActattC
IWGS.CSS.1DL_scaff.2265172	Cadenza2092	9094	C	T	hom	hom	tgaJGTCatttgtttctatcagC	tgaJGTCatttgtttctatcagT	agtgtcaacttccGttcatC
IWGS.CSS.2AL_scaff.643867	Cadenza2092	16201	G	A	hom	hom	ttttctgTactttaacgtcaattgaC	ttttctgTactttaacgtcaattgaT	gtgaggaatgaggtgaagacC
IWGS.CSS.2AL_scaff.6439430	Cadenza2092	25101	C	T	het	—	caagaaggcCagCtCagC	caagaaggcCagCtCagT	tcGtAcTcttctActgggaa
IWGS.CSS.2DL_scaff.9760848	Cadenza2092	4733	C	T	het	hom	gcacattgggtctcaggtaC	gcacattgggtctcaggtaT	taagtcagtttGCTCtGTCG
IWGS.CSS.3AL_scaff.4407012	Cadenza2092	2785	C	T	het	hom	acatatAggtgtcttctaccacatC	acatatAggtgtcttctaccacatT	acctctcatgttaataggtttG
IWGS.CSS.3AS_scaff.3441108	Cadenza2092	541	G	A	het	het	GttagtgaactttgagacGgaG	GttagtgaactttgagacGgaA	aggcaTgacaaCgcgaA
IWGS.CSS.3B_scaff.10449827	Cadenza1551	4779	G	A	hom	hom	ggcagggtcnaagaaacGgtT	ggcagggtcnaagaaacGgtT	aCagaGtgggttagaggcaG
IWGS.CSS.3B_scaff.10550638	Cadenza1551	3250	C	T	het	het	ctctctactttgtcggT	ctctctactttgtcggT	gnaacAtTtgaactgcaagG
IWGS.CSS.3DL_scaff.6945816	Cadenza1551	589	C	T	hom	hom	agcatctcactgtcaacCaataT	agcatctcactgtcaacCaataT	TtggcccTtgaAttttcaTG
IWGS.CSS.3DL_scaff.6954177	Cadenza1551	3508	C	T	het	het	tgtagcatcacatfaactttcttA	tgtagcatcacatfaactttcttA	gcttggtataaacCttacgacA
IWGS.CSS.4AS_scaff.5938272	Cadenza1551	19080	G	A	hom	hom	agAcCcgAtcgccatgA	agAcCcgAtcgccatgA	GggAgatAcaggtaaaActcTtcG
IWGS.CSS.4AS_scaff.5937594	Cadenza1551	11092	C	T	het	het	gctttagtcgaacacaaA	gctttagtcgaacacaaA	ggctctcagctctgcA
IWGS.CSS.5AL_scaff.2671035	Cadenza1551	5859	C	T	het	het	cggtgatattTttagacttcgagC	cggtgatattTttagacttcgagT	ggcagttcagcGaccatT
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IWGS.CSS.5AL_scaff.2794167	Cadenza2088	13162	G	A	het	—	agtattcaggcaagcatCttCaG	agtattcaggcaagcatCttCaA	caatgaacacctcgaagaaGaG
IWGS.CSS.5BL_scaff.10889232	Cadenza2088	3885	G	A	het	het	cTcaaccacatgggcaAatC	cTcaaccacatgggcaAatT	tcttcatcaatcaatgttgG
IWGS.CSS.5BL_scaff.2267405	Cadenza2088	11113	C	T	hom	hom	ctttgagatctaggcctctTG	ctttgagatctaggcctctTA	tgtatttgtTtgggtAgagtttGA
IWGS.CSS.3B_scaff.10475354	Cadenza1409	2203	G	A	hom	hom	agCgaacaagagGtcaaacG	agCgaacaagagGtcaaacA	ctgaacacaCtagaCAattAccG
IWGS.CSS.3B_scaff.10674115	Cadenza1409	4555	C	T	het	het	gcttcagtcagcttcaG	gcttcagtcagcttcaA	cttcaacccGagataatGtattG
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IWGS.CSS.4DL_scaff.14314966	Cadenza1409	2010	G	A	het	hom	gtagggtccctctCAGgG	gtagggtccctctCAGgA	cgsggTcaaaAggttgCcT
IWGS.CSS.4DS_scaff.2324074	Cadenza1409	7606	G	A	het	het	tGatgaanaatgtGcaGaG	tGatgaanaatgtGcaGaA	ggggaAgttcAaaactGaag'gaaG
IWGS.CSS.5AS_scaff.1517889	Cadenza1409	3561	G	A	het	het	tctcgacatcttccgtgtA	tctcgacatcttccgtgtT	gtgctggcaacattgcttattA
IWGS.CSS.5AS_scaff.1523866	Cadenza1409	8054	G	A	hom	—	gggtgatctaccgcaGgaT	gggtgatctaccgcaGgaT	tcttgagCcTctctcA
IWGS.CSS.5BL_scaff.10917655	Cadenza1409	19073	G	A	hom	hom	caaatgacatgcaaaagaattgC	caaatgacatgcaaaagaattgT	cggttcatcactacaAaataigcT
IWGS.CSS.1AL_scaff.3886649	Cadenza1599	5204	C	T	het	het	tgtatgcaacacacatGcC	tgtatgcaacacacatGcT	ggagtagctgtgaccattatttG
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IWGS.CSS.2BL_scaff.7952427	Cadenza1599	19249	G	A	hom	hom	cgTccctCctcagcagcA	cgTccctCctcagcagcT	aTcacttccattagcgcAG
IWGS.CSS.2DL_scaff.9897981	Cadenza1599	5627	C	T	het	het	cttgggtctTgattgttactC	cttgggtctTgattgttactT	gTtggctCtctctgactTtgtG
IWGS.CSS.3AL_scaff.4446105	Cadenza1599	1765	G	A	hom	—	aaatgcttttcttcaCcgtagtG	aaatgcttttcttcaCcgtagtA	tcttAgaggcaatagctTatagcT

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