# Developing next-generation sequencing technology for *Rosa spp.*

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## Introduction

- Black spot disease of rose is the most important leaf disease of outdoor roses grown in warm humid areas causing roses to defoliate.
- The quantitative trait loci (QTLs) responsible for partial black spot resistance are unidentified.
- Next-generation sequencing can generate abundant single nucleotide polymorphism (SNP) markers for genetic linkage and QTL mapping and the discovery of molecular markers associated with black spot resistance. These markers can accelerate the breeding process significantly.

## Objectives

- Optimize high-throughput sequencing for rose and generate SNP-based diploid maps for *Rosa*
- Conduct QTL discovery analysis: SNP markers and phenotypic data (lab and field) will be used to map QTLs conditioning black spot partial resistance.

## Materials

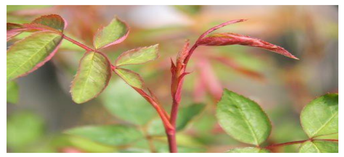
- Four diploid rose populations created from the crosses of black spot resistant breeding lines derived from *R. wichurana* 'Basye's Thornless' and susceptible commercial cultivars ('Vineyard Song', 'Red Fairy' and 'Little Chief') will be used to establish templates for SNP marker detection (Table 1). New growth tissues were collected for DNA extraction.
- Two methylation sensitive enzymes (FseI and NgoMIV) and one partial methylation sensitive enzyme NheI were used to construct templates for one population.
- Two different DNA extraction methods: modified CTAB and Fastprep kit were used and compared.

Table 1. Rose populations for genotyping. S= susceptible, MR= medium resistance, HR= high resistance.

| Female Parent | Male Parent | Population Size |
|---|---|---|
| J06-20-14-3 (HR) | Vineyard Song (S) | 93 |
| J06-20-14-3 (HR) | Little Chief (S) | 154 |
| Old Blush (MR) | Red Fairy (S) | 158 |
| Old Blush (MR) | J06-30-3-6 (HR) | 112 |

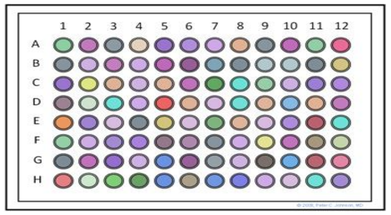
## Methods

### NGS- Next Generation Sequencing



**DNA is extracted from folded young leaves**

1. Plate DNA




2. Digest DNA with restriction enzyme (RE)

3. Ligate barcoded adapters

4. Pool DNAs

5. Shear DNA, select size, fill in 5' adapters sequence, blunt DNA, ligate 'A' and ligate T-tailed adapter

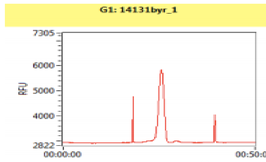
6. Biotinylated Primers

7. PCR

8.Clean up and purify single strand DNA

8. PCR

9. DNA quality evaluation




**10**. Sequence in Illumina HiSeq 2500

**11. Data analysis**

One of the methylation sensitive and partial sensitive REs (FseI, NgoMIV and NheI ) will be used to cut gene rich region and filter out repetitive genomic fraction

## Results

1. DNA templates were successfully constructed with all three FseI, NgoMIV and NheI enzymes (Figure 1-4).
2. Reads generated from Hiseq sequencer were mapped to both rose contigs and strawberry genome (Table 2).

**Figure 1. DNA post shearing**

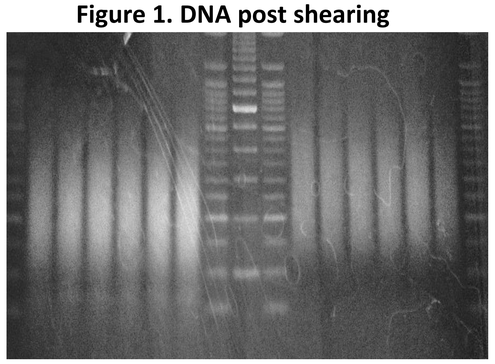


**Figure 2. Fragment size**

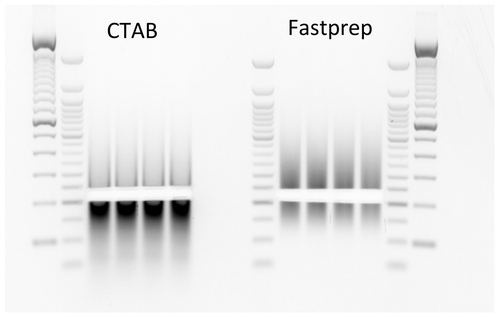



**Figure 3. Pre-selection PCR**

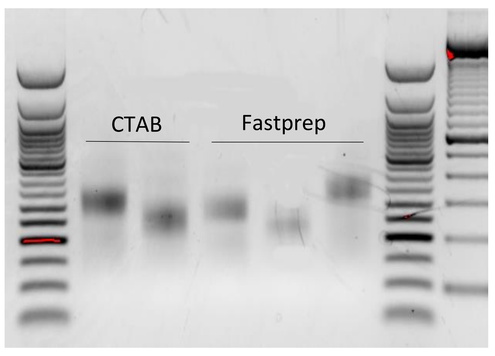



**Figure 4. Final PCR (C= CTAB, F=Fastprep)**

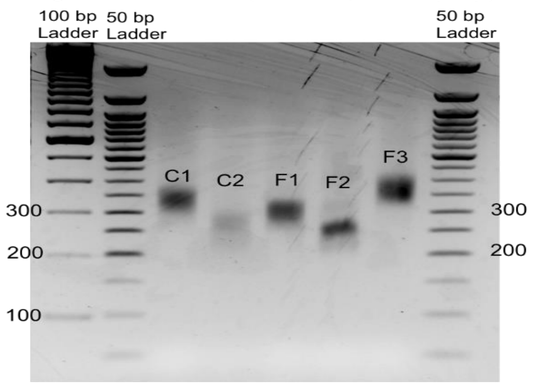



Table 2: Results of reads aligned to rose contigs and strawberry genome

| Enzyme | DNA extraction method | % reads mapped to rose contigs | % reads mapped to strawberry genome |
|---|---|---|---|
| FseI | CTAB+Zymo PCR inhibitor | 89-92% | 35-36% |
| | Fastprep | | 36-37% |
| | | | |
| NgoMIV | CTAB+Zymo PCR inhibitor | | 54-57% |
| | Fastprep | | 59-60% |
| | | | |
| NheI | Fastprep+Zymo clean&con. | | 33-34% |

## Conclusions

1. NgoMIV enzyme preferred over the other two enzymes, because it resulted in more sequences, it is less expensive and generates more reads that map to the strawberry genome.
2. CTAB+Zymo PCR inhibitor works as well as the Fastprep and is preferred as it is less expensive.

## Acknowledgments

Texas A&M Department of Horticultural Sciences
China Scholarship Council
Texas AgriLife Research

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