

# 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 as a 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 where 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 F<sub>2</sub> 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 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

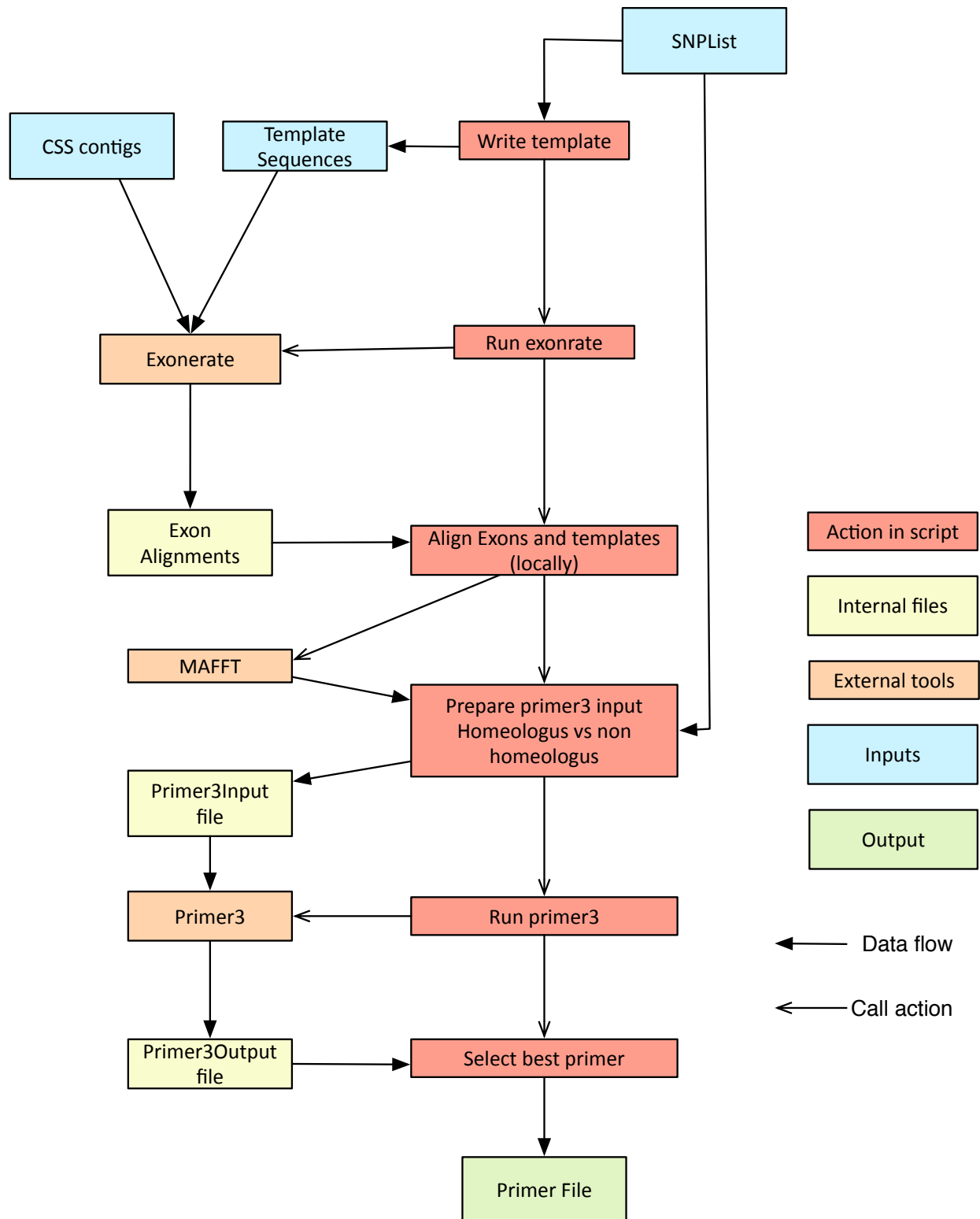


Figure 4.1: Steps and tools called by PolyMarker. The colour of the boxes represent: the step is an action inside the script(red); actions of the script(orange); temporary files(yellow); inputs(blue) and; output(green)



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). 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 Primer design tools

In this section, the principles of *in silico* primer design are discussed, and why not simply selecting a genomic variation is enough (thermal stability, primers folding on themselves)

## 4.3 Primer selection algorithms

Different algorithms to select the best primer:

### 4.3.1 KASP markers

For KASP markers, the product should be as short as possible with the mutation in the first three bases.

## 4.4 Designed markers

Details of the generated primers for the 80k iSelect chip and the 820k axion chip. This section also include counts on how many are genome specific, semi-specific and non specific. Also an analysis of how many are repeated or map to more than one chromosome perfectly.

### 4.4.1 Regular markers

PolyMarker was designed for KASP assays, but it was later extended to produce regular primers, where both primers start with a genome-specific base. This simplifies the design of primers for regular PCR and capillary sequencing.

### 4.4.2 Deletion algorithms

Algorithm to produce KASP for deletions in polyploids.

## 4.5 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.

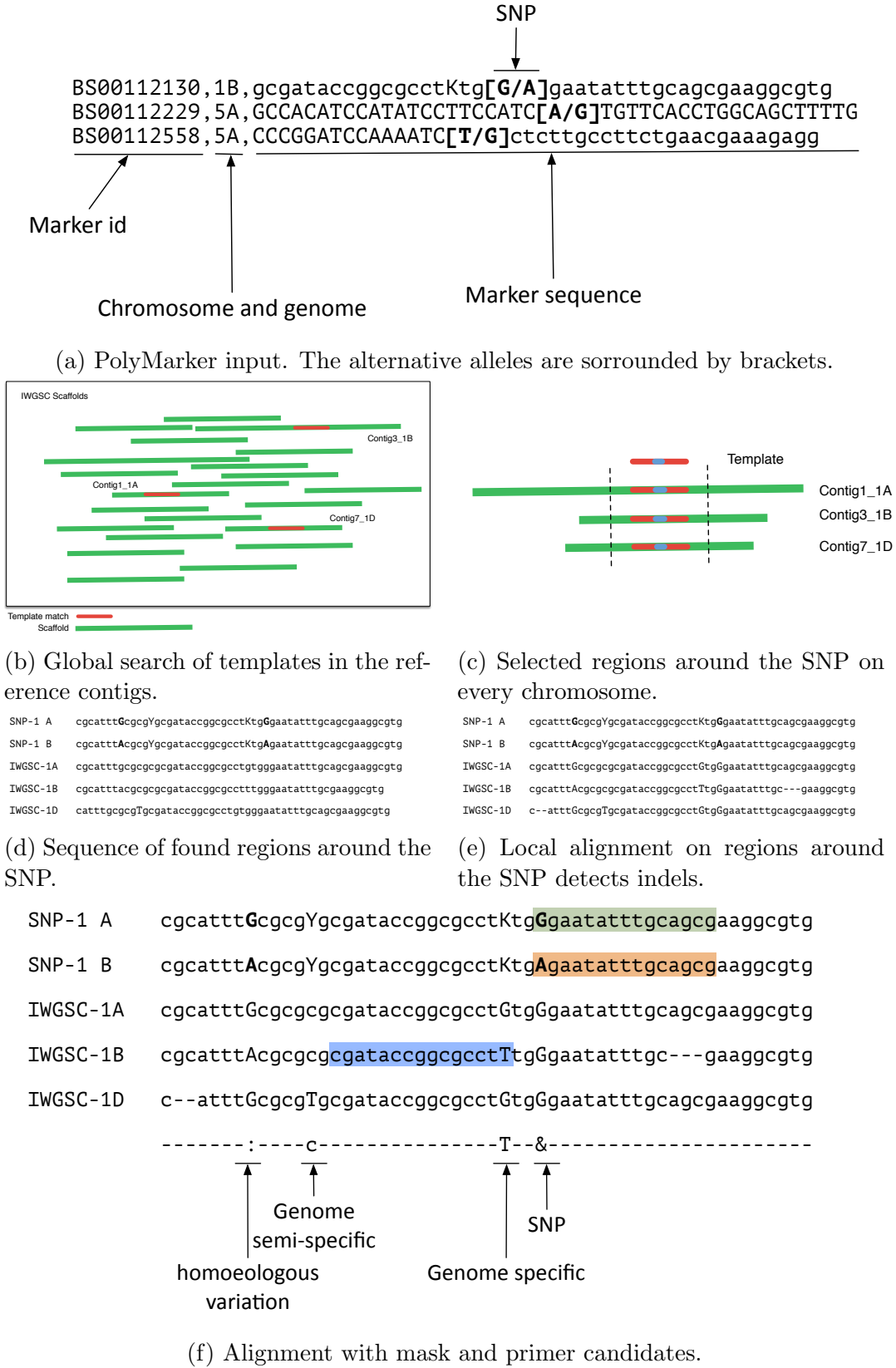


Figure 4.2: Alignments done by PolyMarker.

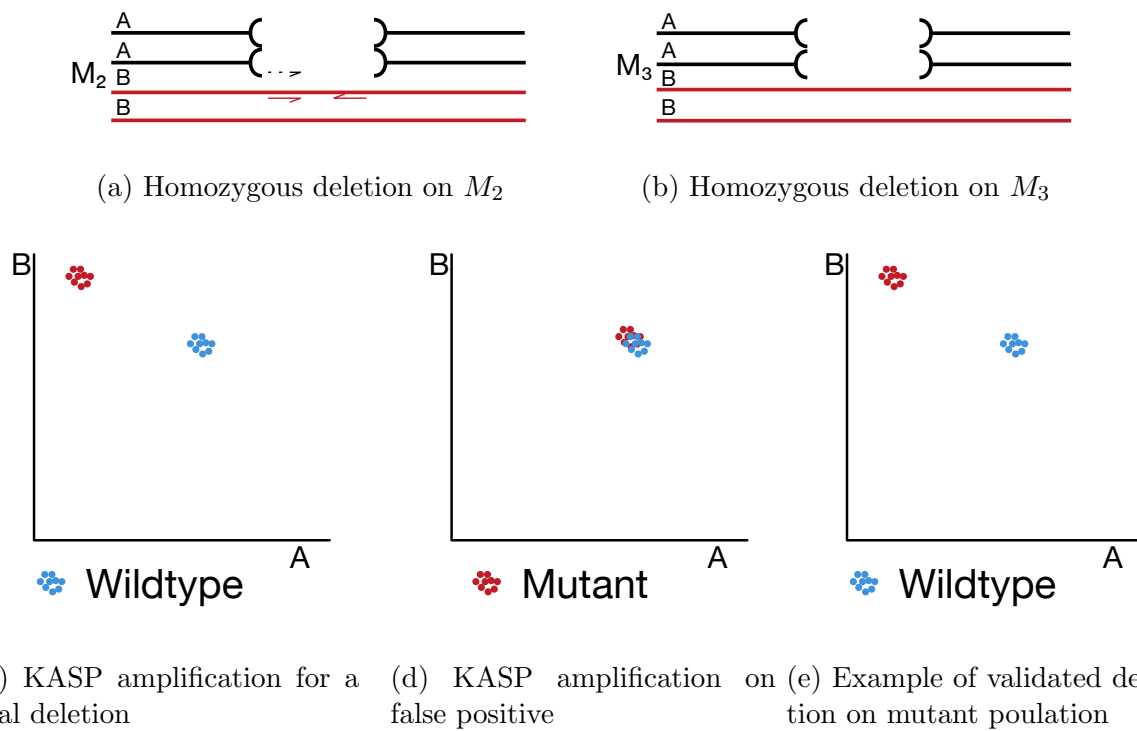


Figure 4.3: PolyMarker used to find primers to detect long deletions in tetraploid wheat.



# 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

## PolyMarker validation

# A.1 Validation of mutations on $M_4$ on Kronos

## APPENDIX A. POLYMARKER VALIDATION

IWGSC contig		Line	Pos	WT	Mut	Predicted	Called on $M_4$	Primer 1 (Kronos)	Primer 2 (mutant)	Common Primer
IWGSC_CSS_1AS.scnaf.3284790	Kronos3085	7449	G	A	Het	Het	Het	ccacactgagctcgC	ccacactgagctcGT	gtgatthgcaaggagA
IWGSC_CSS_1BL.scnaf.3897513	Kronos3085	1515	C	T	Het	Het	Het	gcttcaactggctctC	gcttcaactggctctC	acAagagctgttcgaacG
IWGSC_CSS_2AL.scnaf.6434745	Kronos3085	3424	C	T	Het	Het	Het	ctctGgtttgcaattttatcG	ctctGgtttgcaattttatcG	ggGcaATgcataaanaaA
IWGSC_CSS_3AS.scnaf.3408995	Kronos3085	732	C	T	Het	Het	Het	aggcactttgaaatccG	aggcactttgaaatccG	ggTgtaATccagAcctgTG
IWGSC_CSS_3B.scnaf.10708748	Kronos3085	2675	G	A	Het	Het	Het	gttgcaatcttaccacagG	gttgcaatcttaccacagA	gttaaanctggattgtagaC
IWGSC_CSS_4AL.scnaf.7132733	Kronos3085	1799	G	T	Hom	Hom	Hom	caaccgtgafgagcttC	caaccgtgafgagcttC	acGcctaGaaanaagctC
IWGSC_CSS_5AS.scnaf.1534693	Kronos3085	4605	G	A	Het	Het	Het	ccagcttcaggctcttAtC	ccagcttcaggctcttAtT	gtACctcagAGraATgAG
IWGSC_CSS_6AS.scnaf.4361911	Kronos3085	8857	C	T	Het	Het	Het	tcacgaagagagctctaacC	tcacgaagagagagctctaacC	catgggtgtgcttatccA
IWGSC_CSS_6BS.scnaf.3008326	Kronos3085	1528	G	A	Het	Het	Het	ccatgtgtactggttggtC	ccatgtgtactggttggtT	ggaaagatcagGaaTgA
IWGSC_CSS_7AS.scnaf.4214385	Kronos3085	27835	C	T	Hom	Hom	Hom	cgtaacttcgtttgggaagG	cgtaacttcgtttgggaagA	ctcttggtaagTgtatagA
IWGSC_CSS_1AL.scnaf.33929964	Kronos3191	1336	C	T	Het	Het	Het	tttggcccaactggacaatT	tttggcccaactggacaatT	attggcccaactggacaatT
IWGSC_CSS_1BL.scnaf.3899789	Kronos3191	7925	G	A	Hom	Hom	Hom	actctcacTggcagacG	actctcacTggcagacG	caacgtggtgcccatGtA
IWGSC_CSS_2AL.scnaf.6426728	Kronos3191	1481	G	A	Hom	Hom	Hom	gaatctcagcagctcG	gaatctcagcagcG	ccaGcGctgtgagaaA
IWGSC_CSS_2BL.scnaf.7960273	Kronos3191	690	C	T	Hom	Hom	Hom	gcatcttaactcttagagG	gcatcttaactcttagagT	acatgaatgttggatagG
IWGSC_CSS_3AS.scnaf.3286603	Kronos3191	2975	G	A	Het*	Hom	Hom	ccgtgtgtttgtttggG	ccgtgtgtttgtttggA	gaaaggaagtTcaTgaaG
IWGSC_CSS_5AL.scnaf.2694249	Kronos3191	2399	C	T	Het	Het	Het	gctctcaagatagaccGT	gctctcaagatagaccGT	ggccatcagatattccG
IWGSC_CSS_5BL.scnaf.10923577	Kronos3191	3713	C	T	Het	Het	Het	gtgatthgctgagcttG	gtgatthgctgagcttG	ttgggtcctcttggagC
IWGSC_CSS_6AL.scnaf.5823017	Kronos3191	13225	C	T	Hom	Hom	Hom	ccctttcgagctctggagG	ccctttcgagctctggagA	ttcggaagcccatcgA
IWGSC_CSS_6BS.scnaf.2955394	Kronos3191	1622	C	T	Het*	Hom	Hom	gtggaagatgaagtttagcaG	gtggaagatgaagtttagcaA	gatactgTgcaatgggtT
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IWGSC_CSS_1AS.scnaf.3276389	Kronos3288	9720	C	T	Hom	Hom	Hom	aCcaGcagagccAAatgctC	aCcaGcagagccAAatgctT	atgatgcaactcagcaatT
IWGSC_CSS_2AL.scnaf.6367515	Kronos3288	6976	G	A	Het	Het	Het	caggttcagTgtctccgG	caggttcagTgtctccgA	ggsgtgaCtggaaaggC
IWGSC_CSS_2AL.scnaf.6422019	Kronos3288	4523	G	A	Het	Het	Het	cgctagagctccttagaG	cgctagagctccttagaA	acgCAcgctaaagcgtAC
IWGSC_CSS_3AL.scnaf.4284850	Kronos3288	7901	G	A	Hom	Hom	Hom	ttgctttggacacatcgG	ttgctttggacacatcgA	ttgCAgcatagacagcaG
IWGSC_CSS_4AS.scnaf.5962359	Kronos3288	13049	G	A	Het	Hom	Hom	ccatcaagaagtagagttcgA	ccatcaagaagtagagttcgAT	accatggccagctgtgtCA
IWGSC_CSS_6AL.scnaf.5778773	Kronos3288	6853	G	A	Het	Het	Het	gagtggttcccttccttC	gagtggttcccttccttT	ggaggaagcgtactcgtCT
IWGSC_CSS_6AS.scnaf.4392100	Kronos3288	3434	C	T	Het	Het	Het	atggaagcaagctgtacCG	atggaagcaagctgtacCA	ggAagcgaaagtgaacaaA
IWGSC_CSS_7BL.scnaf.6744240	Kronos3288	9772	G	A	Het	Het	Het	agctgtgtcttcccttaacAG	agctgtgtcttcccttaacA	cangtgccttcttgagctC
IWGSC_CSS_1AL.scnaf.3887185	Kronos3413	9708	C	T	Hom	Hom	Hom	gcaagctttcttagagtaaG	gcaagctttcttagagtaaA	tGAaanaagcagagagA
IWGSC_CSS_2BS.scnaf.3381362	Kronos3413	5160	C	T	Het*	Hom	Hom	caactcttgctcgttagtG	caactcttgctcgttagtA	tGAaanaagcagGcaanaaC
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IWGSC_CSS_3B.scnaf.10693516	Kronos3413	12632	C	T	Het	Het	Het	ctaggcttggacaacagG	ctaggcttggacaacagT	agcttgcctataggatT
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IWGSC_CSS_5BL.scnaf.10856077	Kronos3413	5853	G	A	Het	Het	Het	agactttaccacatcttC	agactttaccacatctT	aagCacatitAatagctgaagC
IWGSC_CSS_6AL.scnaf.5750718	Kronos3413	11046	G	A	Hom	Hom	Hom	caagcTttccgactcttataG	caagcTttccgactcttataA	AgacgatTgtaatggatcag
IWGSC_CSS_7AL.scnaf.4433177	Kronos3413	3511	C	T	Het	Het	Het	GaTgtctcGtcagctG	GaTgtctcGtcagctA	cactatcgacaagctttgG
IWGSC_CSS_7BL.scnaf.6742567	Kronos3413	667	C	T	Het	Het	Het	gttgcgttgctggaagcG	gttgcgttgctggaagT	cattttggaccgttgctTG
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IWGSC_CSS_2BL.scnaf.7882382	Kronos3935	2721	C	T	Het	Het	Het	gcaagcttagaagcttagcagT	gcaagcttagaagcttagcagT	ggcaacagtagaggaagaAT
IWGSC_CSS_3AL.scnaf.4242376	Kronos3935	2340	C	T	Het	Het	Het	agaaaccaaaccgTacttaG	agaaaccaaaccgTacttaA	gtagGtCcatCcaagctttG
IWGSC_CSS_3B.scnaf.10485067	Kronos3935	3349	C	T	Hom	Hom	Hom	gttgagcaaacatctcaactA	gttgagcaaacatctcaactA	gtaattctcttaTccagagT
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IWGSC_CSS_4BL.scnaf.7019402	Kronos3935	9081	C	T	Het	Het	Het	tgcataatgtatgagagctA	tgcataatgtatgagagctT	agacatgatcctagaagCcatatC
IWGSC_CSS_5BL.scnaf.10842786	Kronos3935	3304	G	A	Het	Het	Het	tggttcccGaaagctgaagC	tggttcccGaaagctgaagT	cgcatacttgaacatTgagcAC
IWGSC_CSS_6BS.scnaf.3045205	Kronos3935	2293	G	A	Het	Het	Het	aaggaacaaagcccaactcG	aaggaacaaagcccaactcA	agTgatcgaagcccatgtogA
IWGSC_CSS_7AL.scnaf.455249	Kronos3935	4487	C	T	Het	Het	Het	cAGgtctcgagtagggC	cAGgtctcgagtagggT	cCttgcaaccctctgatT
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IWGSC_CSS_2BL.scnaf.5131713	Kronos4240	5900	G	A	Het	Het	Het	ctttatcaggaanaagagacT	ctttatcaggaanaagagacT	caccattgttaggtttcttThC
IWGSC_CSS_5AL.scnaf.2769540	Kronos4240	9626	C	T	Het	Het	Het	tgCaggtggggaagagG	tgCaggtggggaagagA	catgagTgagatcttctgT
IWGSC_CSS_5BL.scnaf.10871091	Kronos4240	7062	G	A	Het	Het	Het	gcgaagAACataactcG	gcgaagAACataactcT	GcagctttggcAACcagA
IWGSC_CSS_6AL.scnaf.5800333	Kronos4240	2360	G	A	Het	Het	Het	cgacaagatgttggagCgtC	cgacaagatgttggagCgtT	tcaagatgtcgaagattatC
IWGSC_CSS_7BL.scnaf.6716931	Kronos4240	2613	G	A	Het	Het	Het	gGtGgtattTgctttgtaG	gGtGgtattTgctttgtaA	tGtGactcagaChGtA
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IWGSC_CSS_3B.scnaf.10460714	Kronos4346	14359	C	T	Hom	Hom	Hom	ctaccttgcataggagaaA	ctaccttgcataggagaaA	aggacccaagcttttgaG
IWGSC_CSS_4AS.scnaf.5989735	Kronos4346	6404	G	A	Hom	Hom	Hom	agcactgttaacatragcT	agcactgttaacatragcT	actcaagatatacCcgagC
IWGSC_CSS_5BL.scnaf.7648030	Kronos4346	6893	C	T	Het	Het	Het	tacccttccatcttagcagG	tacccttccatcttagcagA	ttttcagaagacacagatataC
IWGSC_CSS_6AL.scnaf.5758540	Kronos4346	778	C	T	Het	Het	Het	atcgatgaatgtgtcagC	atcgatgaatgtgtcagT	acgttgatcAtCaatcAC
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IWGSC_CSS_7AL.scnaf.4542983	Kronos4346	18700	G	A	Het	Het	Het	gcagagctACcgagatC	gcagagctACcgagatC	catctgcGgtttaaactC
IWGSC_CSS_7BS.scnaf.3098098	Kronos4346	5183	C	T	Het	Het	Het	gCgatahgtagcttgaatgaG	gCgatahgtagcttgaatgaA	ttatcttgcttaatGThgCcgG
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IWGSC_CSS_2AL.scnaf.6315418	Kronos4485	10490	G	A	Hom	Hom	Hom	ggccctctcaatCcttcagT	ggccctctcaatCcttcagT	tttcagagctCGaaggaatccC
IWGSC_CSS_2BS.scnaf.5181092	Kronos4485	3742	G	A	Het	Het	Het	TggcagacacactgaG	TggcagacacactgaA	tgcagagatgTtagaAaT
IWGSC_CSS_3B.scnaf.10425015	Kronos4485	2372	C	T	Het	Het	Het	gctactgaagtggCtcG	gctactgaagtggCtcGA	cttcaatcttgggggTtG
IWGSC_CSS_3B.scnaf.10775915	Kronos4485	4701	C	T	Het	Het	Het	ccaagagcttgagagagA	ccaagagcttgagagagA	agactcagatGtctccC
IWGSC_CSS_5AL.scnaf.2754304	Kronos4485	2301	G	A	Het	Het	Het	taaccTggcatcgccG	taaccTggcatcgccA	cattgTccgcaataTgactA
IWGSC_CSS_5BL.scnaf.10919059	Kronos4485	1867	C	T	Hom	Hom	Hom	gatgcctttgtggagagA	gatgcctttgtggagagA	tcttgctccgaanaacttGA
IWGSC_CSS_7AS.scnaf.4246431	Kronos4485	3402	G	A	Hom	Hom	Hom	aagggccttggtgttC	aagggccttggtgttC	agtaagTggaATcagatgtataT
IWGSC_CSS_7BL.scnaf.6667357	Kronos4485	641	C	T	Het	Het	Het	gattcAggtctcattcagG	gattcAggtctcattcagA	ttccctgtcaattgtatgcC

# 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	Cadenzal1772	6019	C	T	het	het	caggatAgtGGagactgtcaaaG	caggatAgtGGagactgtcaaaA	ggagacGGctGggacatT
IWGSC.CSS.3DL_scaff.6955403	Cadenzal1772	2418	C	T	het*	hom	tcagCGgattgtcggagTA	tcagCGgattgtcggagTA	tgic-CatgaaTcttctcacG
IWGSC.CSS.4AL_scaff.7106846	Cadenzal1772	11277	G	A	hom	hom	tggagatcatgctcaactG	tggagatcatgctcaactA	gatggtGgatttgcgcta
IWGSC.CSS.4AS_scaff.5991335	Cadenzal1772	15710	G	A	hom	hom	ctggccctgcgtgctaC	ctggccctgcgtgctaT	gtggaaGhtcagaagacaG
IWGSC.CSS.4BS_scaff.4956646	Cadenzal1772	252	G	A	het*	hom	gcagggtgacttccggaG	gcagggtgacttccggaA	tGaggtacgaGcTaaagAaagC
IWGSC.CSS.4DS_scaff.1715962	Cadenzal1772	1225	G	A	hom	hom	cagctgtggTatctcaactGG	cagctgtggTatctcaactGA	CcCtGaaACACcGtttgggaT
IWGSC.CSS.5AL_scaff.2763407	Cadenzal1772	2119	G	A	hom	hom	ggagCGaacctcagatctGA	ggagCGaacctcagatctGA	gaTggcaATcGTcgtgcA
IWGSC.CSS.5AS_scaff.1548786	Cadenzal1772	12625	G	T	het	het	AtaggcacatigtacatgaG	AtaggcacatigtacatgaA	ggattgggttggcagC
IWGSC.CSS.5BL_scaff.10849226	Cadenzal1772	2289	C	T	het*	hom	cttgacatcatgttcacagTA	cttgacatcatgttcacagT	cactccgaggtgtccatG
IWGSC.CSS.5BS_scaff.2270737	Cadenzal1772	2262	C	A	hom	—	attcCTTgtgttggCaaatgaG	attcCTTgtgttggCaaatgaA	taaGcaaaAccctccagtgG
IWGSC.CSS.1AL_scaff.3022915	Cadenzal1661	891	C	T	hom	hom	ccacgtgagactcctatfagaCG	ccacgtgagactcctatfagaCA	atgctgGatcGtGtGagtcC
IWGSC.CSS.1BL_scaff.3297240	Cadenzal1661	1970	C	T	het	het	catccgccCTttctctC	catccgccGtttctctT	gctccgcatgaagagT
IWGSC.CSS.1BS_scaff.3828996	Cadenzal1661	1340	G	A	hom	hom	agccgagttgtagTtaacT	agccgagttgtagTtaacT	agcagcttgTcgtgtfaaC
IWGSC.CSS.1DS_scaff.1884529	Cadenzal1661	10575	G	A	hom	hom	aCagatacaATgttcacaggT	aCagatacaATgttcacaggT	accitgggTgttccaacttC
IWGSC.CSS.2AL_scaff.6318370	Cadenzal1661	19142	C	T	het	—	cgtggcCGaatCtcGacG	cgtggcCGaatCtcGacA	ttcttggagcggcgC
IWGSC.CSS.2AS_scaff.5213460	Cadenzal1661	1358	G	A	hom	hom	gtcaegaaCCcgctcagG	gtcaegaaCCcgctcagA	aggaaagagggaaanaaGcG
IWGSC.CSS.2BS_scaff.5179331	Cadenzal1661	5604	G	A	het	het	actctcgtaagaactgatacaA	actctcgtaagaactgatacaA	gcaGagaatgttctgcaacT
IWGSC.CSS.3AL_scaff.5341235	Cadenzal1661	4673	G	A	het	het	ggtgaggtatcggagctG	ggtgaggtatcggagctG	ggcggtgtctagagttG
IWGSC.CSS.3AS_scaff.4250995	Cadenzal1661	7046	G	A	hom	hom	cCaagaaacgggtgtgtccaG	cCaagaaacgggtgtgtccaA	ctgcagctgtcccatcagT
IWGSC.CSS.3B_scaff.10404421	Cadenzal1661	4303	G	A	het	het	ctctctcgaCaggacctG	ctctctcgaCaggacctA	GCcagttactCacAtgctctC
IWGSC.CSS.5DL_scaff.2390496	Cadenzal1538	2125	C	T	hom	het	gcagttttatctcagtagcttgG	gcagttttatctcagtagcttGA	ttctgagaaTgtaatgtgcGatG
IWGSC.CSS.6AL_scaff.5753680	Cadenzal1538	3920	C	T	hom	hom	tgtcccaatttggacacaaTaaC	tgtcccaatttggacacaaTaaT	aaaGcaaggsgtaagtitttgT
IWGSC.CSS.6AS_scaff.4425792	Cadenzal1538	4307	G	A	hom	het	agatcgtgtCGgGcaaa	agatcgtgtCGgGcaaa	gctgaagcaacgcgcacaaT
IWGSC.CSS.6BS_scaff.3003630	Cadenzal1538	6933	C	T	het	het	ggcagtaagtgtgtgtgagT	ggcagtaagtgtgtgtgagT	tTgaCttcgtgtgtgtggcA
IWGSC.CSS.6DL_scaff.3246988	Cadenzal1538	9186	G	A	het	het	gctaagaagagcttggagaaatT	gctaagaagagcttggagaaatT	aatttctgaagagaggtgtgtatG
IWGSC.CSS.7AL_scaff.4480114	Cadenzal1538	3446	C	T	het	—	gatatctccacacggcG	gatatctccacacggcA	tgaccactcttcgagttT
IWGSC.CSS.7AS_scaff.4193541	Cadenzal1538	8359	C	T	hom	het	agcaattcttggctatcaatagC	agcaattcttggctatcaatagT	tcactGtcttaacttactctG
IWGSC.CSS.7BL_scaff.6721572	Cadenzal1538	9223	C	T	het	het	gctCaggagggagacaanaaG	gctCaggagggagacaanaaA	tgtctaagaatctcgacctC
IWGSC.CSS.7BS_scaff.3152545	Cadenzal1538	3960	G	A	hom	—	tcagcaaatatcctgcCgT	tcagcaaatatcctgcCgT	gTcgccccatcatgtttaT
IWGSC.CSS.7DS_scaff.3963838	Cadenzal1538	2913	G	A	het	het	tCgttgcgaacCttTgtgtG	tCgttgcgaacCttTgtgtG	agaGttaTcaagTactgtcaA
IWGSC.CSS.1AL_scaff.3903380	Cadenzal1469	6193	G	A	hom	hom	ctcttcAGagatgaacgcgG	ctcttcAGagatgaacgcgA	tcCtGagatgAGtggttGTtA
IWGSC.CSS.1AS_scaff.3287728	Cadenzal1469	3817	C	T	het*	hom	ccgaccaAItcactaacgA	ccgaccaAItcactaacgA	acctcttcccAGactatG
IWGSC.CSS.1BL_scaff.3815304	Cadenzal1469	513	G	A	hom	hom	aacatttgcctTaCcaaaacGT	aacatttgcctTaCcaaaacGT	acacagaactttaatgCAAGC
IWGSC.CSS.1DL_scaff.2266648	Cadenzal1469	5926	C	T	het	het	caacatlgagacacacacttT	caacatlgagacacacacttT	gtcaacgsgtggaggtgtG
IWGSC.CSS.1DS_scaff.1906671	Cadenzal1469	3697	C	T	hom	het	tggTGtagacacttggcggaA	tggTGtagacacttggcggaA	catggcgaccacAcctG
IWGSC.CSS.2AL_scaff.6337088	Cadenzal1469	7334	A	het*	hom	hom	acaatgccAagttgacaggttG	acaatgccAagttgacaggttA	ggaggtgtggttGAgaaacT
IWGSC.CSS.2BL_scaff.7972799	Cadenzal1469	8995	C	T	het	hom	gTgCtctcGcatcttT	gTgCtctcGcatcttT	gatcggCGcaaaactagTG
IWGSC.CSS.2DL_scaff.9832343	Cadenzal1469	3262	G	A	het	het	TtgtcttaAcagacCGcagG	TtgtcttaAcagacCGcagA	agactcgttcagccttctT
IWGSC.CSS.2DS_scaff.5327939	Cadenzal1469	3889	G	A	het	het	ttttTgccttaigtgacttagtaC	ttttTgccttaigtgacttagtaT	gaggccatcacagtagcG
IWGSC.CSS.3B_scaff.10395219	Cadenzal1469	1292	G	A	hom	—	aggtgtcgtgtgtgtG	aggtgtcgtgtgtgtG	cccttctggsggctttaacC
IWGSC.CSS.3BS_scaff.10592217	Cadenzal0580	2994	C	T	het	—	acacgagtatcaagcccttC	acacgagtatcaagcccttC	tgatactgtTggCGgagG
IWGSC.CSS.3DS_scaff.22367081	Cadenzal0580	1037	G	A	het	het	tggttatgCAcagataatCagG	tggttatgCAcagataatCagA	tgacaactgtgatcttaggtT
IWGSC.CSS.4AL_scaff.7973953	Cadenzal0580	9881	C	T	hom	hom	CacaggaagccgttaacaC	CacaggaagccgttaacaT	cttcAGcagcatggagT
IWGSC.CSS.4BL_scaff.7037448	Cadenzal0580	1837	C	T	hom	hom	CgttgaanaaGctgaagaacttaaC	CgttgaanaaGctgaagaacttaaT	cagttcttcTtCaGagcagataT
IWGSC.CSS.4BS_scaff.4920479	Cadenzal0580	10668	G	A	hom	—	tggatttcccgcactgtTC	tggatttcccgcactgtT	gtaaacaaggcatttcaagagtcA
IWGSC.CSS.4DL_scaff.14359838	Cadenzal0580	1408	G	A	hom	—	gCtCAItcaggagTGTcCtaTatG	gCtCAItcaggagTGTcCtaTatA	tgaCagaacagtttggtcatacT
IWGSC.CSS.4DS_scaff.2276484	Cadenzal0580	8034	G	A	hom	hom	gccgtgtgtgaggtAGaG	gccgtgtgtgaggtAGaA	cgtcagatattacgacttgcA
IWGSC.CSS.5AL_scaff.2756579	Cadenzal0580	5278	G	A	het	het	tgaatgatttttctgtccgttC	tgaatgatttttctgtccgtT	ggAATCC/TATgCAgaAGAaaCTG
IWGSC.CSS.5BL_scaff.10787208	Cadenzal0580	10627	G	A	het	—	gcctctcacatgggagaC	gcctctcacatgggagat	accgatgtcAGgtggCcgT
IWGSC.CSS.5BS_scaff.2292179	Cadenzal0580	5267	G	A	het	hom	tgatgggtacagcgtG	tgatgggtacagcgtT	tggcgcccttgaAAtcC
IWGSC.CSS.5DL_scaff.4498073	Cadenzal0423	4937	C	T	hom	hom	gcacctctggttgggtcatC	gcacctctggttgggtcatT	tgagacgaAAngcagccG
IWGSC.CSS.5DS_scaff.2738970	Cadenzal0423	2319	C	T	het	—	ctgtgaggtggsgtattgT	ctgtgaggtggsgtattgT	tggaaactgttacctcaggtTC
IWGSC.CSS.6AL_scaff.5757109	Cadenzal0423	2788	G	A	hom	hom	caggaGcctggcgcaataaaGA	caggaGcctggcgcaataaaGA	ctttcGcagctctttagttcG
IWGSC.CSS.6AS_scaff.4387871	Cadenzal0423	2543	G	A	hom	hom	gcagtctaacagcggcaaaagA	gcagtctaacagcggcaaaagA	ctcatctctctcttcttaaggtT
IWGSC.CSS.6BL_scaff.4271391	Cadenzal0423	4660	C	T	hom	hom	tacgttgcagtgtgtgtgtgtaC	tacgttgcagtgtgtgtgtgtaT	gtttgaagtgcacagatTaaCa
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IWGSC.CSS.7AS_scaff.4227506	Cadenzal0423	952	C	A	het	—	ccaatgtttccaatgttagagC	ccaatgtttccaatgttagagT	tgccttagctgtgtgtgtT
IWGSC.CSS.7BL_scaff.6681872	Cadenzal0423	1486	C	A	hom	hom	agtaagCGtgcagcaatgggG	agtaagCGtgcagcaatgggA	AtgctTtgGtggaaagtacatA
IWGSC.CSS.7BS_scaff.3160328	Cadenzal0423	7801	C	T	het	het	tgttaaatGatacagCctgagT	tgttaaatGatacagCctgagT	tggaaagtgtGtgtgtttT
IWGSC.CSS.7DS_scaff.407428	Cadenzal0423	2051	G	A	het	het	gtcCGcctctctgacaA	gtcCGcctctctgacaA	actcatcAGgtcagcccaA
IWGSC.CSS.3AL_scaff.442479	Cadenza0364	3198	C	T	het	het	gagtcATaaagtgtgaattgtG	gagtcATaaagtgtgaattgtG	GCaCaTaaCaacagatcacG
IWGSC.CSS.3AS_scaff.4447942	Cadenza0364	11917	G	A	het	het	gtcaaaagatgtcctgtgaaG	gtcaaaagatgtcctgtgaaA	ctcGgatgtgggaggaagA
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IWGSC.CSS.3AS_scaff.2648747	Cadenza0364	2688	G	A	het	het	tggAagCAaaggggccC	tggAagCAaaggggccT	GccgcagatggagactG
IWGSC.CSS.3AS_scaff.3304956	Cadenza0364	1017	G	A	het	het	gtcccttgcacacagctttG	gtcccttgcacacagctttA	ctctgctgaactacaacttaacT
IWGSC.CSS.3AS_scaff.3321091	Cadenza0364	4585	C	T	het	het	caagaatGATgctgatgttggaa	caagaatGATgctgatgttggaa	acaatgtgaatccgcgaatC
IWGSC.CSS.3AS_scaff.3371333	Cadenza0364	538	G	A	het	het	gggaaacGAgAcagagcG	gggaaacGAgAcagagcA	ccgtgcttctctaccT
IWGSC.CSS.3AS_scaff.3371815	Cadenza0364	1061	C	T	het	het	atccccagggcacagagG	atccccagggcacagagA	aAtggcccttgggtgaticC
IWGSC.CSS.3AS_scaff.3440912	Cadenza0364	4498	G	A	het	het	ccgtaaactttctgtgtgtG	ccgtaaactttctgtgtgtT	aAtGcgcaactacatgatgtG
IWGSC.CSS.3B_scaff.10343586	Cadenza0364	2242	G	A	het	—	ggttcTgtTcctcttccactG	ggttcTgtTcctcttccactA	tgtgtgaacccgcaagcA

IWGSC contig	Line	Pos	WT	Mut	Predicted	Called on $M_4$	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGSQ_CSS_3AL.scnaf.442479	Cadenza0364	3198	C	T	het	het	gagtaCTaagtgtgtaagattggC	gagtaCTaagtgtgtaagattgT	GCaGaThaCaacagatcAG
IWGSQ_CSS_3AL.scnaf.4447942	Cadenza0364	11917	G	A	het	het	gtataaagattgtctccgttgaAG	gtataaagattgtctccgttgaAG	ctcGgatgtgtggaagA
IWGSQ_CSS_3AS.scnaf.1557483	Cadenza0364	2547	C	A	het	het	aaagatcaatcattgtaccataaAG	aaagatcaatcattgtaccataaA	cgaatccagatccatcA
IWGSQ_CSS_3AS.scnaf.2648747	Cadenza0364	2688	G	A	het	het	tggAagCAcaaggsgcCT	tggAagCAcaaggsgcCT	GccgcgagatccatcA
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IWGSQ_CSS_3AS.scnaf.33781815	Cadenza0364	1061	C	T	het	het	atcccacggcacagagA	atcccacggcacagagA	aatgtccctgttgatTC
IWGSQ_CSS_3AS.scnaf.3440912	Cadenza0364	4498	G	A	het	het	ccgttaaaactttgtgtcttG	ccgttaaaactttgtgtcttA	atActggaacatcattgATG
IWGSQ_CSS_3B.scnaf.10343586	Cadenza0364	2242	G	A	het	—	ggcttCTgtTctctctccatG	ggcttCTgtTctctctccatA	tgtgtggaacagcagA
IWGSQ_CSS_5DL.scnaf.242342	Cadenza0281	2433	C	T	hom	hom	catggCGacagctGtccTG	catggCGacagctGtccA	aaCcccatTTtgCTACTCT
IWGSQ_CSS_5DL.scnaf.4538822	Cadenza0281	1208	C	A	hom	—	acgtcgaacacgcgtttgaC	acgtcgaacacgcgtttgaT	ttaaatTggttgctgaC
IWGSQ_CSS_6AL.scnaf.3513297	Cadenza0281	4532	C	T	hom	—	ggagagggagacgtctgG	ggagagggagacgtctgA	tcttcgtccacgattccG
IWGSQ_CSS_6AL.scnaf.3513297	Cadenza0281	6748	C	T	hom	hom	cccgagttctctcttttTC	cccgagttctctcttttCT	caagtataaanaaagaagTgT
IWGSQ_CSS_6AS.scnaf.4378990	Cadenza0281	5426	C	T	het	het	aCtaccatagcttCGgttgaG	aCtaccatagcttCGgttgaA	tcaagtacaagTtcaagatT
IWGSQ_CSS_6BL.scnaf.4360781	Cadenza0281	3808	G	A	hom	hom	gttctctgtatgagacagC	gttctctgtatgagacagC	ggctcttttctgcttA
IWGSQ_CSS_7AL.scnaf.4488310	Cadenza0281	9232	G	A	het	het	gctcgaagGgttgaagaAagG	gctcgaagGgttgaagaAagA	ggcttGAgtctGaggtT
IWGSQ_CSS_7BS.scnaf.3143575	Cadenza0281	1866	C	T	het	het	agatgttAgaggggcttTC	agatgttAgaggggcttTT	ggttgaATgttgagagT
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IWGSQ_CSS_7DS.scnaf.3933917	Cadenza0281	1243	C	T	het	het	tgtCTgagCttTcaccttG	tgtCTgagCttTcaccttG	agaggtttgtttccatGG
IWGSQ_CSS_3B.scnaf.10626860	Cadenza0148	7847	G	A	het	het	ggagctctgggaagagG	ggagctctgggaagagA	gtttaaAgtaCTctctagcG
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IWGSQ_CSS_4AS.scnaf.5950625	Cadenza0148	10590	C	T	het	het	agaTatCaaATcggtAgttgC	agaTatCaaATcggtAgttgT	ccgtCtccctcagTC
IWGSQ_CSS_4AS.scnaf.5967119	Cadenza0148	11626	C	T	hom	hom	cgTgacacccagagctA	cgTgacacccagagctA	ggcgaagcactcagacG
IWGSQ_CSS_4DL.scnaf.14455742	Cadenza0148	1946	C	T	hom	hom	gCctgagagacagctgG	gCctgagagacagctgT	aaacggGAaCTGgGcA
IWGSQ_CSS_4DS.scnaf.2318993	Cadenza0148	4000	C	T	hom	hom	tccagtttgaacagattggaagG	tccagtttgaacagattggaagA	tgaGaTctgtttcttcaatG
IWGSQ_CSS_5AL.scnaf.2750707	Cadenza0148	4603	G	A	het	het	ccctgtgttagccattcaagTaaG	ccctgtgttagccattcaagTaa	ccagagTtgcgttgaattcaAG
IWGSQ_CSS_5BL.scnaf.10794137	Cadenza0148	9235	C	T	hom	hom	gaagctgtcttgcgttG	gaagctgtcttgcgttA	agatctctccatataagcattG
IWGSQ_CSS_5BS.scnaf.1646558	Cadenza0148	2916	C	T	het	het	gctGtaacatcactAlcccttG	gctGtaacatcactAlcccttA	gcaATgttcaatAlaccctT
IWGSQ_CSS_1AL.scnaf.3883106	Cadenza0110	27326	C	T	het	het	accttccatcactgtctG	accttccatcactgttGA	gtgaaagaaacaggttgaAG
IWGSQ_CSS_1BL.scnaf.3812829	Cadenza0110	10770	G	A	het*	hom	cccccatcattccagG	cccccatcattccagA	gGatgttctgtctggaC
IWGSQ_CSS_1DL.scnaf.2266648	Cadenza0110	6156	G	A	het	het	acgtgcgttAgtttggagcC	acgtgcgttAgtttggagcT	gGatgttctgtctggaC
IWGSQ_CSS_1DS.scnaf.1889435	Cadenza0110	8826	C	T	hom	het	aaactgaattactcggagagG	aaactgaattactcggagacT	gacctgaagattgaacacA
IWGSQ_CSS_2AS.scnaf.5268634	Cadenza0110	4636	G	A	het	het	gatacagtgattgtgcatgttG	gatacagtgattgtgcatgttA	gctcatcgtgattgcatgttA
IWGSQ_CSS_2BL.scnaf.7965110	Cadenza0110	15801	C	T	hom	hom	cattgagcAtaacAatugcAaC	cattgagcAtaacAatugcAaT	attttgagcAtaacAatugcAaT
IWGSQ_CSS_2DL.scnaf.9852812	Cadenza0110	13788	G	A	hom	hom	attttgtatggttcaatcttgcC	attttgtatggttcaatcttgcT	attttgatggttcaatcttgcT
IWGSQ_CSS_2DS.scnaf.5371379	Cadenza0110	2166	C	T	hom	hom	agacacaacacagTgatggTC	agacacaacacagTgatggTC	agacacaacacagTgatggTC
IWGSQ_CSS_3AL.scnaf.4384278	Cadenza0110	1276	C	T	het	het	agCTgaactgcctTgtaAG	agCTgaactgcctTgtaA	atcttAgttgttgcacatA
IWGSQ_CSS_3AS.scnaf.3340122	Cadenza0110	1467	C	T	hom	hom	attcttAgttgttgcacatG	attcttAgttgttgcacatA	gttgcctctcaanaagaaatA
IWGSQ_CSS_5DL.scnaf.454222	Cadenza0103	6528	C	T	het*	hom	ggcgcctcaanaagaaatG	ggcgcctcaanaagaaatG	gactgcctcaanaagaaatG
IWGSQ_CSS_6AL.scnaf.5838640	Cadenza0103	7346	C	T	hom	hom	agaanaagccacaatggtttTC	agaanaagccacaatggtttCT	agaanaagccacaatggtttCT
IWGSQ_CSS_6AS.scnaf.4429974	Cadenza0103	3867	G	A	hom	hom	GagatgaAttatattgagcattggC	GagatgaAttatattgagcattggT	tgcaatgaAttatattgagcattggT
IWGSQ_CSS_6DL.scnaf.3307626	Cadenza0103	4970	G	A	hom	hom	tgcagatgttctcgttgaAG	tgcagatgttctcgttgaA	tgcagatgttctcgttgaA
IWGSQ_CSS_6DS.scnaf.2059604	Cadenza0103	5224	G	A	het	—	gctcaatgactCTgagtgG	gctcaatgactCTgagtgA	gctcaatgactCTgagtgA
IWGSQ_CSS_7AL.scnaf.4552322	Cadenza0103	1412	C	T	het	het	gcaagagCTgacttccacaagG	gcaagagCTgacttccacaagA	gcaagagCTgacttccacaagA
IWGSQ_CSS_7BS.scnaf.3147455	Cadenza0103	4607	G	A	het	—	gcaacttagagattgagCTaatG	gcaacttagagattgagCTaatG	gcaacttagagattgagCTaatG
IWGSQ_CSS_7DL.scnaf.3382467	Cadenza0103	3473	C	T	hom	—	GGTtctgCaGTTCTATTAcatcC	GGTtctgCaGTTCTATTAcatcT	GGTtctgCaGTTCTATTAcatcT
IWGSQ_CSS_3B.scnaf.10457010	Cadenza0277	10599	G	A	het	het	aaacttggcgcagaaacA	aaacttggcgcagaaacA	aaacttggcgcagaaacA
IWGSQ_CSS_3B.scnaf.10593852	Cadenza0277	10124	C	T	het	het	tgcagggggcgtatatacG	tgcagggggcgtatatacA	tgcagggggcgtatatacA
IWGSQ_CSS_3DS.scnaf.2583390	Cadenza0277	663	G	A	hom	hom	agcgtcatcacaacAattChgC	agcgtcatcacaacAattChgT	actgactcatcacaacAattChgT
IWGSQ_CSS_4AL.scnaf.7093953	Cadenza0277	10004	C	T	hom	hom	cttgtatattacagaTgtTtttG	cttgtatattacagaTgtTtttGA	cttgtatattacagaTgtTtttGA
IWGSQ_CSS_4AL.scnaf.7176064	Cadenza0277	6220	C	T	het	het	gtgcgtatTtCCgcttgG	gtgcgtatTtCCgcttgA	gtgcgtatTtCCgcttgA
IWGSQ_CSS_4DL.scnaf.14122349	Cadenza0277	1010	C	T	hom	hom	gtgcgtctgChttggaA	gtgcgtctgChttggaA	gtgcgtctgChttggaA
IWGSQ_CSS_4AL.scnaf.2736916	Cadenza0277	4296	G	A	het	het	agaactATgAaAGtaacacagC	agaactATgAaAGtaacacagC	agaactATgAaAGtaacacagC
IWGSQ_CSS_5BL.scnaf.10883744	Cadenza0277	2080	G	A	hom	hom	gctcttctChgtTAgctcagG	gctcttctChgtTAgctcagA	gctcttctChgtTAgctcagA
IWGSQ_CSS_2DL.scnaf.538086	Cadenza0548	11765	C	T	hom	hom	accgcaacCCaagagagG	accgcaacCCaagagagA	accgcaacCCaagagagA
IWGSQ_CSS_1BS.scnaf.3417505	Cadenza0548	373	C	T	het	het	gtgtgagagCGgttgGagG	gtgtgagagCGgttgGagA	gtgtgagagCGgttgGagA
IWGSQ_CSS_2AS.scnaf.5305619	Cadenza0548	2786	C	T	hom	hom	atacagatgacctAAgtgtTtC	atacagatgacctAAgtgtTtC	atacagatgacctAAgtgtTtC
IWGSQ_CSS_2AS.scnaf.5306489	Cadenza0548	46953	T	G	het	wt	aggttccagatcatalagagGT	aggttccagatcatalagagG	aggttccagatcatalagagG
IWGSQ_CSS_2BL.scnaf.7984123	Cadenza0548	11660	G	A	het	het	catgttggcatagatcatalagAG	catgttggcatagatcatalagA	catgttggcatagatcatalagA
IWGSQ_CSS_2DL.scnaf.5907477	Cadenza0548	1363	C	A	hom	hom	tgcctcccttggcagagC	tgcctcccttggcagagC	tgcctcccttggcagagC
IWGSQ_CSS_2DS.scnaf.5330886	Cadenza0548	5449	G	A	hom	hom	gcatgtcctttactaagGgtG	gcatgtcctttactaagGgtA	gcatgtcctttactaagGgtA
IWGSQ_CSS_3AL.scnaf.4449951	Cadenza0548	633	C	T	het	het	tccaacactcaagcttacaacAG	tccaacactcaagcttacaacA	tccaacactcaagcttacaacA
IWGSQ_CSS_3B.scnaf.10479889	Cadenza0097	3339	C	T	hom	—	tgtTtttCGagaaagagCA	tgtTtttCGagaaagagCA	tgtTtttCGagaaagagCA
IWGSQ_CSS_3B.scnaf.10562262	Cadenza0097	7819	C	T	het	het	agaagggttgcatacatAttgG	agaagggttgcatacatAttgA	agaagggttgcatacatAttgA
IWGSQ_CSS_4AL.scnaf.7040796	Cadenza0097	10772	G	A	hom	het	acacacatggccacagA	acacacatggccacagA	acacacatggccacagA
IWGSQ_CSS_4AL.scnaf.7063488	Cadenza0097	6360	C	T	het	het	gctctcacaCTtAattgaagttC	gctctcacaCTtAattgaagttC	gctctcacaCTtAattgaagttC
IWGSQ_CSS_4AL.scnaf.7091701	Cadenza0097	5050	G	A	het	het	catgtgagcatgggaggaatAG	catgtgagcatgggaggaatA	catgtgagcatgggaggaatA
IWGSQ_CSS_4DS.scnaf.1845841	Cadenza0097	7110	G	A	hom	hom	aatgTAgctcccatcAGgG	aatgTAgctcccatcAGgA	aatgTAgctcccatcAGgA
IWGSQ_CSS_5AL.scnaf.2767581	Cadenza0097	3757	G	A	het	het	gagagggtcccatcAlctggC	gagagggtcccatcAlctggT	gagagggtcccatcAlctggT
IWGSQ_CSS_5BL.scnaf.10784643	Cadenza0097	1568	C	T	hom	hom	agaaATAcagattggatgagCG	agaaATAcagattggatgagCA	agaaATAcagattggatgagCA

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	—	tgagtagaagaattgacagtgG	tgagtagaagaattgacagtgG	tgccaccattgacatgagaG
IWGS.CSS.1BL_scaff.3858008	Cadenza2092	10278	G	A	hom	hom	tfttgagcagcaggatcG	tfttgagcagcaggatcG	actcagcctatcActattC
IWGS.CSS.1DL_scaff.2265172	Cadenza2092	9094	C	T	hom	hom	tfttgTactttgtcttctacG	tgaTGTactttgtcttctacG	aggtccactatccGtctatC
IWGS.CSS.2AL_scaff.6439430	Cadenza2092	16201	G	A	hom	hom	tftctgTactttacgtcaattgaC	tftctgTactttacgtcaattgaC	gtagagtagatgagtagaacC
IWGS.CSS.2DL_scaff.9760848	Cadenza2092	25101	C	T	het	—	caagaaggGagCtCagC	caagaaggGagCtCagC	tcGttAcTcttcActgttgaa
IWGS.CSS.3AL_scaff.4407012	Cadenza2092	4733	C	T	het	het	gcaccatgggtctcagtaC	gcaccatgggtctcagtaC	taagttagtttCCTCtgTCTG
IWGS.CSS.3AS_scaff.3441108	Cadenza2092	2785	C	T	hom	hom	acatatAggtttctctaccatC	acatatAggtttctctaccatC	acctctcagtttaagtgttgT
IWGS.CSS.3BS_scaff.1049827	Cadenza2092	541	G	A	het	het	GtgcagctctgagacGgaA	GtgcagctctgagacGgaA	aggcaTgacaaCgagcaA
IWGS.CSS.3BL_scaff.1050638	Cadenza1551	4779	G	A	hom	hom	ggcaaggcgaagaacGgtT	ggcaaggcgaagaacGgtT	aCagaGgggttagaggcaG
IWGS.CSS.3DL_scaff.6945816	Cadenza1551	3250	C	T	het	het	ctctctcactgtttggcC	ctctctcactgtttggcC	gcaacATrIgatactgaaagG
IWGS.CSS.3DL_scaff.6954177	Cadenza1551	589	C	T	hom	hom	agcatctcactgtcaacCaataC	agcatctcactgtcaacCaataC	TgtgcccTtgaAattttcaTG
IWGS.CSS.4AS_scaff.5938272	Cadenza1551	3508	C	T	het	het	tgtagcatcacataactttctG	tgtagcatcacataactttctA	gcttggtataaacCttacgacA
IWGS.CSS.4AS_scaff.5977594	Cadenza1551	19080	G	A	hom	hom	agAcCcgATgcacatG	agAcCcgATgcacatG	GggAgatAcaggtaaaActcTtcG
IWGS.CSS.5AL_scaff.2671035	Cadenza1551	11092	C	T	het	het	gcttgatcgggaacaaacC	gcttgatcgggaacaaacC	ggtctctcagttcagcaA
IWGS.CSS.5BL_scaff.10889480	Cadenza1551	5859	C	T	het	het	gggtgataTTttagacttgcagC	gggtgataTTttagacttgcagC	ggcagttcagcGacccatT
IWGS.CSS.3B_scaff.10528396	Cadenza2088	2530	G	A	hom	hom	gagcttaactcagatggag	gagcttaactcagatggag	tcctatgCAacGctttgT
IWGS.CSS.3B_scaff.10637573	Cadenza2088	8059	G	A	het	—	cttttcctcgttaagcaataG	cttttcctcgttaagcaataA	gtgcactgttccagcctgA
IWGS.CSS.4AL_scaff.7086469	Cadenza2088	6697	G	A	het	het	agcaagcttaacGgtctgC	agcaagcttaacGgtctgT	cgagcAactacagcagctT
IWGS.CSS.4AL_scaff.7126302	Cadenza2088	3627	G	A	het	het	gctgtctactcaacgC	gctgtctactcaacgC	ccaGaggttgtTGcatttT
IWGS.CSS.4BL_scaff.7041808	Cadenza2088	10234	G	A	hom	hom	gttcaaaaacagggtcctAatttgC	gttcaaaaacagggtcctAatttgT	cacaaggatagaagcTctctagA
IWGS.CSS.5AL_scaff.2794167	Cadenza2088	13162	G	A	het	—	tcaatggatgaagggtcttC	tcaatggatgaagggtcttT	ccatagcagcatcagccacA
IWGS.CSS.5BL_scaff.10889232	Cadenza2088	3885	G	A	het	het	agattcaggacaagcatCttCaG	agattcaggacaagcatCttCaA	caatgaacacctcagaagaaG
IWGS.CSS.5BS_scaff.2267405	Cadenza2088	11113	C	T	hom	hom	cTcaaccacatgggcaAatC	cTcaaccacatgggcaAatT	tccttcataatcatcaattgtG
IWGS.CSS.3B_scaff.10475354	Cadenza1409	2203	G	A	hom	hom	ctttgagctctaggcctctTG	ctttgagctctaggcctctTA	ctgatttgtTtggtTAgagtttGA
IWGS.CSS.3B_scaff.10674115	Cadenza1409	4555	C	T	het	het	agCgaacaagagGtcaaacG	agCgaacaagagGtcaaacA	gtgaacaacaGtagCAaattAocG
IWGS.CSS.4AL_scaff.7153568	Cadenza1409	13073	C	T	hom	hom	tcgacagcAtcaaccttgG	tcgacagcAtcaaccttgA	gaccggaactcctcggC
IWGS.CSS.4DL_scaff.14314966	Cadenza1409	2010	G	A	het	hom	gtaggttccctcctCAGgaA	gtaggttccctcctCAGgaA	cgggcTcaaaAggttgCcT
IWGS.CSS.4DS_scaff.2324074	Cadenza1409	7606	G	A	het	het	tGatgaanaattgtGcaGag	tGatgaanaattgtGcaGaa	gggtcAgttcAaaactGaaagtgaG
IWGS.CSS.5AS_scaff.1517889	Cadenza1409	3561	G	A	het	het	tctcgacatcttccgtgtaC	tctcgacatcttccgtgtaT	gtgcctggaacatgcttattA
IWGS.CSS.5AS_scaff.1523866	Cadenza1409	8054	G	A	hom	—	gggtatctaccgcaGgaC	gggtatctaccgcaGgaT	tcttgagCcTctctcA
IWGS.CSS.5BL_scaff.10917655	Cadenza1599	19073	C	T	het	hom	caaatgacatgcaanaagaattgC	caaatgacatgcaanaagaattgT	cgcttcactactacaAaata'grcT
IWGS.CSS.1BL_scaff.3886649	Cadenza1599	5204	C	T	het	het	tgatgcaaccacatGcT	tgatgcaaccacatGcT	ggacatgactgtgaccattttaG
IWGS.CSS.1DL_scaff.3810267	Cadenza1599	6634	C	T	hom	hom	ccCaggaatgagcactC	ccCaggaatgagcactC	cgaggcgaagatggtgaTtG
IWGS.CSS.1DL_scaff.2291677	Cadenza1599	12856	C	T	hom	hom	GgtagaacagtgcgcaA	GgtagaacagtgcgcaA	ctctctctcaacGCcG
IWGS.CSS.2AL_scaff.6354492	Cadenza1599	7566	G	A	het	het	gGagaatgaCAgtAacTtctgG	gGagaatgaCAgtAacTtctgA	tccggaagaaccacaTctTG
IWGS.CSS.2AS_scaff.5282937	Cadenza1599	9736	G	A	het	het	gctgtagattttatagctgctagC	gctgtagattttatagctgctagT	caacCagaatttgtCactgatttTC
IWGS.CSS.2BL_scaff.7952427	Cadenza1599	19249	G	A	hom	hom	cgTccctCcttagcagcG	cgTccctCcttagcagcG	aTcaactccattagcagAG
IWGS.CSS.2DL_scaff.9897981	Cadenza1599	5627	C	T	het	het	cttggtgctTgatt'gcttactC	cttggtgctTgatt'gcttactC	gTtgttCtctctgattCtTgtG
IWGS.CSS.3AL_scaff.4446105	Cadenza1599	1765	G	A	hom	—	aaatgcttttctcaCcgctagT	aaatgcttttctcaCcgctagA	tcttAgaggcaatagctTatatgcT



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