

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

Explain how the SNP markers are designed without the tool and an overview.

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:

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.

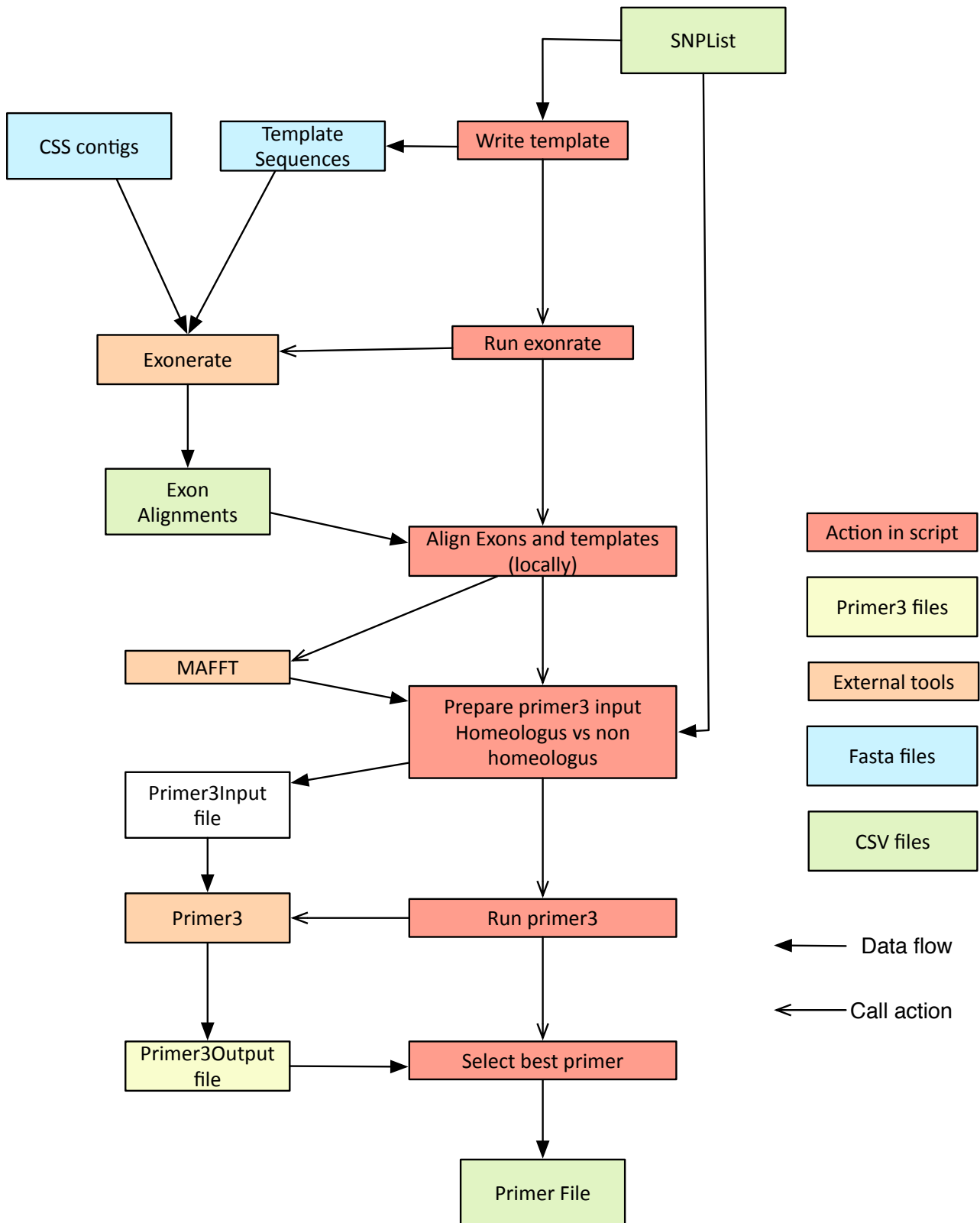


Figure 4.1: PolyMarker Pipeline

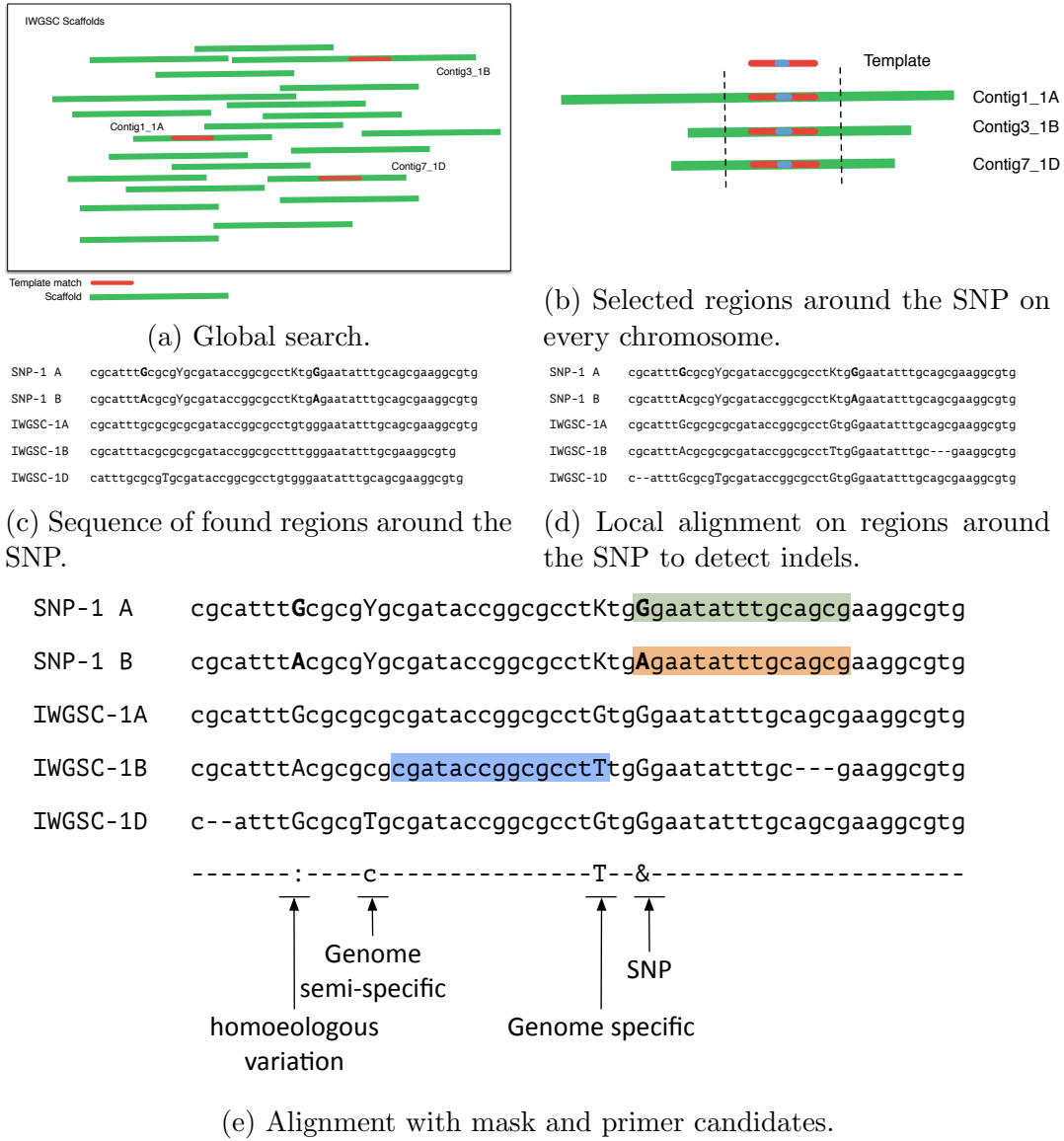


Figure 4.2: Alignments done by PolyMarker.

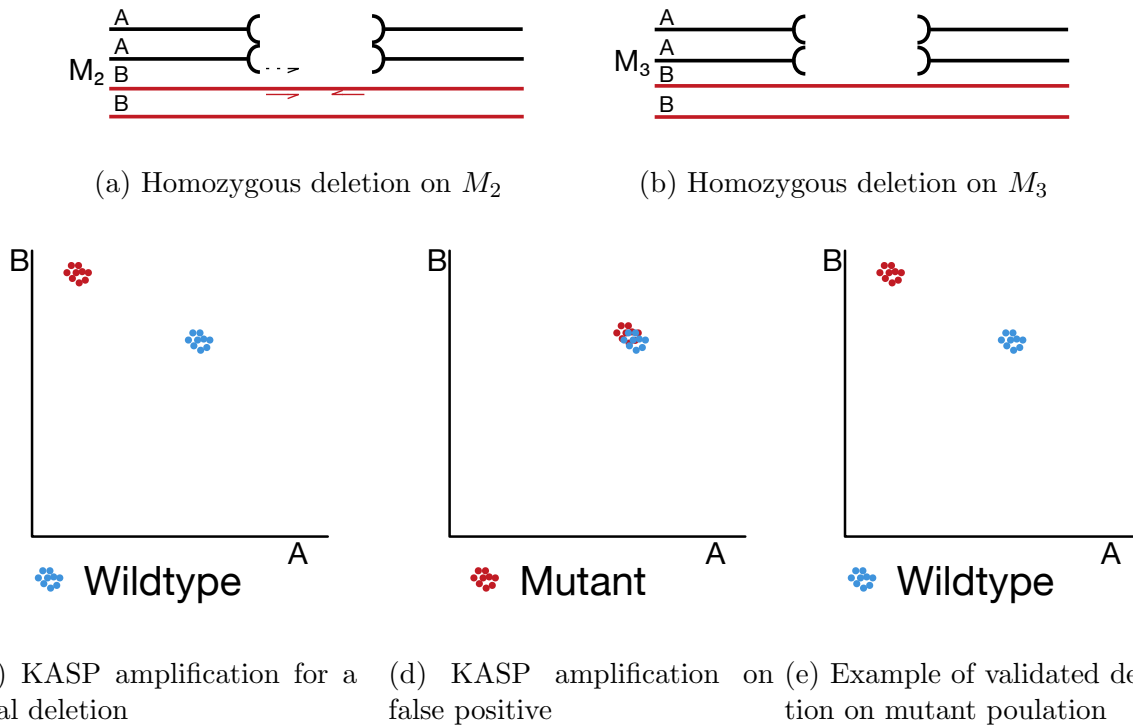


Figure 4.3: PolyMarker used to find primers to detect long deletions in tetraploid wheat.

4.6.1 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	gtgatthgccaaggagA
IWGSQ.CSS.1BL.scnaf.3897513	Kronos3085	1515	C	T	Het	Het	Het	gcttccacttggtctcC	gcttccactGgtgtcGT	acAagagctgcttaagacC
IWGSQ.CSS.2AL.scnaf.6434745	Kronos3085	3424	C	T	Het	Het	Het	ctctGgtttgcaattttatcC	ctctGgtttgcaattttatcGT	ggGcaATgctataaanaaA
IWGSQ.CSS.3AS.scnaf.3408995	Kronos3085	732	C	T	Het	Het	Het	aggcactttgtaattccG	aggcacttttogaattccGT	ggTgtaATccagAacctgTG
IWGSQ.CSS.3B.scnaf.10708748	Kronos3085	2675	G	A	Het	Het	Het	ggttgacgtctaccacagG	ggttgacgtctctaccacagA	gtaaaacttggtttgtagacC
IWGSQ.CSS.4AL.scnaf.7132733	Kronos3085	1799	G	T	Hom	Hom	Hom	caaccgttgatgacctC	caaccgttgatgacctT	acCcctaGaaagaaatctC
IWGSQ.CSS.5AS.scnaf.1534693	Kronos3085	4805	G	T	Het	Het	Het	ccagcttcaggacctcttAtC	ccagcttcaggacctcttAtT	gtaCctcagcAgtaATgagAG
IWGSQ.CSS.6AS.scnaf.4361911	Kronos3085	8657	C	A	Het	Het	Het	tcacgaagagagctcttaacC	tcacgaagagagagctcttaC	catgggtgtgcttatctcaA
IWGSQ.CSS.6BS.scnaf.3008326	Kronos3085	1528	G	A	Het	Het	Het	ccatgtgtactggttggtC	ccatgtgtactggttggtGT	ggaaagatcagCgaagtgaA
IWGSQ.CSS.7AS.scnaf.4214385	Kronos3085	27835	C	T	Hom	Hom	Hom	cgtaacttcgtttgggaagG	cgtaacttcgtttgggaagA	ctcttgtaagTgtatagacT
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IWGSQ.CSS.6AL.scnaf.5823017	Kronos3191	13225	C	T	Hom	Hom	Hom	ccctttcgagctctcgagG	ccctttcgagctctcgagA	ttcggaaggcccatcgA
IWGSQ.CSS.6BS.scnaf.2955394	Kronos3191	1622	C	T	Het*	Hom	Hom	gtggaagatgaagtttagcaG	gtggaagatgaagtttagcaA	gatactgtTgcaatgggtGT
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IWGSQ.CSS.3AL.scnaf.4284850	Kronos3288	7901	G	A	Hom	Hom	Hom	ttgctttggacacacatcgG	ttgctttggacacacatcgA	ttgcaAgcataagacagcG
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IWGSQ.CSS.7AS.scnaf.4246431	Kronos4485	3402	G	A	Hom	Hom	Hom	aaggcgccttggtgttctC	aaggcgccttggtgttctT	agtaagtaggaACagacttgataT
IWGSQ.CSS.7BL.scnaf.6667357	Kronos4485	641	C	T	Het	Het	Het	gaticAgctgtctatcagG	gaticAgctgtctatcagA	ttccctgtcaatttgatgcC

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.5BL_scaff.10849226	Cadenzal1772	2289	C	T	het*	hom	cttgacatcatgtttcacgAT	cttgacatcatgtttcacgAT	cactccgagggttccatG
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	catccgccCgtttctcT	catccgccCgtttctcT	gctccgcatgaagagT
IWGSC.CSS.1BS_scaff.3828996	Cadenzal1661	1340	G	A	hom	hom	agccggtgttagTgttaacT	agccggtgttagTgttaacT	agcagcttgTgcgtfAAC
IWGSC.CSS.1DS_scaff.1884529	Cadenzal1661	10575	G	A	hom	hom	aCagatacaATgttcacgagT	aCagatacaATgttcacgagT	accitgggTgttccaatctC
IWGSC.CSS.2AL_scaff.6318370	Cadenzal1661	19142	C	T	het	—	cgtggcCGaatCtcGacG	cgtggcCGaatCtcGacA	ttcttggagcggcgC
IWGSC.CSS.2AS_scaff.5213460	Cadenzal1661	1358	G	A	hom	hom	gtacgaaCCcgctcagG	gtacgaaCCcgctcagA	aggaaagagggaaanaaGcG
IWGSC.CSS.2BS_scaff.5179331	Cadenzal1661	5604	G	A	het	het	actctgtcgaagaactgatacaA	actctgtcgaagaactgatacaA	gcaGagaatgtcttgcacT
IWGSC.CSS.3AL_scaff.4250995	Cadenzal1661	4673	G	A	het	het	ggtgaggtatccggactG	ggtgaggtatccggactG	ggcggtgtctacagttG
IWGSC.CSS.3BL_scaff.10404421	Cadenzal1661	7046	G	A	hom	hom	cCaagaaacgggtgtgtccaG	cCaagaaacgggtgtgtccaA	ctgcagctgtcccatcagT
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IWGSC.CSS.6AL_scaff.5753680	Cadenzal1538	2125	C	T	hom	het	gcagttttatctcagtagcttgG	gcagttttatctcagtagcttgaA	ttctgagaaTgtaatgtcGatG
IWGSC.CSS.6AS_scaff.4425792	Cadenzal1538	3920	C	T	hom	hom	tgctccaaattgtgacacaaTaaC	tgctccaaattgtgacacaaTaaT	aaaGcaaggsgtaagtitttgT
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IWGSC.CSS.9AL_scaff.3246988	Cadenzal1538	3446	C	T	het	—	gatatcccaacggcgG	gatatcccaacggcgA	tgaccactcttcgagttT
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IWGSC.CSS.2AL_scaff.6337088	Cadenzal1469	7334	A	het*	hom	hom	acaatgccAagttgacagttG	acaatgccAagttgacagttA	ggaggtgtgggtCagaacaT
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IWGSC.CSS.3B_scaff.10395219	Cadenzal1469	2994	C	T	het	—	acacgagtatcaagccctC	acacgagtatcaagccctT	tgatactgtTggCggagG
IWGSC.CSS.3BS_scaff.10592217	Cadenzal0580	2994	C	T	het	—	tggttatGCAaggataatCagA	tggttatGCAaggataatCagA	tgacaattgtgatcttaggT
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IWGSC.CSS.4DS_scaff.2276484	Cadenzal0580	8034	G	A	hom	hom	tgaaTgatttttctcccttC	tgaaTgatttttctcccttT	ggAATCCATATgCAgaAGaaCTG
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IWGSC.CSS.3AS_scaff.4447942	Cadenza0364	11917	G	A	het	het	gtcataaagatgtcctgtgaaG	gtcataaagatgtcctgtgaaA	ctcGgatgtgggaggaA
IWGSC.CSS.3AS_scaff.1557483	Cadenza0364	2547	C	T	het	het	aaagtacatcatgcttaccataaA	aaagtacatcatgcttaccataaA	cgaaatccaacgctcatcA
IWGSC.CSS.3AS_scaff.2648747	Cadenza0364	2688	G	A	het	het	tggaAagCAaaggggccC	tggaAagCAaaggggccC	GccgcagatggagactC
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IWGSC.CSS.3AS_scaff.3371333	Cadenza0364	538	G	A	het	het	gggaaacGAgAcagcggA	gggaaacGAgAcagcggA	ccgtgctctctaccT
IWGSC.CSS.3AS_scaff.3371815	Cadenza0364	1061	C	T	het	het	atccccaggcacagagG	atccccaggcacagagA	aATggcccttgggtgaticC
IWGSC.CSS.3AS_scaff.3440912	Cadenza0364	4498	G	A	het	het	ccgtaaactttcttggttG	ccgtaaactttcttggttG	atACgcaactacatgatgtcC
IWGSC.CSS.3B_scaff.10343586	Cadenza0364	2242	G	A	het	—	ggttcTgTctctcttccactG	ggttcTgTctctcttccactA	tggttgaacccgcaagA

IWGSC contig		Line	Pos	WT	Mut	Predicted	Called on M_4	Primer 1 (Cadenza)	Primer 2 (mutant)	Common Primer
IWGSC_CSS_3AL.scnaf.442479		Cadenza0364	3198	C	T	het	het	gagtaCTaagtgtgtaagtggC	gagtaCTaagtgtgtaagtgtT	GCaGaThaCaacagatcAG
IWGSC_CSS_3AL.scnaf.4447942		Cadenza0364	11917	G	A	het	het	gtataaagattgtctccgttgaG	gtataaagattgtctccgttgaG	ctcGgatgtgtgagagA
IWGSC_CSS_3AS.scnaf.1557483		Cadenza0364	2547	C	A	het	het	aaagatcaatcattgtaccataaG	aaagatcaatcattgtaccataaA	cgaatccatcattcattcA
IWGSC_CSS_3AS.scnaf.2648747		Cadenza0364	2688	G	A	het	het	tggAagcAcaaggsgcCT	tggAagcAcaaggsgcCT	GccgcgacatccattcA
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IWGSC_CSS_3AS.scnaf.3321091		Cadenza0364	4585	C	T	het	het	caagaaAgATgctgattgttgaG	caagaaAgATgctgattgttgaA	acatgtcgatgcgcgaATC
IWGSC_CSS_3AS.scnaf.3371833		Cadenza0364	538	C	A	het	het	agcaaaCgAgAcagagG	agcaaaCgAgAcagagCA	ccgtgtccctccaccCT
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IWGSC_CSS_6AL.scnaf.3513297		Cadenza0281	1208	C	A	hom	—	acgtcgaacacgctttgaC	acgtcgaacacgctttgaT	ttaaatTggttgctgcacC
IWGSC_CSS_6AL.scnaf.3513297		Cadenza0281	4532	C	T	hom	—	ggagagggagacgtctgG	ggagagggagacgtctgA	tcttcctgcacgattccC
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IWGSC_CSS_7BS.scnaf.3143575		Cadenza0281	1866	C	T	het	het	agatgttgagggsgcctTC	agatgttgagggsgcctTT	ggttgaATgttgagagT
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IWGSC_CSS_5BS.scnaf.1646558		Cadenza0148	2916	C	T	het	het	gctGtaccatcacATcccttTG	gctGtaccatcacATcccttA	gcaATgttccatATaccctT
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IWGSC_CSS_5DL.scnaf.4542222		Cadenza0103	6528	C	T	het*	hom	ggcgcctcaaaagaaacaaattG	ggcgcctcaaaagaaacaaattG	aTtccacaatCGaTtttgctacC
IWGSC_CSS_6AL.scnaf.5838640		Cadenza0103	7346	C	T	hom	hom	agaanaagccacaatggtttTC	agaanaagccacaatggtttCT	aCTcgtTAggtttccacC
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IWGSC_CSS_7AL.scnaf.4552322		Cadenza0103	1412	C	T	het	het	gcaaaagcTgtaactccaagG	gcaaaagcTgtaactccaagA	ggcAAGcCaAgttaaaagAG
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IWGSC_CSS_2DL.scnaf.5330886		Cadenza0548	11765	C	T	hom	hom	accgcaacCcaagagaaG	accgcaacCcaagagaaA	cccatthaGccgTgcAacG
IWGSC_CSS_1BS.scnaf.3417505		Cadenza0548	373	C	T	het	het	gtgtgagagCGgttgGaaG	gtgtgagagCGgttgGaaA	tgttgTGccagtgttgA
IWGSC_CSS_2AS.scnaf.5305619		Cadenza0548	2786	C	T	hom	hom	atacagaagccctAAtgtgtTC	atacagaagccctAAtgtgtTC	ggaaagaATGctccagtaC
IWGSC_CSS_2AS.scnaf.5306489		Cadenza0548	46953	T	G	het	wt	aggttccaatgatacagaagGT	aggttccaatgatacagaagG	aggcttCAgaccttgcTAC
IWGSC_CSS_2BL.scnaf.7984123		Cadenza0548	11660	G	A	het	het	catltgtgcatgatacagaagG	catltgtgcatgatacagaagA	aatctattgtgatacagaagC
IWGSC_CSS_2DL.scnaf.5330886		Cadenza0548	1363	C	A	hom	hom	tgcctccctttcgcaagC	tgcctccctttcgcaagT	ggcacaactgattgttgacC
IWGSC_CSS_2DS.scnaf.5330886		Cadenza0548	5449	G	A	hom	hom	gcattctctttattactgaGtG	gcattctctttattactgaGtA	catgtcgcattcttgcacC
IWGSC_CSS_3AL.scnaf.4449951		Cadenza0548	633	C	T	het	het	tccaanaactaaagcttaacataG	tccaanaactaaagcttaacataA	gttcgtagTGCcattgtC
IWGSC_CSS_3B.scnaf.10479889		Cadenza0097	3339	C	T	hom	—	tgtTttcGgagaaagcCG	tgtTttcGgagaaagcCA	ggctgtccatcaacCGcA
IWGSC_CSS_3B.scnaf.10562262		Cadenza0097	7819	C	T	het	het	agaagggttgcatacatAttgG	agaagggttgcatacatAttgA	agcgaatgagagagagC
IWGSC_CSS_4AL.scnaf.7040796		Cadenza0097	10772	G	A	hom	hom	acacacattgccacagA	acacacattgccacagA	CaatGcattgtgttctatC
IWGSC_CSS_4AL.scnaf.7063488		Cadenza0097	6360	C	T	het	het	gcctctcaacCttAattgaagttC	gcctctcaacCttAattgaagttG	aggcaggtgagattgtgaattT
IWGSC_CSS_4AL.scnaf.7091701		Cadenza0097	5050	G	A	het	het	catgtgagcattgggagaaatG	catgtgagcattgggagaaatA	agcaagagCAatagaagagA
IWGSC_CSS_5AL.scnaf.1845841		Cadenza0097	7110	G	A	het	het	aatgTAgctcccatcCGg	aatgTAgctcccatcCGa	actgaatcTgaactcgtTtaagA
IWGSC_CSS_5BL.scnaf.10784643		Cadenza0097	3757	G	A	het	het	gagaggctcccatcATccgG	gagaggctcccatcATccgT	cgTtaccacaatattgttgG
IWGSC_CSS_5BL.scnaf.10784643		Cadenza0097	1568	C	T	hom	hom	agaaATAcattgattggatgCG	agaaATAcattgattggatgCA	catctcCtttccCGgaaag

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	actcagcgctatataCctattC
IWGC.CSS.1DL_scaff.2265172	Cadenza2092	9094	C	T	hom	hom	tfttgTtactttgtctatcagC	tgaTGTtactttgtctatcagT	aggtccactatccGttcatC
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	taagttagtttCCTCtgTCTG
IWGC.CSS.3AS_scaff.5938272	Cadenza2092	2785	C	T	hom	hom	acatatAggtttctatcaccatC	acatatAggtttctatcaccatT	acctctcagtttaaggtttgT
IWGC.CSS.3BS_scaff.3441108	Cadenza2092	541	G	A	het	het	GtgcagctttgagacGgaC	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	ctctctactgtttggcC	ctctctactgtttggcT	gcaacATtTgatactgcaagG
IWGC.CSS.3DL_scaff.6945816	Cadenza1551	589	C	T	hom	hom	agcatctcactgtcaacAaatC	agcatctcactgtcaacAaatT	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	gtgcactgttcaggcctgA
IWGC.CSS.3B_scaff.10637573	Cadenza2088	16815	G	A	het	het	agcaagcttaccGgtctgC	agcaagcttaccGgtctgT	cgagcAactacagcagctT
IWGC.CSS.4AL_scaff.7086469	Cadenza2088	6697	G	A	het	het	gcgcttacttcaacgC	gcgcttacttcaacgA	ccaGaggttgtTGcatittT
IWGC.CSS.4AL_scaff.7126302	Cadenza2088	3627	G	A	hom	hom	gttcaaaaacagggtcctAatttgC	gttcaaaaacagggtcctAatttgT	cacaaggatatgaagcTctctagA
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	ctgatttgtTtggtTAgagtttGA
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	cgggcTcaaaAggttgCcT
IWGC.CSS.4DS_scaff.2324074	Cadenza1409	7606	G	A	het	het	tGatgaataatgtGcaGag	tGatgaataatgtGcaGaa	ggggaAgttcAaaactGaagtgaagG
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	caaatgacatgcaaaagaagtigC	caaatgacatgcaaaagaagtigT	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	GgtagaacagtgcgcaG	GgtagaacagtgcgcaA	ctctctctcaacGCcG
IWGC.CSS.2AL_scaff.6354492	Cadenza1599	7566	G	A	het	het	gGagaatgaCAgtAacTtctgG	gGagaatgaCAgtAacTtctgA	tccggaagaaccacaTtcTG
IWGC.CSS.2AS_scaff.5282937	Cadenza1599	9736	G	A	het	het	gctgtagattttatagctgctagC	gctgtagattttatagctgctagT	caaCagaatttgttCactgatttTC
IWGC.CSS.2BL_scaff.7952427	Cadenza1599	19249	G	A	hom	hom	cgTccctCcttagcacgC	cgTccctCcttagcacgT	aTcaactccattagcgagAG
IWGC.CSS.2DL_scaff.9897981	Cadenza1599	5627	C	T	het	het	cttggtgctTgatt'gttactC	cttggtgctTgatt'gttactT	gTtgttCtctctgattCtTgtG
IWGC.CSS.3AL_scaff.4446105	Cadenza1599	1765	G	A	hom	—	aaatgcttttctcaCcgctagT	aaatgcttttctcaCcgctagA	tcttAgaggcaatagctTatatgcT

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