Investigation of promoter regions in Scots pine combining linear DNA amplification with massive parallel sequencing

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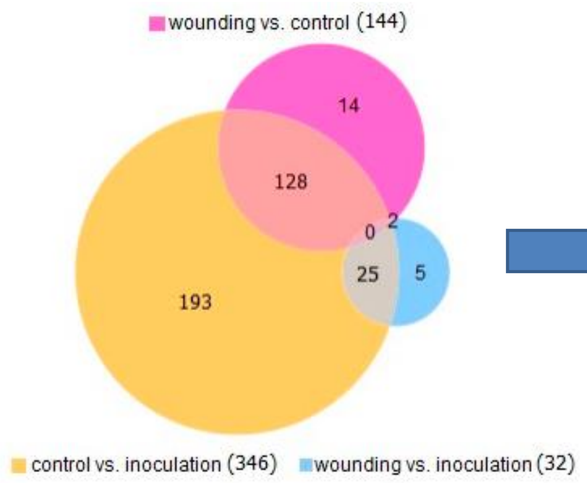
Before the experiment



Changes in transcriptome in response to inoculation with *Heterobasidion annosum* identified

How are these genes regulated? What are the structures of their promoter regions?

Problem: no reference genome, limited possibilities for primer design to clarify 5' flanking regions

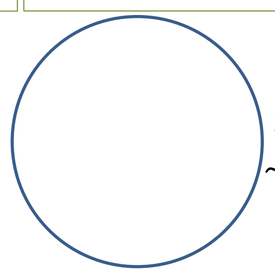
Workflow, approach I

Extract HMW DNA

Make sure it's HMW

Multiplex linear DNA amplification

Circular ligation of ssDNA molecules



ssDNA ~1000 nt

Enzymatic degradation of remaining ssDNA

Circular ligation of products that long is possible but with **low** efficiency

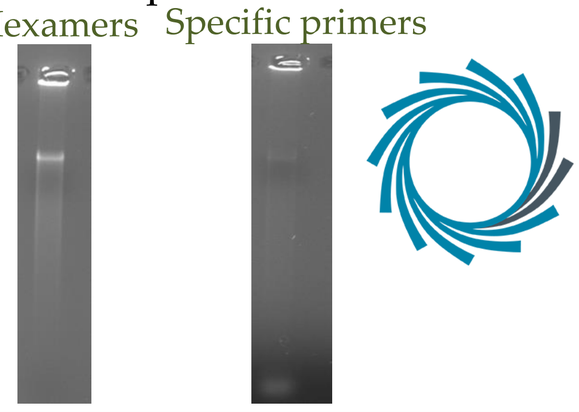
RCA with specific primers

Preparation for Ion Torrent and Oxford Nanopore sequencing

Fragmentation + Swift BIOSCIENCES™ Accel-NGS® DNA Library Kit for the Ion Torrent™ Platform

Amplification of RCA product

Hexamers Specific primers



Result

2 out of 100 targeted sequences identified. Rest of the sequences from Scots pine but not what expected. Despite low efficiency, in principle the method is working and, with optimization, results could be improved.

Workflow, approach II

Extract HMW DNA

Make sure it's HMW

Product shouldn't be visible

Singleplex linear DNA amplification

Modified TTAS method

Jiang et al., 2019

https://doi.org/10.1007/s13238-018-0540-9

Oxford Nanopore sequencing result

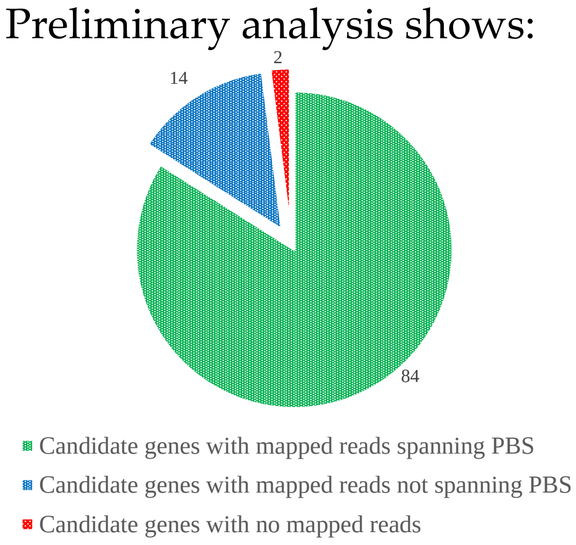
Primer binding sites found in sequences but adjacent sequences don't match the reference (transcriptome from Wachowiak et al., 2015) used for primer design

Whole genome sequencing

and initial findings

To clarify the reasons for lack of success with the modified TTAS method, we chose to perform low coverage WGS of *P. sylvestris*.

Preliminary analysis shows:



PBS - primer binding site

~ 867 k reads
(L ≥ 30 kb, q ≥ 10)

As an example, for a gene encoding a UDP-glycosyltranferase, a 15kb long sequence downstream the PBS was obtained.

Some clues for SNPs in PBS also obtained.

PostDoc Latvia
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IEGULDĪJUMS TAVĀ NĀKOTNĒ
Investigation of regulatory regions of Scots pine disease resistance genes 1.1.1.2/VIAA/3/19/510