Masterproef industriële ingenieurswetenschappen

Application of an Ion-Torrent sequencing approach for genotyping Leguminosae species

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Introduction

The genetic diversity of *Pisum sativum* and *Vicia faba* has to be investigated. That is done by conducting a phylogenetic analysis based upon the sequence of single nucleotide polymorphisms (SNP's), linked to possible resistance genes against high salinity and the fungal disease Ascochyta blight. To conduct the analysis a method has to be developed and optimised to determine the sequence of chosen SNP's, which is the aim of this thesis.

Methods

- To determine the sequence of the SNP's the following steps are executed:
  1) Choosing SNP's,
  2) Designing primer sets to amplify fragments containing the SNP's;
  3) Performing genomic PCR to amplify fragments (fig. 1);
  4) Performing barcoding PCR to add adaptor and key sequence (fig. 2);
  5) Preparing equimolar fragment library;
  6) Performing emulsion PCR to hybridize fragment to a bead (fig. 3);
  7) Performing enrichment of beads;
  8) Sequencing in Ion Torrent next generation sequencing.

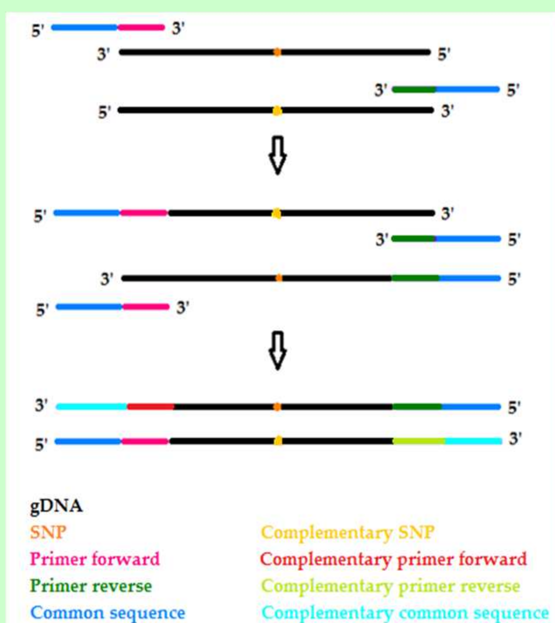

Figure 1: Genomic PCR

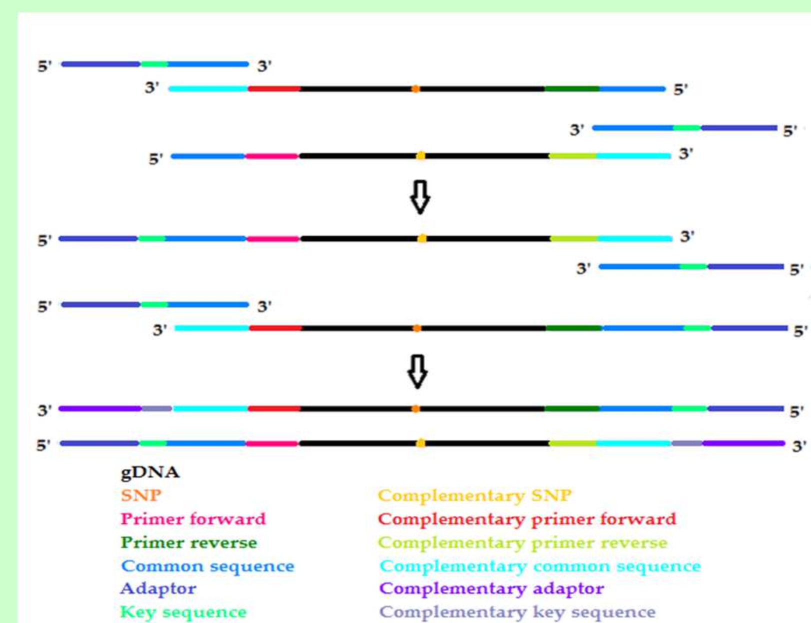

Figure 2: Barcoding PCR

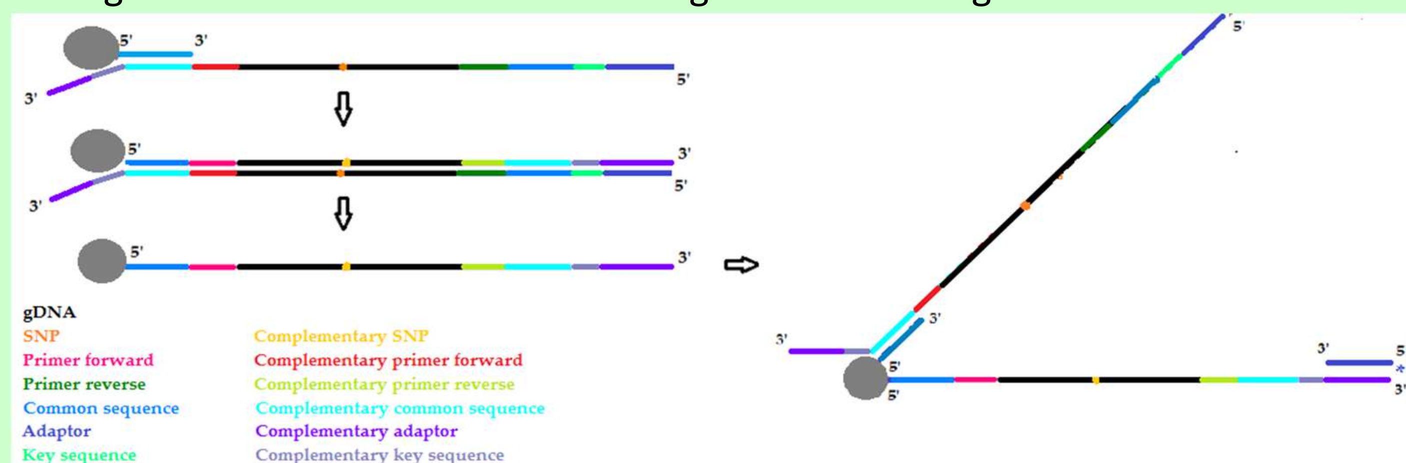

Figure 3: Emulsion PCR

- Optimisation consists of finding the right PCR conditions and the ideal concentration of the fragment library.

Results

- Design of specific part forward and reverse primer:

	Min	Optimum	Max
Melting temperature (°C)	49	50	51
Number of base pares	14	15	16
%GC (%)	40	/	60

Table 1: Primer design

- Determination of PCR conditions:

Thermocyclers	DNA polymerase kit
GeneAmp PCR System 9700 (Applied Biosystems)	One*Taq* DNA Polymerase (New Englands BioLabs)
Robocycler Gradient 96 (Stratagene)	PrimeSTAR GXL (TaKaRa Bio)
TProfessional thermocycler (Biometra)	

Table 2: PCR conditions

- Choosing fragment library concentration:

Concentration (pM)	Templated Ion Speres (%)
26	82,03
13	54,58
6,5	81,39

Table 3: Fragment library concentration

Discussion

71,7 % of the designed primer sets could amplify their SNP fragment for *Vicia faba* and 64,4 % for *Pisum sativum*. The PCR's work best when using the TProfesional thermocyler in combination with One*Taq* DNA Polymerase kit. The ideal fragment library concentration is 13 pM.

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