

# Chapter 1

## Introduction

A general overview



## Chapter 2

### Literature review



## Chapter 3

### Genetic mapping of *Yr15*



# Chapter 4

## PolyMarker





# Chapter 5

## expVIP



# Chapter 6

## Conclusions

The proposed the following outline for my PhD thesis:

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.
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 an the concept of gene, both as a locus in the genome (Quantitative Trait Locus, QTL) and an specific transcript (central dogma of molecular biology). Finally, it discusses traditional Mendelian inheritance and the effect of polyploidy.
  - 2.3. **Wheat Genomics.** A description of the current status of the wheat genome (Mayer et al. [2014], Chapman et al. [2015]), the different available assemblies and 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.
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. diccoides*).
  - 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 thef 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 techinque 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.
4. **PolyMarker** A fast polyploid primer design pipeline

- 4.1. **Manual process of primer design(Introduction)** Explain how the SNP markers are designed without the tool.
- 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. For KASP markers, the product should be as short as possible with the mutation in the first three bases.
  - 4.5.2. Algorithm to select the two primers with a genome-specific variation. For amplicons/capillary sequencing.
  - 4.5.3. Algorithm to produce KASP for deletions in polyploids.
- 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.
- 4.7. **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]
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.
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.



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