

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 whet genetics and genomics. MAS wheat, CeralsDB, 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 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

4.1 Introduction

One of the main challenges of working with polyploid species is the design of genome specific molecular markers. This is particularly true when targetting conserved homoeologous 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 physico-chemical 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 Primer3Rozen 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 in polyploids.

4.2 Global alignment

Search of the contigs with the sequence in the CSS reference and the importance of being able to distinguish between homoeologous regions.

4.3 Local alignment

Once the region with the primer has been selected, make a local alignment. This section discusses why the local alignment is needed.

4.4 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)

4.5 Primer selection algorithms

Different algorithms to select the best primer:

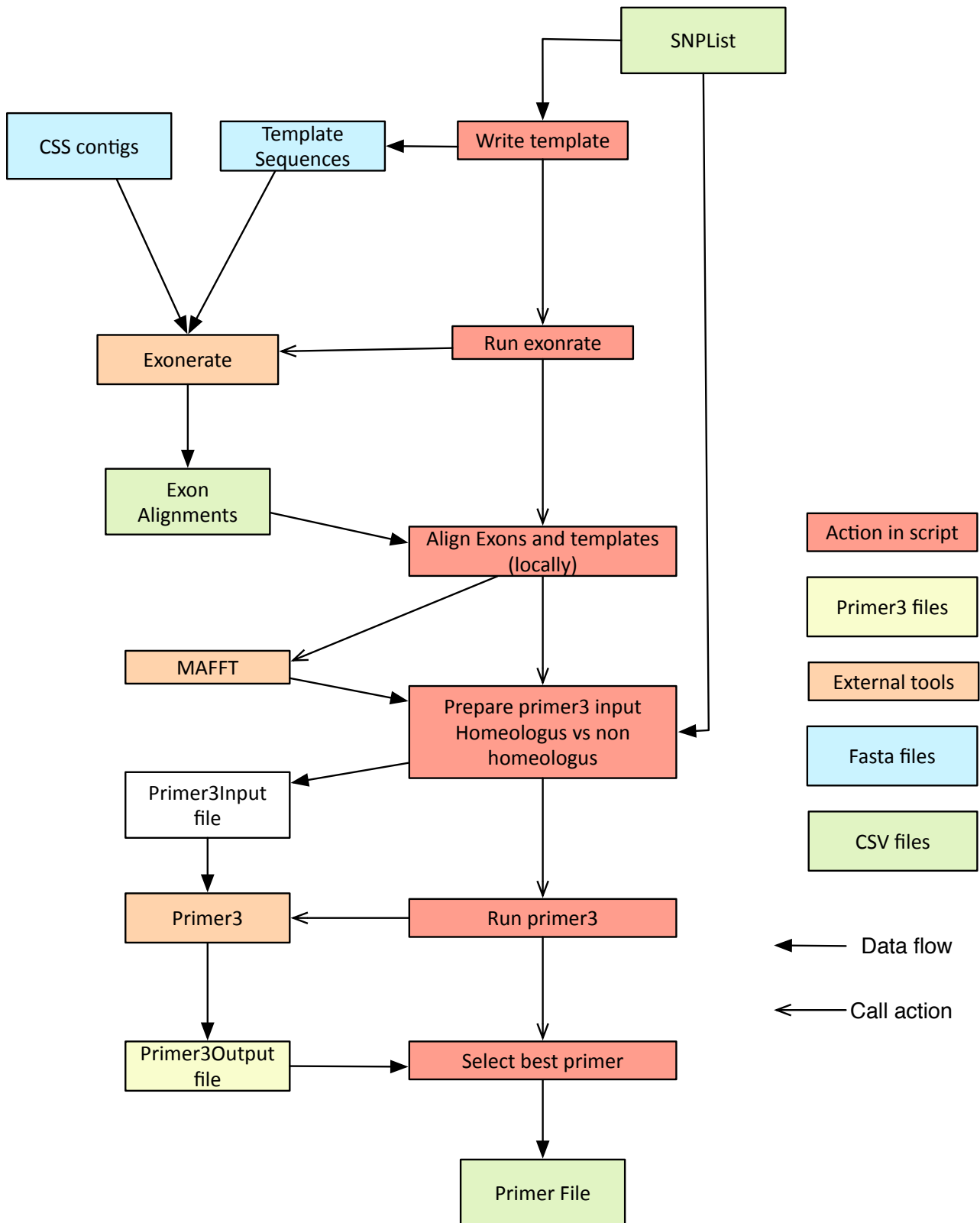


Figure 4.1: PolyMarker Pipeline

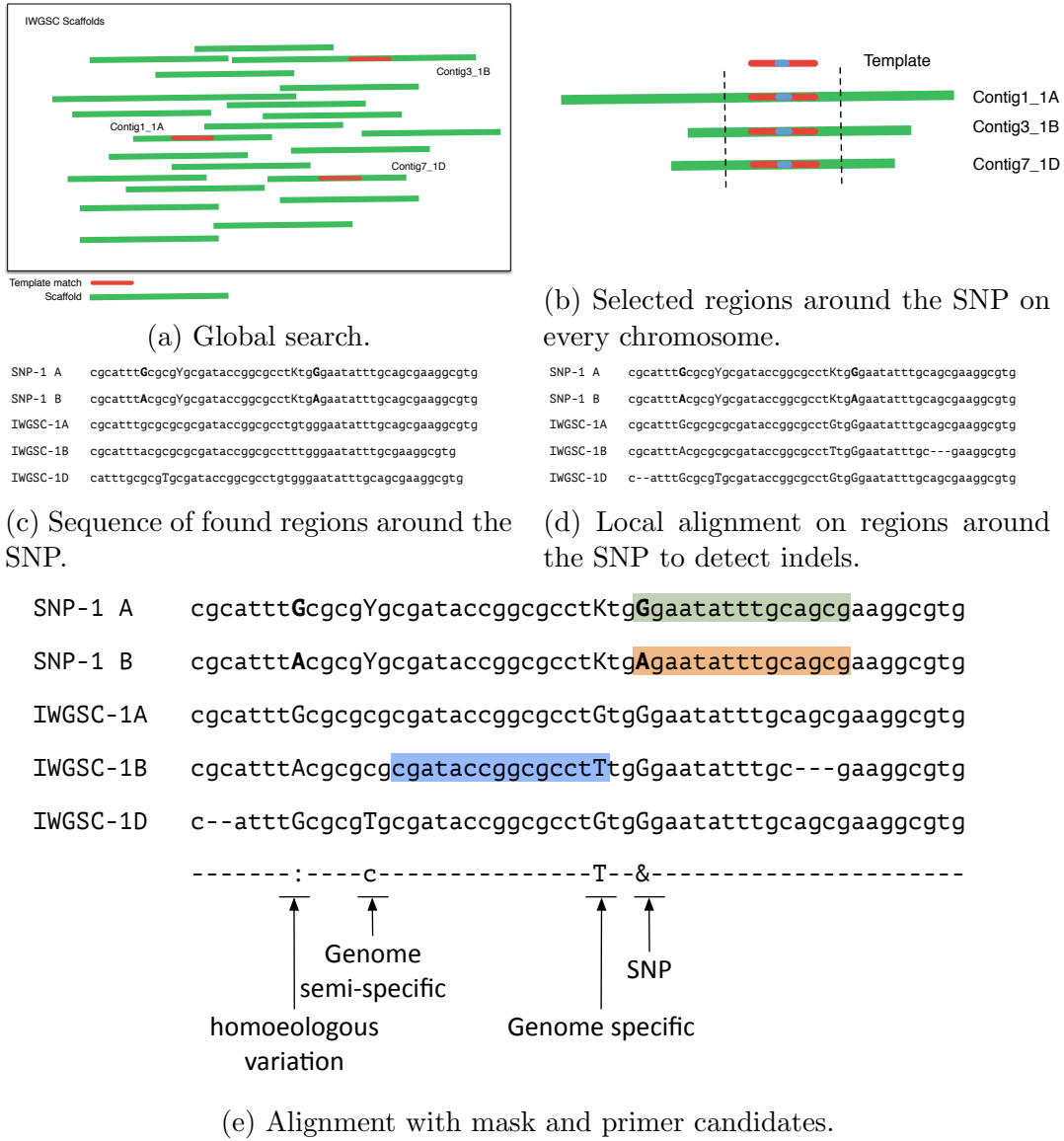


Figure 4.2: Alignments done by PolyMarker.

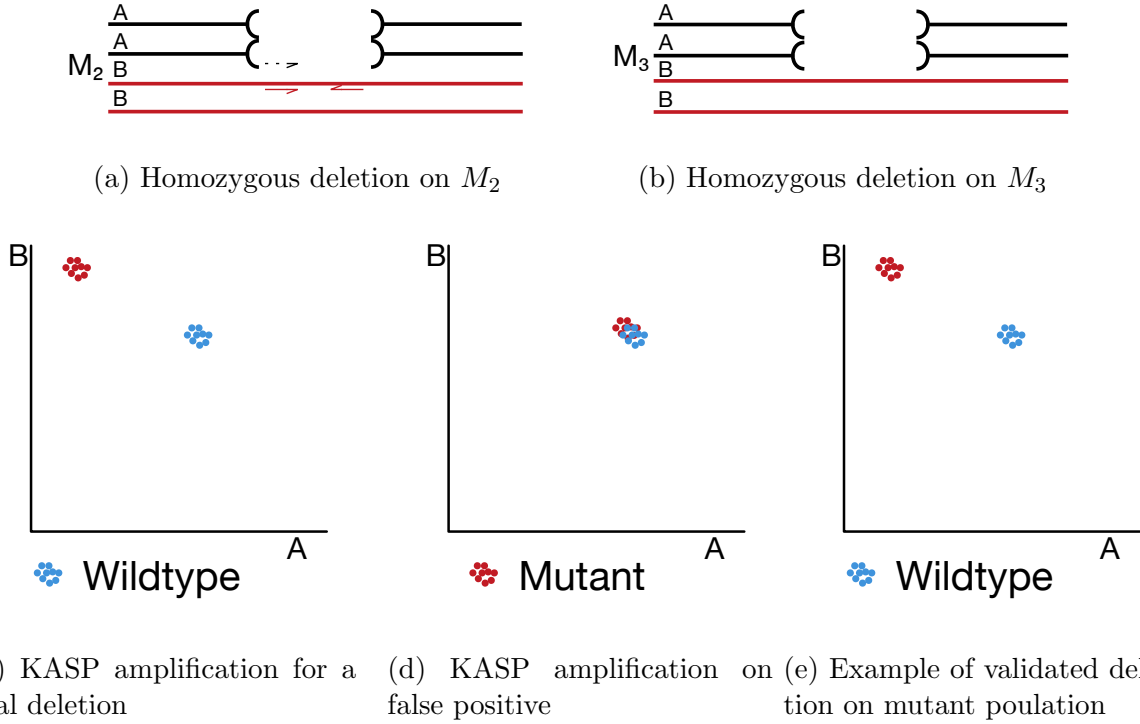


Figure 4.3: PolyMarker used to find primers to detect long deletions in tetraploid wheat.

4.5.1 KASP markers

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

4.6 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.

4.6.1 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.

4.6.2 Deletion algorithms

Algorithm to produce KASP for deletions in polyploids.

4.7 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 succesfully 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

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
IWGSQ.CSS.1AS.scnaf.3284790	Kronos3085	7449	G	A	Het	Het	Het	ccacacttgagctcgC	ccacacttgagctcGT	gtgatthgcaaggagA
IWGSQ.CSS.1BL.scnaf.3897513	Kronos3085	1515	C	T	Het	Het	Het	gcttcaacttggtcttC	gcttcaacttggtcttGT	acAagagctgcttaagacC
IWGSQ.CSS.2AL.scnaf.6434745	Kronos3085	3424	C	T	Het	Het	Het	ctctGgtttgcaattttatC	ctctGgtttgcaattttatGT	ggGcaATgctataaanaaA
IWGSQ.CSS.3AS.scnaf.3408995	Kronos3085	732	C	T	Het	Het	Het	aggcactttgtaattccG	aggcactttgtaattccGT	ggTgtaATccagAacctgTG
IWGSQ.CSS.3B.scnaf.10708748	Kronos3085	2675	G	A	Het	Het	Het	ggttgacgtctaccacagG	ggttgacgtctaccacagA	gtaaanaacttggtttgtagaC
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IWGSQ.CSS.6AL.scnaf.5778773	Kronos3288	6853	G	A	Het	Het	Het	gagtggttcccttcctttC	gagtggttcccttcctttGT	ggagagacagctactcgtCT
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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	caggatAgtGggactgtcaaaG	caggatAgtGggactgtcaaaA	ggagacGGctGggacatT
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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	atgtctgaticGtGtGagtcC
IWGSC.CSS.1BL_scaff.3297240	Cadenzal1661	1970	C	T	het	het	catccgccctGtttctcT	catccgccctGtttctcT	gctccgcatgaagagctT
IWGSC.CSS.1BS_scaff.3828996	Cadenzal1661	1340	G	A	hom	hom	agccgagttgtatgttaacT	agccgagttgtatgttaacT	agcagcttgTcgtgtfaaC
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IWGSC.CSS.2AS_scaff.5213460	Cadenzal1661	1358	C	A	hom	hom	gtcaegaaCcgctcagG	gtcaegaaCcgctcagA	aggaaagagggaaagaGcG
IWGSC.CSS.2BS_scaff.5179331	Cadenzal1661	5604	G	A	het	het	actctgtcaagaactgatacaA	actctgtcaagaactgatacaA	gcaGagaatgtcttgcacT
IWGSC.CSS.3AL_scaff.4250995	Cadenzal1661	4673	G	A	het	het	ggtgaggtatcggagctG	ggtgaggtatcggagctG	ggcgggtctacaggttG
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IWGSC.CSS.7AS_scaff.4193541	Cadenzal1538	8359	C	T	hom	het	agcaattcttggctatcaatagC	agcaattcttggctatcaatagT	tcactGtcttaacttactctG
IWGSC.CSS.1BL_scaff.3815304	Cadenzal1469	513	C	A	hom	hom	aacatttgcctTaCcaaaacGT	aacatttgcctTaCcaaaacGT	acacagaagttataatgCAAGC
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IWGSC.CSS.2BL_scaff.7972799	Cadenzal1469	8995	C	T	het	hom	gTgCtctcGcatcttT	gTgCtctcGcatcttT	gatcggCGcaaaactagTG
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IWGSC.CSS.3B_scaff.10395219	Cadenzal1469	1292	G	A	hom	—	agggtctgtgtgtctgG	agggtctgtgtgtctgA	ccctcttgggggcttataC
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IWGSC.CSS.4BL_scaff.7037448	Cadenzal0580	1837	C	T	hom	hom	CgttgaanaaGctgaagaacttaaC	CgttgaanaaGctgaagaacttaaT	cagttcttcTtCaGagcagataT
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IWGSC.CSS.3AS_scaff.3371333	Cadenza0364	538	G	A	het	het	gggaaaCgAgAcagcggA	gggaaaCgAgAcagcggA	ccgtgcttcttcaccT
IWGSC.CSS.3AS_scaff.3371815	Cadenza0364	1061	C	T	het	het	atccccagggcacagagC	atccccagggcacagagA	aATggcccttgggtgaticC
IWGSC.CSS.3AS_scaff.3440912	Cadenza0364	4498	G	A	het	het	ccgtaaaactttcttggttG	ccgtaaaactttcttggttG	atACgcaactacatcatgatgC
IWGSC.CSS.3B_scaff.10343586	Cadenza0364	2242	G	A	het	—	ggttcTgTctctcttccactG	ggttcTgTctctcttccactA	tggttgaacccgcaagcA

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	ctcGgatgtgtgagagA
IWGSQ_CSS_3AS.scnaf.1557483	Cadenza0364	2547	C	A	het	het	aaagatcaatcattgtaccataaAG	aaagatcaatcattgtaccataaA	cgaatcgaatccatcA
IWGSQ_CSS_3AS.scnaf.2648747	Cadenza0364	2688	G	A	het	het	tggAagCAcaaggsgcCT	tggAagCAcaaggsgcCT	GccgcgagatccatcA
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IWGSQ_CSS_3AS.scnaf.3371333	Cadenza0364	538	C	A	het	het	agaaacCGgAgAcagagAG	agaaacCGgAgAcagagCA	ccgttcggtccctaccCT
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IWGSQ_CSS_3AS.scnaf.3440912	Cadenza0364	4498	G	A	het	het	ccgttaaaactttgtgtcttG	ccgttaaaactttgtgtcttA	atActggaacatacattgATG
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IWGSQ_CSS_2DL.scnaf.9852812	Cadenza0110	13788	G	A	hom	hom	attttgtatgcttcaatcttgcC	attttgtatgcttcaatcttgcT	gaaagctTtacttctgacttG
IWGSQ_CSS_2DS.scnaf.5371379	Cadenza0110	2166	C	T	hom	hom	agacacaacacagTgatggCT	agacacaacacagTgatggCT	gctgtcgtgcaattgtTtatttG
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IWGSQ_CSS_6AL.scnaf.5833640	Cadenza0103	7346	C	T	hom	hom	agaanaagccacaatggtttcCT	agaanaagccacaatggtttcCT	aCTcgtTAgagtttccagC
IWGSQ_CSS_6AS.scnaf.4429974	Cadenza0103	3867	G	A	hom	hom	GagatgaAttatttAgagcatgttG	GagatgaAttatttAgagcatgttG	ggttcagagaggttattgttgaCT
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IWGSQ_CSS_6DS.scnaf.2059604	Cadenza0103	5224	G	A	het	—	gctcaatgcatcCTgaggtG	gctcaatgcatcCTgaggtA	tgtcaagattatttcttctcgtG
IWGSQ_CSS_7AL.scnaf.4552322	Cadenza0103	1412	C	T	het	het	gcaagagcTgtaactccaagAG	gcaagagcTgtaactccaagA	ggcAAAGcCaAgtttaaagAG
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IWGSQ_CSS_4DL.scnaf.14122349	Cadenza0277	1010	C	T	hom	hom	gtgcgtcgtChgttgaAG	gtgcgtcgtChgttgaA	ggttcgagagggagagAG
IWGSQ_CSS_4AL.scnaf.2736916	Cadenza0277	4296	G	A	het	het	agaactATgAaaGtaacacagAC	agaactATgAaaGtaacacagAC	ttcCtTtThagCGatChcG
IWGSQ_CSS_5BL.scnaf.10883744	Cadenza0277	2080	C	T	hom	hom	gctcttctChgtTAgctcAG	gctcttctChgtTAgctcAA	cgcaagatgtgtgtgattTgCA
IWGSQ_CSS_5DL.scnaf.3932013	Cadenza0548	11765	C	T	hom	hom	accgcaacCCaagagagAG	accgcaacCCaagagagAG	cccttcaagCCgtTgcAacG
IWGSQ_CSS_1AS.scnaf.3417505	Cadenza0548	373	C	T	het	het	gtgtgagagCGgttgaAG	gtgtgagagCGgttgaGA	tgtgtGCGagtgttgaA
IWGSQ_CSS_2AS.scnaf.5305619	Cadenza0548	2786	C	T	hom	hom	atacagatgacctAAgtgtTtC	atacagatgacctAAgtgtTtC	ggaaagaAAGctccagagAC
IWGSQ_CSS_2AS.scnaf.5306489	Cadenza0548	46953	T	G	het	wt	aggttccaagctatagagGT	aggttccaagctatagagGT	aggctcTtAgacctcTtACAgT
IWGSQ_CSS_2BL.scnaf.7984123	Cadenza0548	11660	G	A	het	het	catgttggcatgatacagtaagAG	catgttggcatgatacagtaagAG	aatattacttggatcattacagccC
IWGSQ_CSS_2DL.scnaf.5330886	Cadenza0548	1363	C	A	hom	hom	tgcctcccttgcagagAC	tgcctcccttgcagagAC	ggcacaactggtgtgtcctG
IWGSQ_CSS_2DS.scnaf.5330886	Cadenza0548	5449	G	A	hom	hom	gcattctcttattactgaAG	gcattctcttattactgaAG	catgttcctctcttgcagCC
IWGSQ_CSS_3AL.scnaf.4449951	Cadenza0548	633	C	T	het	het	tccaacctaaagcttacaacatAG	tccaacctaaagcttacaacatA	gcttcagatTGCaatgtcG
IWGSQ_CSS_3B.scnaf.10479889	Cadenza0097	3339	C	T	hom	—	tgtTtctCGagagagagCG	tgtTtctCGagagagagCA	ggctgtcatcaacCGCA
IWGSQ_CSS_3B.scnaf.10562262	Cadenza0097	7819	C	T	het	het	agaggggttgcatacatAttgG	agaggggttgcatacatAttgA	agcgaTgagagagcttC
IWGSQ_CSS_4AL.scnaf.7040796	Cadenza0097	10772	G	A	hom	het	acacacatgcccacagAA	acacacatgcccacagAA	CAatGatattgttcttAtcC
IWGSQ_CSS_4AL.scnaf.7063488	Cadenza0097	6360	C	T	het	het	gctctcacaCttAattgaaggtC	gctctcacaCttAattgaaggtT	aggcagagagagattgtgaagT
IWGSQ_CSS_4AL.scnaf.7091701	Cadenza0097	5050	G	A	het	het	catgtgagcatggaggaanaatG	catgtgagcatggaggaanaatA	agcagaagcAAtaagaaggaAA
IWGSQ_CSS_5AL.scnaf.1845841	Cadenza0097	7110	G	A	het	het	aatgTAgctcccatcAGg	aatgTAgctcccatcAGgA	actgaacatCgaatcgtTtaagGA
IWGSQ_CSS_5BL.scnaf.10784643	Cadenza0097	3757	G	A	het	het	gagaggttccctacATccgtC	gagaggttccctacATccgtT	cgTtcaacaaatattgtctggG
IWGSQ_CSS_5BL.scnaf.10784643	Cadenza0097	1568	C	T	hom	hom	agaaATAcagattggttgaAG	agaaATAcagattggttgaCA	catctcCtcttccCGgaaagG

IWGC contig	Line	Pos	WT	Mut	Predicted	Called on <i>M4</i>	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGC.CSS.1AL_scaff.3952258	Cadenza2092	8107	C	T	het	—	tgagtagaagaattgacagtgG	tgagtagaagaattgacagtgG	tgccaccattgacatgagaG
IWGC.CSS.1BL_scaff.3858008	Cadenza2092	10278	G	A	hom	hom	tfttgagcagcaggatcgC	tfttgagcagcaggatcgT	actcagcgctatacActattC
IWGC.CSS.1DL_scaff.2265172	Cadenza2092	9094	C	T	hom	hom	tfttgTtactttgtctatcagC	tgaTGTtactttgtctatcagT	aggtccactatccGtctatC
IWGC.CSS.2AL_scaff.6439430	Cadenza2092	16201	G	A	hom	hom	tftctgTacttaacgtcaattgaC	tftctgTacttaacgtcaattgaT	gtagagtagatgagtagaacC
IWGC.CSS.2DL_scaff.9760848	Cadenza2092	25101	C	T	het	—	caagaaggGagCtCagC	caagaaggGagCtCagT	tcGttAcTcttcActggtgaa
IWGC.CSS.3AL_scaff.4407012	Cadenza2092	4733	C	T	het	het	gcaccatgggtctcagtaC	gcaccatgggtctcagtaT	tcagtcagtttCCTCtgTCTG
IWGC.CSS.3AS_scaff.5977594	Cadenza2092	2785	C	T	hom	hom	acatatAggtttctatcaccatC	acatatAggtttctatcaccatT	acctctcagtttaaggtttgT
IWGC.CSS.3BS_scaff.3441108	Cadenza2092	541	G	A	het	het	GtgcagctttgagacGgaA	GtgcagctttgagacGgaA	aggcaTgacaaCgagcaA
IWGC.CSS.3BL_scaff.10449827	Cadenza1551	4779	G	A	hom	hom	ggcaggcgaagaacGgtC	ggcaggcgaagaacGgtT	aCagaGtgggttagaggcaG
IWGC.CSS.3BL_scaff.1050638	Cadenza1551	3250	C	T	het	het	ctctctcactttgtggC	ctctctcactttgtggT	gcaacATtTgatactgcaagG
IWGC.CSS.3DL_scaff.6945816	Cadenza1551	589	C	T	hom	hom	agcatctcacttgcgaAaataC	agcatctcacttgcgaAaataT	TgtggccTtTgaAattttcaTG
IWGC.CSS.3DL_scaff.6954177	Cadenza1551	3508	C	T	het	het	tgtagcatcacataactttctG	tgtagcatcacataactttctA	gcttggtataaacCttacgacA
IWGC.CSS.4AS_scaff.5938272	Cadenza1551	19080	G	A	hom	hom	agAcCcgATgcacatG	agAcCcgATgcacatG	GggAgatAcaggtaaaActcTtcG
IWGC.CSS.4AS_scaff.5977594	Cadenza1551	11092	C	T	het	het	gcttgatcgggaacaaacC	gcttgatcgggaacaaacT	ggtctctcagtcagcaA
IWGC.CSS.5AL_scaff.2671035	Cadenza1551	5859	C	T	het	het	cggTgataTTttagacttgcagC	cggTgataTTttagacttgcagT	ggcagttcagcGacccatT
IWGC.CSS.5BL_scaff.10889480	Cadenza1551	2530	G	A	hom	hom	gagcttaactcagatggag	gagcttaactcagatggaa	tcctatgCAacGctttgT
IWGC.CSS.3B_scaff.10528396	Cadenza2088	8059	G	A	hom	—	cttttcctcgttaagcaataG	cttttcctcgttaagcaataA	gtgcactgttgcagcctgA
IWGC.CSS.3B_scaff.10637573	Cadenza2088	16815	G	A	het	het	agcaagcttaccGgtctgC	agcaagcttaccGgtctT	cgagcAactacgagcagctT
IWGC.CSS.4AL_scaff.7086469	Cadenza2088	6697	G	A	het	het	gcgcttacttcaacgC	gcgcttacttcaacgA	ccaGaggttGTGcatTTT
IWGC.CSS.4AL_scaff.7126302	Cadenza2088	3627	G	A	hom	hom	gttcaaaaacagggtcctAatttgC	gttcaaaaacagggtcctAatttgT	cacaaggatagaagcTctctagA
IWGC.CSS.4BL_scaff.7041808	Cadenza2088	10234	G	A	hom	hom	tcaatggatgaagggtcttC	tcaatggatgaagggtcttT	ccatagcagcatcagccacA
IWGC.CSS.5AL_scaff.2794167	Cadenza2088	13162	G	A	het	—	agTattcaggacaagcatCttCaG	agTattcaggacaagcatCttCaA	caatgaacacctcagaagaaGaG
IWGC.CSS.5BL_scaff.10889232	Cadenza2088	3885	G	A	het	het	cTcaaccacatgggcaAatC	cTcaaccacatgggcaAatT	tccttcatcaatcatcaattgtgG
IWGC.CSS.5BS_scaff.2267405	Cadenza2088	11113	C	T	hom	hom	ctttgagcatctaggcctctTG	ctttgagcatctaggcctctTA	ctgatttggTCtggttAgagtttGA
IWGC.CSS.3B_scaff.10475354	Cadenza1409	2203	G	A	hom	hom	agCgaacaagagGtcaaacG	agCgaacaagagGtcaaacA	gtgaacaacaGtagaCAaattAocG
IWGC.CSS.3B_scaff.10674115	Cadenza1409	4555	C	T	het	het	gcttcagtgcaagccttcaG	gcttcagtgcaagccttcaA	cttcaaccccGagataatGtattG
IWGC.CSS.4AL_scaff.7153568	Cadenza1409	13073	C	T	hom	hom	tcgacagcAftcaacttgG	tcgacagcAftcaacttgA	gaccggaactcctcgC
IWGC.CSS.4DL_scaff.14314966	Cadenza1409	2010	G	A	het	hom	gtaggttccctcctCAGga	gtaggttccctcctCAGga	cgggcTcaaaAggttCcT
IWGC.CSS.4DS_scaff.2324074	Cadenza1409	7606	G	A	het	het	tGatgaanaattgtGcaGaa	tGatgaanaattgtGcaGaa	ggggaAgttcAaaactGaaagtgaG
IWGC.CSS.5AS_scaff.1517889	Cadenza1409	3561	G	A	het	het	tctcgacatcttccgtgtaC	tctcgacatcttccgtgtaT	gtgcctggaacatgcttatttA
IWGC.CSS.5AS_scaff.1523866	Cadenza1409	8054	G	A	hom	—	ggTgatctaccgcaGgaC	ggTgatctaccgcaGgaT	tcttgagCcTctctcaA
IWGC.CSS.5BL_scaff.10917655	Cadenza1409	19073	C	T	hom	hom	caaaTgacatgcaanaagaattgC	caaaTgacatgcaanaagaattgT	cgcttcatcactacaAaata'grcT
IWGC.CSS.1AL_scaff.3886649	Cadenza1599	5204	C	T	het	het	tgatgcaaccacaaatGcT	tgatgcaaccacaaatGcT	ggacatgactgctgaccattttaG
IWGC.CSS.1BL_scaff.3810267	Cadenza1599	6634	C	T	hom	hom	ccCaggaaatgagcactC	ccCaggaaatgagcactT	cgaggcggaagtgtgaTtG
IWGC.CSS.1DL_scaff.2291677	Cadenza1599	12856	C	T	hom	hom	GgtagaagaatgcgcgaA	GgtagaagaatgcgcgaA	ctctctctcaacGCcG
IWGC.CSS.2AL_scaff.6354492	Cadenza1599	7566	G	A	het	het	gGagaatgaCAgtAacTtctgG	gGagaatgaCAgtAacTtctgA	tccggaagaaccacaTctTG
IWGC.CSS.2AS_scaff.5282937	Cadenza1599	9736	G	A	het	het	gctgtagattttatagctgctagC	gctgtagattttatagctgctagT	caaCagaattgttCactgatttTC
IWGC.CSS.2BL_scaff.7952427	Cadenza1599	19249	G	A	hom	hom	cgTccctCcttagcagcG	cgTccctCcttagcagcT	aTcaactccattagcagAG
IWGC.CSS.2DL_scaff.9897981	Cadenza1599	5627	C	T	het	het	cttggtgctTgatt'gttactC	cttggtgctTgatt'gttactT	gTtggctCtctctgattCtTgtG
IWGC.CSS.3AL_scaff.4446105	Cadenza1599	1765	G	A	hom	—	aaatgcttttctcaCcgctagT	aaatgcttttctcaCcgctagA	tcttAgaggcaatagctTatatgcT

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