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Title: Deploying RLK/ RLP enrichment sequencing to accelerate mapping surface immune receptor in potato

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Applicant: Xiao Lin, Wageningen University, Plant Breeding Group, Wageningen(NL) , xiao.lin@wur.nl

Host: Ingo Hein, The James Hutton Institute, Dundee(UK), ingo.hein@hutton.ac.uk

Background:

The potato (*Solanum tuberosum* L.) is one of the three most consumed crops worldwide. The most devastating disease of potato is late blight, which is caused by the oomycete *Phytophthora infestans*. The most sustainable strategy to manage late blight is to breed broad-spectrum disease resistance into potato. However, traditional disease resistance breeding that exploits cytoplasmic resistance genes (*R* genes) has been of limited success, as *P. infestans* has a remarkable capacity to rapidly adapt to resistant plants. Another, yet unexploited layer of immunity occurs at the surface of plant cells. This apoplastic immunity has generally a broader spectrum and is based on recognition of conserved proteins of pathogens. To obtain novel potato pattern recognition receptors (PRRs) that can recognise oomycete apoplastic effectors, we are deploying an effectoromics approach. A variety of predicted oomycete apoplastic effectors are subjected to functional screens on almost 100 wild potato species. One of the selected effectors is SCR74, a 74 amino-acid *s*ecreted *c*ysteine *r*ich protein that is highly polymorphic in *P. infestans*. Various *Solanum* genotypes were identified that showed specific responses to SCR74. A map-based cloning approach was initiated to identify the gene that mediates response to SCR74. F1 populations were generated, and the receptor is mapped to Chromosome 9 by SSR markers. Alternatively, a improved

Resistance gene enrichment sequencing (RenSeq) that has been used for more rapid mapping of SCR74 receptor in the segregating populations.

Objectives/ Purpose of the visit:

Our plan is to adopt the Resistance gene enrichment and Sequencing (RenSeq) strategy to isolate the receptor of SCR74 from *S. microdontum*. First we will adapt RenSeq for RLP/RLK, prediction of RLP/ RLK genes from potato and tomato genomes, and design the RLP/ RLK-specific RenSeq bait library based the predicted genes. Meantime, we will expand the mapping population in Wageningen. Then we will perform the RLP/ RLK enrichment in James Hutton Institute with Ingo Hein. After that we will sequence the pools of responding and non-responding progenies and analysis the data to obtain linkage SNPs makers. Using the RenSeq markers and other molecular markers, obtain the receptor and explore the durable resistance to potato late blight. Finally, our goal is to develop a new strategy to rapid cloning novel plant immune receptors.

Description of the work:

1. Isolate DNA from SCR74 response parents GIG362-6, MCD360-1, F1 segregation population 7026-15 to 7026-69, DM and Heiz-1706;
2. Quantify DNA from all samples by Nanodrop and Qubit;
3. Pool the samples to 8 bulks-> DNA fragments by Covaris -> AMPure XP Beads purification -> Measure DNA by Qubit;
4. NEBNext End Prep-> Adaptor ligation-> AMPure XP Beads purification-> PCR with index -> AMPure XP Beads purification;
5. Using Bioanalyzer to analyze the size of DNA fragments;
6. SureSelect enrichment-> hybridization overnight for 24 hours;
7. Wash the enrichment beads-> post enrichment PCR-> XP Beads purification -> Qubit quantify;

8. qPCR for post-capture PCR samples (Bulk1 and Buik2, 10 cycles);
9. MiSeq pair-end sequencing.

Main results:

1. Bulks after fragmentation

Bulk number	Bulk	concentration by Qibut (high sensitive kit, ng/ml)	ng/ul	*55ul (ng)	for 1ug (add water to 55.5ul)
1	Scr74-R pool	394	39.4	2167	25.4
2	Scr74-N pool	392	39.2	2156	25.5
3	INF1-R pool	405	40.5	2227.5	24.7
4	INF1-N pool	382	38.2	2101	26.2
5	MCD360-1	366	36.6	2013	27.4
6	GIG362-6	358	35.8	1969	28
7	DM	171	17.1	940.5	55.5
8	Heiz-1706	349	34.9	1919.5	28.7

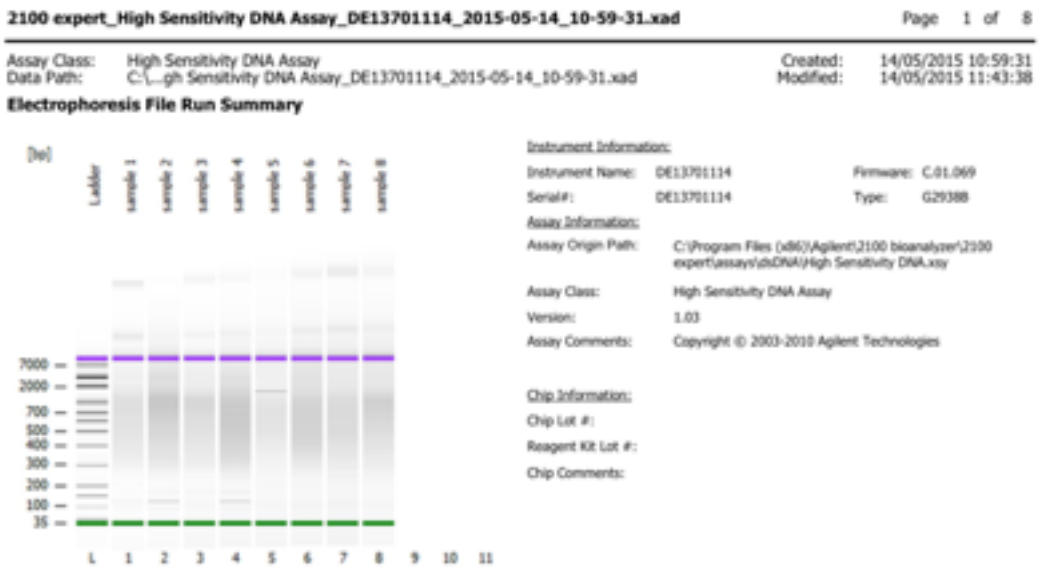
The DNA samples from 7026 population were bulked based on the response/ no reponse to apoplastic receptor SCR74 and INF1. The DNA amount were tested by Qubit high sensitive kit and the results was shown in table above.

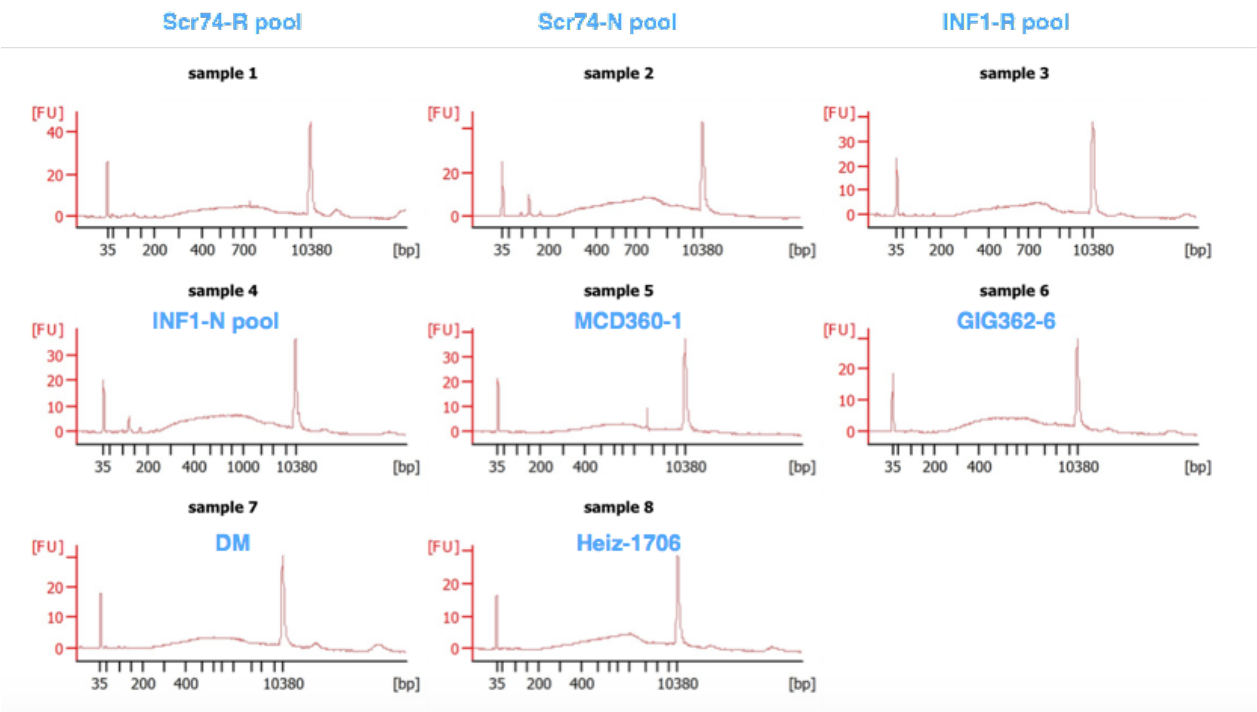
2. Bulks with adaptor ligation and index

Bulk number	Bulk	before PCR (ng/ml)	ng/ul	amount (23ul) (ng)	After PCR (ng/ml)	ng/ul	amount (40ul) (ng)
1	Scr74-R pool	127	25.4	**584.2**	58.9	11.78	**459.42**
2	Scr74-N pool	188	37.6	**864.8**	42.9	8.58	**334.62**
3	INF1-R pool	65.6	13.12	**301.76**	62.9	12.58	**490.62**
4	INF1-N pool	151	30.2	**694.6**	60.1	12.02	**468.78**
5	MCD360-1	151	30.2	**694.6**	61.7	12.34	**481.26**
6	GIG362-6	223	44.6	**1025.8**	54	10.8	**421.2**
7	DM	174	34.8	**800.4**	93.4	18.68	**728.52**
8	Heiz-1706	178	35.6	**818.8**	61.3	12.26	**478.14**
9	negative control				0		

After fragments by Covaris, adaptor and index were added on the each bulks, and the amount of DNA was measured by Qubit.

3. Bioanalyzer-high sensitivity DNA chip for 8 bulks





A Bioanalyzer high sensitivity DNA chip was used for measuring the size of DNA after fragmentation. The mean size ranges are from 700bp to 1000bp, this range is slightly bigger then expecting size 650bp (with adaptors). A size selection may perform after enrichment.

4. Pool the 8 bulks to 2 pools for further enrichment

Bulk number	Bulk	
1	Scr74-R pool	Pool1 (750ng DNA)
2	Scr74-N pool	
3	INF1-R pool	
4	INF1-N pool	
5	MCD360-1	Pool2 (750ng DNA)
6	GIG362-6	
7	DM	
8	Heiz-1706	
9	negative control	

5. Post-capture PCR

Bulk number	Bulk		Cycle	Output DNA by Qubit (ng/ml)	ng/ul
1	Scr74-R pool	Pool1 (750ng DNA)	10	53.4	2.136
2	Scr74-N pool		12	140	5.6
3	INF1-R pool		14	344	13.76
4	INF1-N pool		16	274	10.96
5	MCD360-1	Pool2 (750ng DNA)	10	74.9	2.996
6	GIG362-6		12	267	10.68
7	DM		14	388	15.52
8	Heiz-1706		16	296	11.84
9	negative control				

For minimized the PCR induced mutation, we performed a preliminary PCR with cycle 10, 12, 14 and 16. The result indicates 10 cycles is enough for the further sequencing.

6. qPCR shows the target genes are successfully enriched.

		mean ΔCT	mean fold enrichment
ELR-primer1	Pool1	6.13332653	70.1964677
	Pool2	6.396722794	84.25689193
ELR-primer2	Pool1	6.382049561	83.40428268
	Pool2	6.898123741	119.2730047

For testing the enrichment efficiency, qPCR were used for comparing the library before and after enrichment. Two primer pairs designed based on a known RLP gene— elicitin receptor ELR. The mean ΔCT is 6-7 for both pool, the fold enrichment is from 70 to 120. This result indicates the libraries are successfully enriched.

7. Post-capture PCR (10 cycles)

Bulk number	Bulk		Cycle	Output DNA by Qubit (ng/ml)	ng/ul	*33 (ng)
1	Scr74-R pool	Pool1 (750ng DNA)	10	36.6	3.66	120.1
2	Scr74-N pool					
3	INF1-R pool					
4	INF1-N pool					
5	MCD360-1	Pool2 (750ng DNA)	10	65	6.5	214.5
6	GIG362-6					
7	DM					
8	Heiz-1706					

Future collaboration with the host institution (if applicable):

1. After MiSeq sequencing, assemble the reads and mapped the reads to DM genome.
2. SNP/ Indel calling, RenSeq based Marker development for fine mapping.
3. Using the customized RLK/ RLP library on other population, mapping other potato receptors.
4. Using other enrichment sequencing strategies for fast mapping.