# Masterproef industriële ingenieurswetenschappen

# *Development of a high-throughput PCR & sequencing approach for SNP identification in Leguminosae species*

**Ine Maes**

Academiejaar: 2014-2015

## Introduction & goals

One of the goals of the research project 'Eurolegume' is to identify genotypes of *Pisum sativum* and *Vicia faba* with a high protein content and resistance against diseases, heat and drought. The genotypes may differ because of point mutations in the genome, single nucleotide polymorphisms (SNP's). 88 genotypes of *Pisum sativum* and 116 of *Vicia faba* should be examined to find the ones corresponding to the desired phenotype. This work can be minimalized by making a phylogenetic analysis based on SNP's in order to evaluate genetic variability. The goal of this master's thesis is to optimize PCR and sequencing conditions to identify SNP's in the genome.

## Materials & methods

### 1) Primer design:

60 SNP sequences for *Vicia faba* and 45 for *Pisum sativum* are selected and primerssets are designed. All the primers have an additional common sequence at the 5'end.

### 2)Optimization PCR conditions:

Three PCR kits are tested:

- BIOLINE → MyTaq™ DNA Polymerase
- NEW ENGLAND BioLabs Inc. → OneTaq™ DNA Polymerase
- Clontech A Takara Bio Company → Primestar GXL

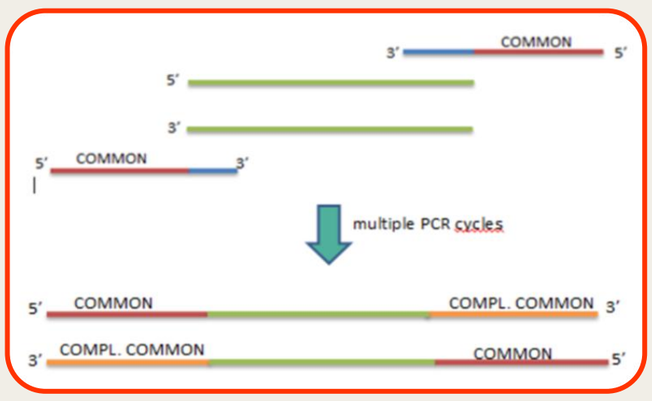
Three thermocyclers are tested:

- Agilent Technologies → Stratagene Robocycler
- Biometra An Analytik Jena Company → Tprofessional Thermocycler
- applied biosystems part of life technologies → GeneAmp® 9700

### 3)PCR's & DNA sequencing:

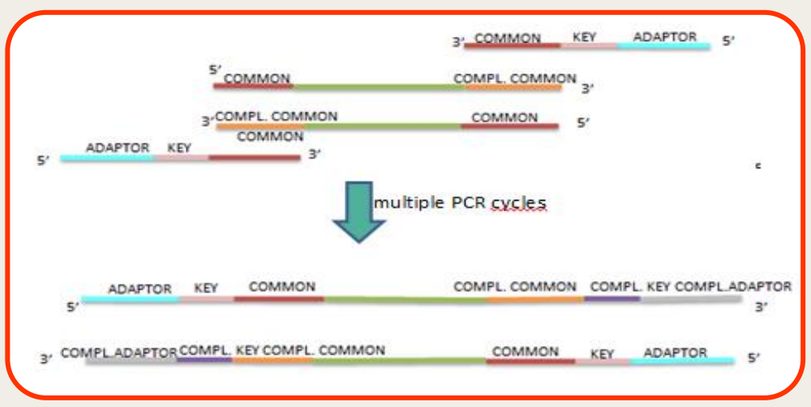
#### Genomic PCR

- Amplification DNA fragment with chosen SNP
- Elongation DNA fragment with common sequence



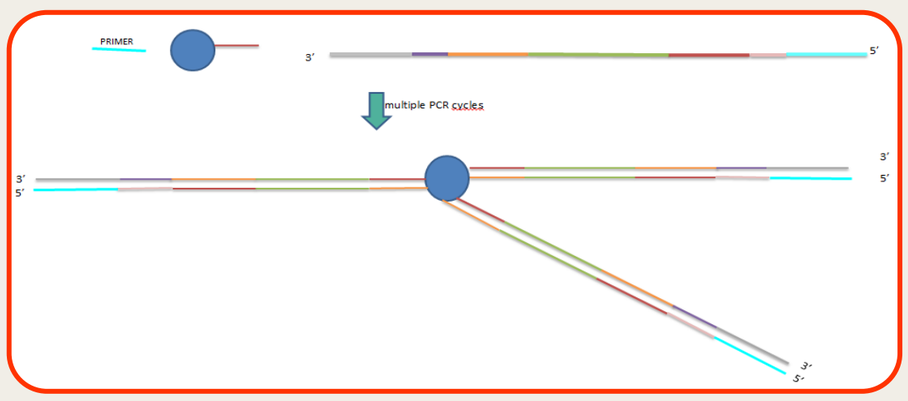

#### Barcoding PCR

- Elongation DNA fragment with adaptor sequence and key sequence




#### Emulsion PCR

- Preparation for Ion Torrent sequencing
- Amplification DNA fragments on beads
- Testing three amplicon library concentrations (26 pM, 13pM and 6,5 pM)




## Discussion

Optimal PCR conditions are obtained with the TProfessional Thermocycler and OneTaq DNA Polymerase kit. 35,5 % of the primers for *Pisum sativum* and 28,3 % for *Vicia faba* did not work and should be redesigned. The ideal concentration for the emPCR is 13 pM. A combination of using the TProfessional Thermocycler and OneTaq DNA Polymerase kit for the first two PCR's and an amplicon library concentration of 13 pM for the emulsion PCR gives the most ideal template for Ion Torrent sequencing.

Promotoren / Copromotoren: ir. Myriam Meyers
Prof. Dr. Julia Weiss
Prof. Dr. Marcos Egea Gutiérrez-Cortines

Universidad Politécnica de Cartagena

EUROLEGUME 2014-2017

KU LEUVEN | universiteit hasselt