

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 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.

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.

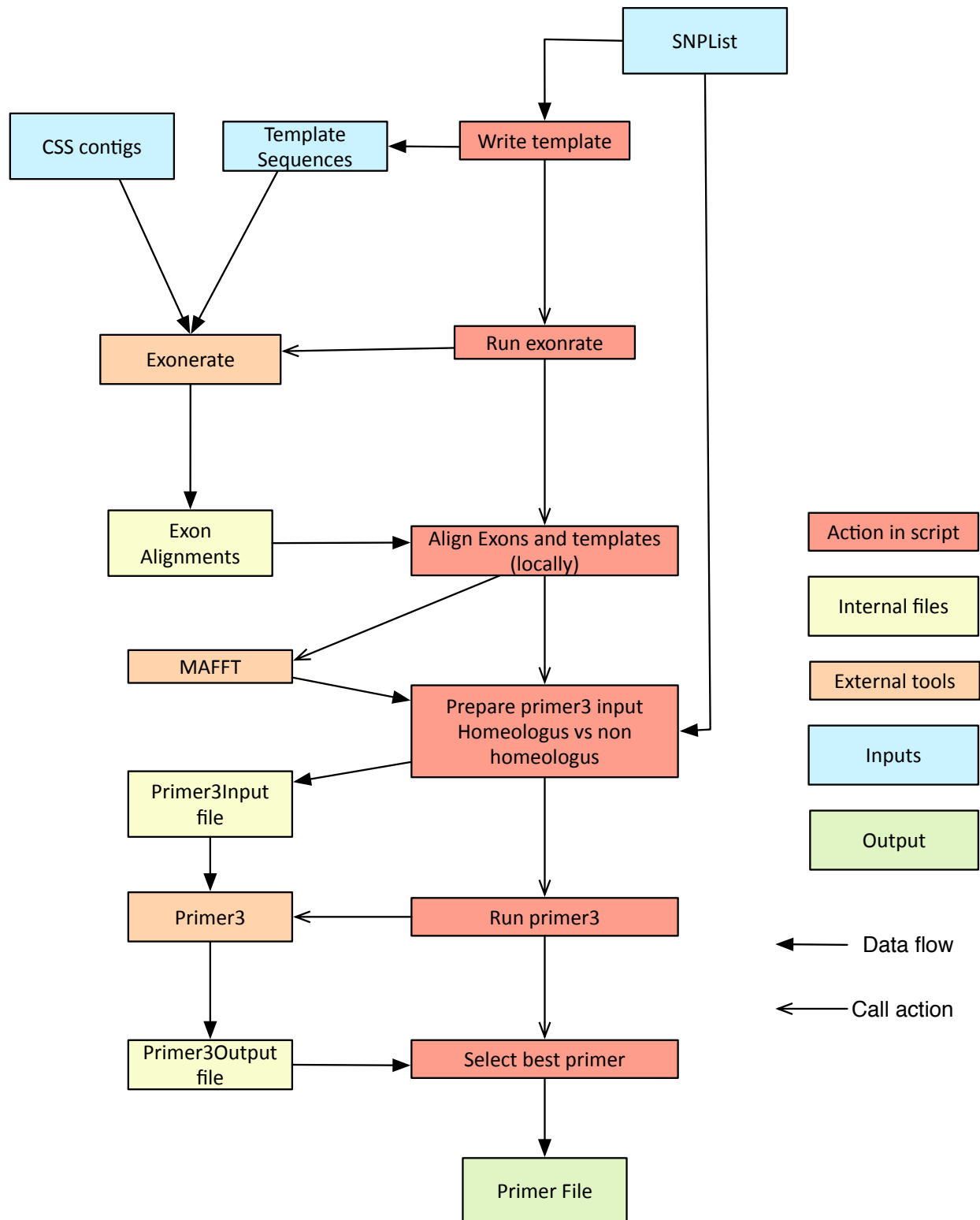


Figure 4.1: Steps and tools called by PolyMarker. The colour of the boxes represent: the step is an action inside the script(red); actions of the script(orange); temporary files(yellow); inputs(blue) and; output(green)

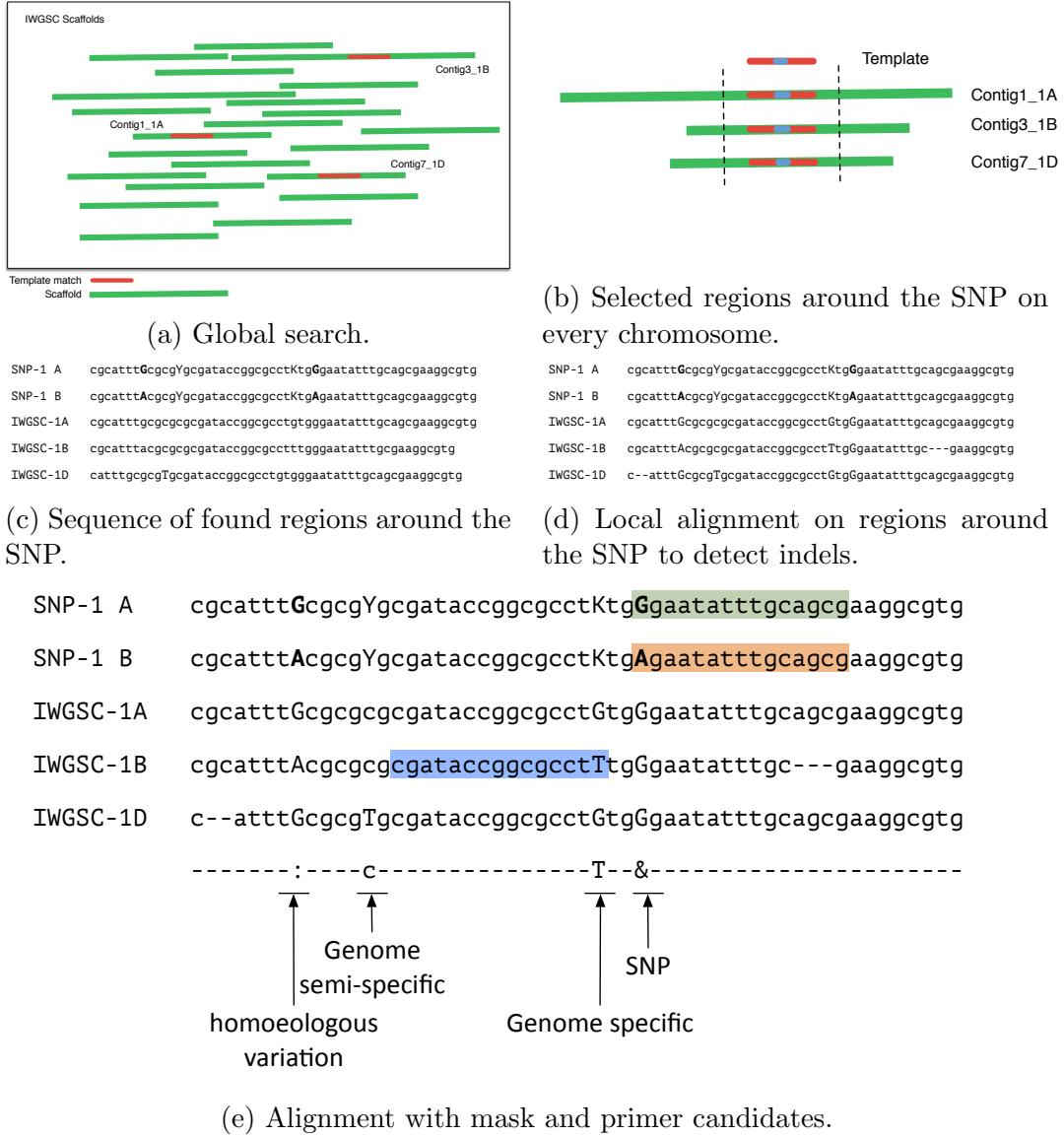


Figure 4.2: Alignments done by PolyMarker.

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.

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 successfully to design genome-specific primers in several projects.

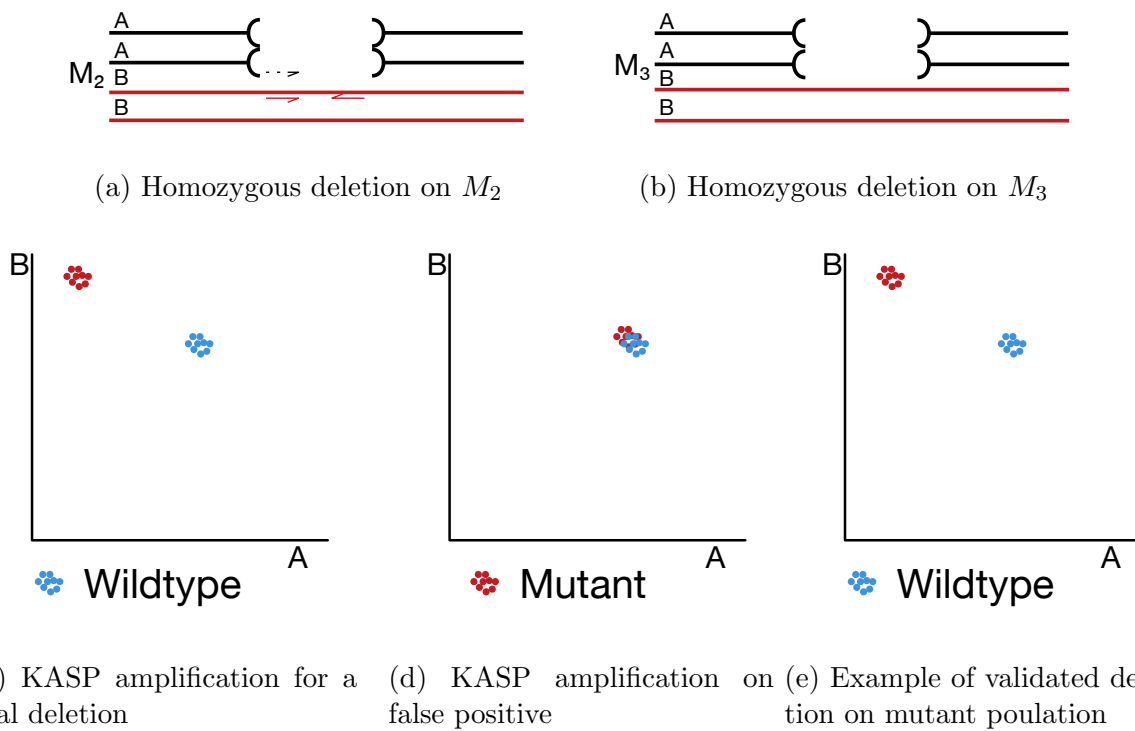


Figure 4.3: PolyMarker used to find primers to detect long deletions in tetraploid wheat.

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
IWGSQ.CSS.1AS.scaff.3284790	Kronos3085	7449	G	A	Het	Het	Het	ccacacttgagctcG	ccacacttgagctcGT	gtgatthgcagggagA
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IWGSQ.CSS.6AS.scaff.4361911	Kronos3085	8857	C	A	Het	Het	Het	tcacgaagagagctctaacC	tcacgaagagagagctcttC	catgggtggtcttctcttA
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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	caggatAgtGGagactgtcaaaG	caggatAgtGGagactgtcaaaA	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	atgctgGatcGtGtGagtcC
IWGSC.CSS.1BL_scaff.3297240	Cadenzal1661	1970	C	T	het	het	catccgccCTttctctC	catccgccCTttctctC	gctccgcatgaagagT
IWGSC.CSS.1BS_scaff.3828996	Cadenzal1661	1340	G	A	hom	hom	agccgagttgtagTtaacT	agccgagttgtagTtaacT	agcagcttgTcgtgtfaaC
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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
IWGSC_CSS_3AL.scnaf.442479		Cadenza0364	3198	C	T	het	het	gagtaCTaagtgtgtaagattggC	gagtaCTaagtgtgtaagattgT	GCaGaThaCaacagatcAG
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IWGSC_CSS_7AL.scnaf.4552322		Cadenza0103	1412	C	T	het	het	gcaagagCTgacttccacaagG	gcaagagCTgacttccacaagA	ggcCAAGcctgtaaaagaaGC
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IWGSC_CSS_4AL.scnaf.7176064		Cadenza0277	6220	C	T	het	het	gtgcgtatTtCCgctcgG	gtgcgtatTtCCgctcgA	ggttcggagggagggG
IWGSC_CSS_4DL.scnaf.14122349		Cadenza0277	1010	C	T	hom	hom	gtgcgtcgCTgttggaA	gtgcgtcgCTgttggaA	ggttcggagggagggG
IWGSC_CSS_4AL.scnaf.2736916		Cadenza0277	4296	G	A	het	het	agaactATgAaaGtaacacagAC	agaactATgAaaGtaacacagAT	ttcCtTtThagCGatChcG
IWGSC_CSS_5BL.scnaf.10883744		Cadenza0277	2080	C	T	hom	hom	gctctttChgtTAgctcagG	gctctttChgtTAgctcagA	cgcaagatgtgtgtgATgCA
IWGSC_CSS_2DL.scnaf.538086		Cadenza0548	11765	C	T	hom	hom	accgcaacCCaagagagA	accgcaacCCaagagagA	cccttaagCCagTgcAacG
IWGSC_CSS_1BS.scnaf.3417505		Cadenza0548	373	C	T	het	het	gtgtgagagCGgttgaG	gtgtgagagCGgttgaA	tggtgCGcaggttggaA
IWGSC_CSS_2AS.scnaf.5305619		Cadenza0548	2786	C	T	hom	hom	atacagatgacctAAgtgtTtC	atacagatgacctAAgtgtTtT	ggaaagaAAGctccagatcAC
IWGSC_CSS_2AS.scnaf.5306489		Cadenza0548	46953	T	G	het	wt	aggttccaagctatagagGT	aggttccaagctatagagGT	aggctCTAgtctcTtACAgT
IWGSC_CSS_2BL.scnaf.7984123		Cadenza0548	11660	G	A	het	het	catgttgcatagatcaagtaagAG	catgttgcatagatcaagtaagA	aatctactgttgatcgaatcAG
IWGSC_CSS_2DL.scnaf.538086		Cadenza0548	1363	C	A	hom	hom	tgcttccctttcgcaagAC	tgcttccctttcgcaagAT	ggcacaactggtgtgtcCT
IWGSC_CSS_2DS.scnaf.538086		Cadenza0548	5449	G	A	hom	hom	gcatctctttattactgaGtG	gcatctctttattactgaGtA	catgtcgtctcttgcagC
IWGSC_CSS_3AL.scnaf.4449951		Cadenza0548	633	C	T	het	het	tcnaaacatcaagcttcaacatAG	tcnaaacatcaagcttcaacatA	gcttcgagTGCcattgtC
IWGSC_CSS_3B.scnaf.10479889		Cadenza0097	3339	C	T	hom	—	tgtTttcCGagaaagagCA	tgtTttcCGagaaagagCA	gggtgtcatcaacCGcA
IWGSC_CSS_3B.scnaf.10562262		Cadenza0097	7819	C	T	het	het	agaggggttgctatcattAttgA	agaggggttgctatcattAttgA	agcgaTgacatcagcttC
IWGSC_CSS_4AL.scnaf.7040796		Cadenza0097	10772	G	A	hom	het	acacacatgcccacagA	acacacatgcccacagA	CAatGatgttgcTtaccC
IWGSC_CSS_4AL.scnaf.7063488		Cadenza0097	6360	C	T	het	het	gctctcaacCttAattgaagttC	gctctcaacCttAattgaagttT	aggcaggtgagattgtgaattT
IWGSC_CSS_4AL.scnaf.7091701		Cadenza0097	5050	G	A	het	het	catgtgagcatgggaggaatAG	catgtgagcatgggaggaatA	agcaagagCAatagaaggaA
IWGSC_CSS_4DS.scnaf.1845841		Cadenza0097	7110	C	A	hom	hom	aatgTAgcttcccatcCGg	aatgTAgcttcccatcCGgA	actgaacCTgaactcgtTtaagGA
IWGSC_CSS_5AL.scnaf.2767581		Cadenza0097	3757	G	A	het	het	gagaggttccctacATccgCT	gagaggttccctacATccgCT	cgTtaccacaatattgtctggG
IWGSC_CSS_5BL.scnaf.10784643		Cadenza0097	1568	C	T	hom	hom	agaaATAcagattggatgagCA	agaaATAcagattggatgagCA	catctcCtttccCGgaaagG

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	tfttgagcagcaggatcgC	tfttgagcagcaggatcgT	actcagcgctatataCctattC
IWGS.CSS.1DL_scaff.2265172	Cadenza2092	9094	C	T	hom	hom	tfttgTtGtattgtcttattagC	tgaTGTtattgtcttattagT	aggtccactatccGttcatC
IWGS.CSS.2AL_scaff.6439430	Cadenza2092	16201	G	A	hom	hom	tftctgTacttaacgtcaattgaC	tftctgTacttaacgtcaattgaT	gtagagtagatgagtagaacC
IWGS.CSS.2DL_scaff.9760848	Cadenza2092	25101	C	T	het	—	caagaagaaggCagCtCagT	caagaagaaggCagCtCagT	tcGttAcTcttcActgttgaa
IWGS.CSS.3AL_scaff.4407012	Cadenza2092	4733	C	T	het	het	gcaccatgggtctcagtaC	gcaccatgggtctcagtaT	taagttagtttCCTCtgTCTG
IWGS.CSS.3AS_scaff.3441108	Cadenza2092	2785	C	T	hom	hom	acatatAggttctctaccatC	acatatAggttctctaccatT	acctctcagtttaagtgttgT
IWGS.CSS.3BS_scaff.1049827	Cadenza2092	541	G	A	het	het	GtgcagctctgagacGgaC	GtgcagctctgagacGgaA	aggcaTgacaaCgagcaA
IWGS.CSS.3BL_scaff.1050638	Cadenza1551	4779	G	A	hom	hom	ggcaaggcgaagaacGgtC	ggcaaggcgaagaacGgtT	aCagaGtgggttagaggcaG
IWGS.CSS.3DL_scaff.6945816	Cadenza1551	3250	C	T	het	het	ctctctcactgtttggcC	ctctctcactgtttggcT	gcaacATtTgatactgcaagG
IWGS.CSS.3DL_scaff.6945816	Cadenza1551	589	C	T	hom	hom	agcatctcactgtcaacCaataC	agcatctcactgtcaacCaataT	TgtgcccTtTgaAattttcaTG
IWGS.CSS.3DL_scaff.6954177	Cadenza1551	3508	C	T	het	het	tgtagcatcacataactttctG	tgtagcatcacataactttctA	gcttggtataaacCttacgacA
IWGS.CSS.4AS_scaff.5938272	Cadenza1551	19080	G	A	hom	hom	agAcCccgATgcacatG	agAcCccgATgcacatG	GggAgatAcaggtaaaActcTtcG
IWGS.CSS.4AS_scaff.5977594	Cadenza1551	11092	C	T	het	het	gctctgattcggaacaaaaC	gctctgattcggaacaaaaT	ggtctctcagttcagcaA
IWGS.CSS.5AL_scaff.2671035	Cadenza1551	5859	C	T	het	het	cggTgataattTttagacttgcagC	cggTgataattTttagacttgcagT	ggcagttcagcGacccatT
IWGS.CSS.5BL_scaff.10889480	Cadenza1551	2530	G	A	hom	hom	gagcttaactcgcagatggaG	gagcttaactcgcagatggaA	tcctatgCAacGctttgT
IWGS.CSS.3B_scaff.10528396	Cadenza2088	8059	G	A	hom	—	cttttcctcgttaagcaataG	cttttcctcgttaagcaataA	gtgcactgttccagcctgA
IWGS.CSS.3B_scaff.10637573	Cadenza2088	16815	G	A	het	het	agcaagcttaccGgtctgC	agcaagcttaccGgtctgT	cgagcAactacagcagctT
IWGS.CSS.4AL_scaff.7086469	Cadenza2088	6697	G	A	het	het	gcgcTctactcaacgC	gcgcTctactcaacgA	ccaGaggttgtTGcatittT
IWGS.CSS.4AL_scaff.7126302	Cadenza2088	3627	G	A	hom	hom	gttcaaaaaaagggtctAatttgC	gttcaaaaaaagggtctAatttgT	cacaaggatatgaagcTcttctagA
IWGS.CSS.4BL_scaff.7041808	Cadenza2088	10234	G	A	hom	hom	tcaattgagtagagggtcttC	tcaattgagtagagggtcttT	ccatagcagcatcagccacA
IWGS.CSS.5AL_scaff.2794167	Cadenza2088	13162	G	A	het	—	agTattcaggacaagcatCttCaG	agTattcaggacaagcatCttCaA	caatgaacacctctegaagaaG
IWGS.CSS.5BL_scaff.10889232	Cadenza2088	3885	G	A	het	het	cTcaaccacatgggcaAatC	cTcaaccacatgggcaAatT	tccttcatcaatcatcaattgtG
IWGS.CSS.5BS_scaff.2267405	Cadenza2088	11113	C	T	hom	hom	ctttgagctctaggcctctTG	ctttgagctctaggcctctTA	tgatttgtTtggtTAgagtttGA
IWGS.CSS.3B_scaff.10475354	Cadenza1409	2203	G	A	hom	hom	agCgaacaagagGtcaaacG	agCgaacaagagGtcaaacA	ctgaacaacaGtagaCAattAocG
IWGS.CSS.3B_scaff.10674115	Cadenza1409	4555	C	T	het	het	gcttcagtgcaagccttcaG	gcttcagtgcaagccttcaA	cttcaaccccGagataatGtattG
IWGS.CSS.4AL_scaff.7153568	Cadenza1409	13073	C	T	hom	hom	tcgcagcATcaaccttgG	tcgcagcATcaaccttgA	gaccggaactctctggcC
IWGS.CSS.4DL_scaff.14314966	Cadenza1409	2010	G	A	het	hom	gtaggttccctctCAGgaG	gtaggttccctctCAGgaA	cgggcTcaaaAggttgCcT
IWGS.CSS.4DS_scaff.2324074	Cadenza1409	7606	G	A	het	het	tGatgaataatgtGcaGag	tGatgaataatgtGcaGaa	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	—	ggTgatctaccgcaGgaC	ggTgatctaccgcaGgaT	tcttgagCcTctctcaA
IWGS.CSS.5BL_scaff.10917655	Cadenza1409	19073	C	T	hom	hom	caaatgacatgcaaaagaagtG	caaatgacatgcaaaagaagtT	cgcttcatcactacaAaata'grcT
IWGS.CSS.1AL_scaff.3886649	Cadenza1599	5204	C	T	het	het	tgtatgcaaccacatGcT	tgtatgcaaccacatGcT	ggacatgactgtgaccattttaG
IWGS.CSS.1BL_scaff.3810267	Cadenza1599	6634	C	T	hom	hom	ccCaggaatgagcactC	ccCaggaatgagcactT	cgaggcggaagtgtgaTtG
IWGS.CSS.1DL_scaff.2291677	Cadenza1599	12856	C	T	hom	hom	GgtagaagaatgcgcgaA	GgtagaagaatgcgcgaA	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	gctgtagattttatagctgtagC	gctgtagattttatagctgtagT	caaCagaatttgttCactgatttTC
IWGS.CSS.2BL_scaff.7952427	Cadenza1599	19249	G	A	hom	hom	cgTccctCcttagcagcG	cgTccctCcttagcagcT	aTcaactccattagcgAG
IWGS.CSS.2DL_scaff.9897981	Cadenza1599	5627	C	T	het	het	cttggtgctTgatt'gttactC	cttggtgctTgatt'gttactT	gTtgttCtctctgattCtTgtG
IWGS.CSS.3AL_scaff.4446105	Cadenza1599	1765	G	A	hom	—	aaatgcttttctcaCcgtagtA	aaatgcttttctcaCcgtagtA	tcttAgaggcaatagctTatatgcT

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