

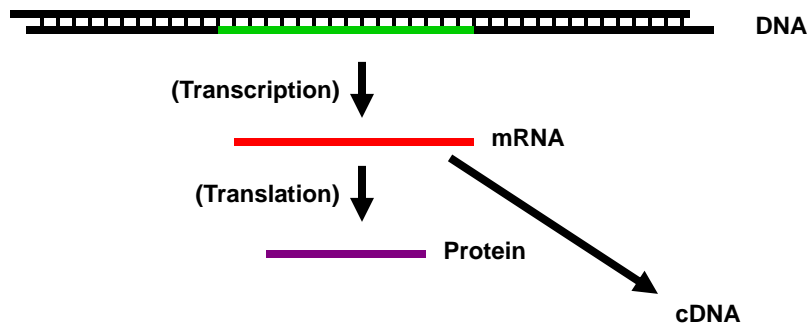
# **Developing expressed sequence tags to study the genetics of the *Pisum sativum-Sclerotinia sclerotiorum* host-pathogen interaction**

**Martin Chilvers – Michigan State University  
Tobin Peever – Washington State University  
Tristan Coram – North Carolina State University  
Kevin McPhee – North Dakota State University  
Weidong Chen – USDA-ARS/Washington State University**

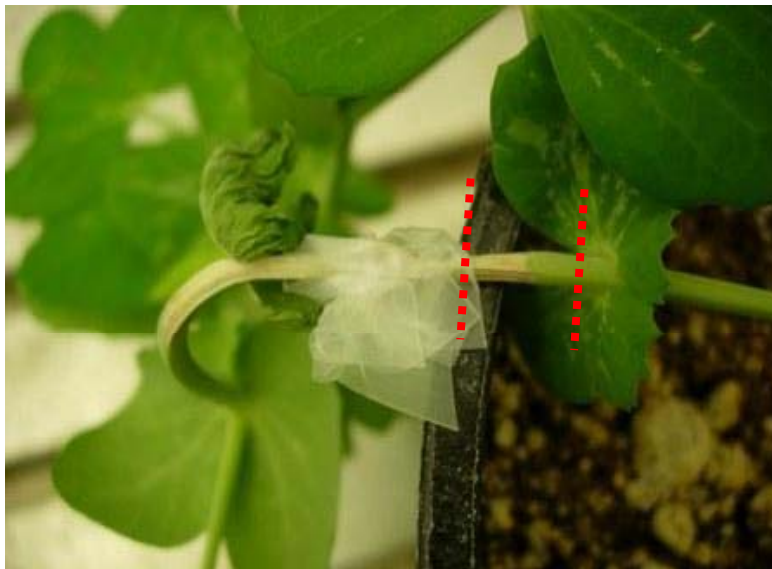
## **Objective**

**Develop expressed sequence tags (ESTs) for both  
*S. sclerotiorum* and pea for genome wide  
expression profiling studies**

## Express Sequence Tag

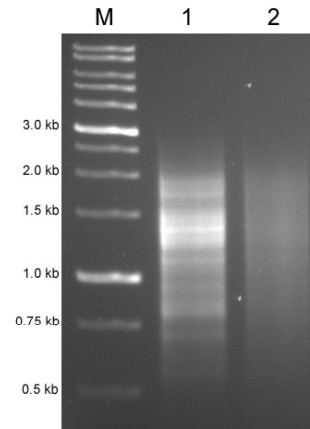


## RNA collection

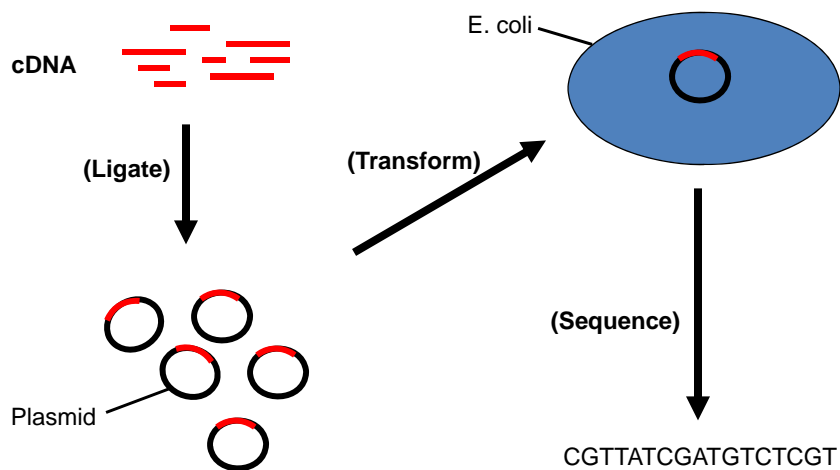


## Normalize cDNA

- Reduce high abundant transcripts
- Increase chances of sequencing rare transcripts



## Traditional cDNA (EST) library



## Next generation sequencing

- Massively parallel



## Conventional vs. Next generation sequencing technology

### Sanger

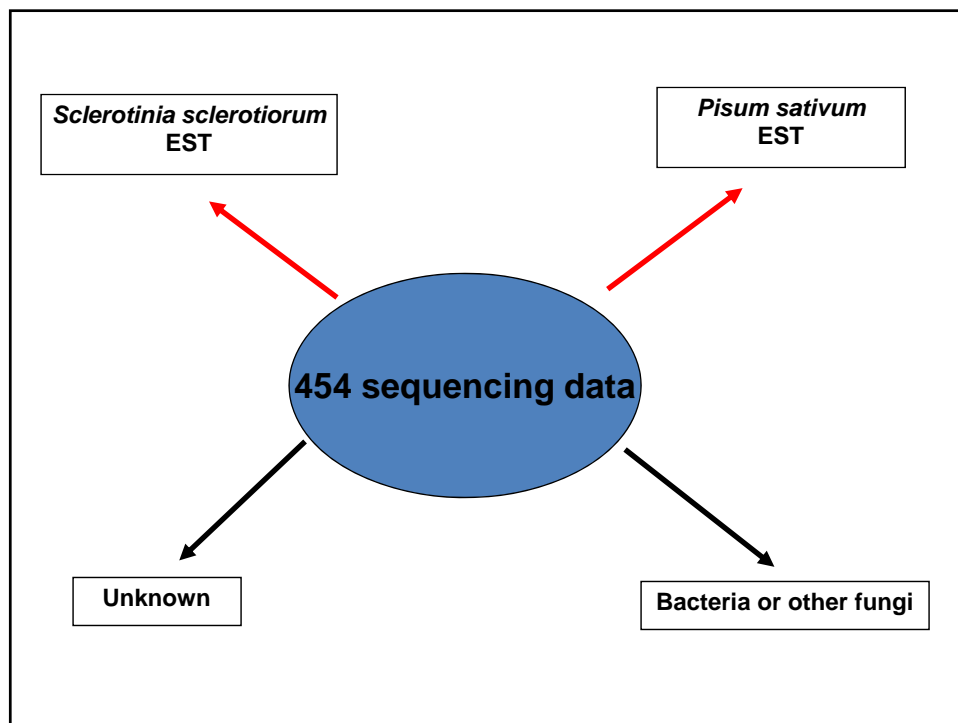
- read length ~ 800 bp
- Low throughput
  - 96 reads per run
- ~ \$2-6 per read

### 454 GS FLX (Roche)

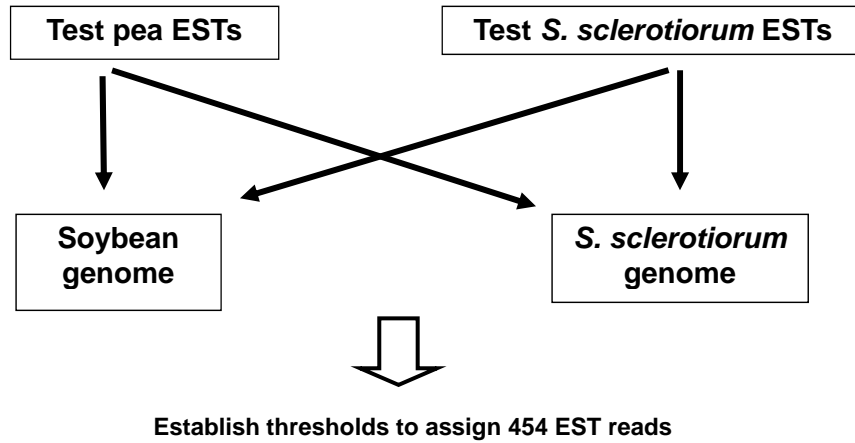
- read length ~240 bp
- High throughput
  - 400,000 reads per run
- ~ \$0.03-0.06 per read

## 454 sequence data

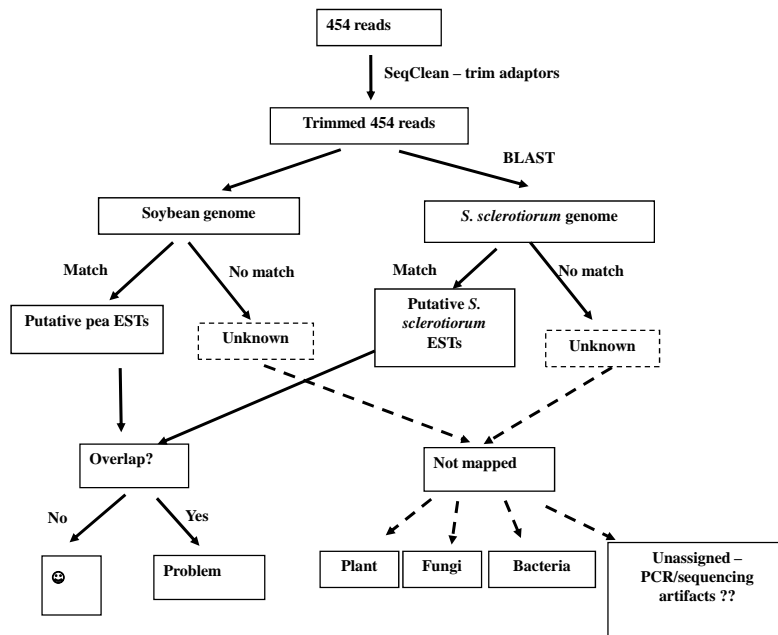
- 35 Mb (35,000,000) of normalized cDNA pool
- 162,729 reads
- Average length 215 nucleotides
- $\approx$  45,000 traditional EST clones



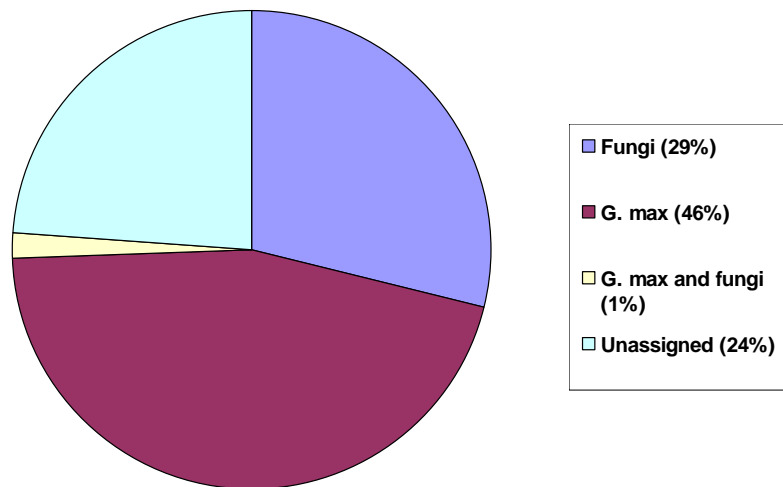
# Species assignment thresholds



# Processing and analysis workflow



## Assignment of ESTs



## Outcomes and future work

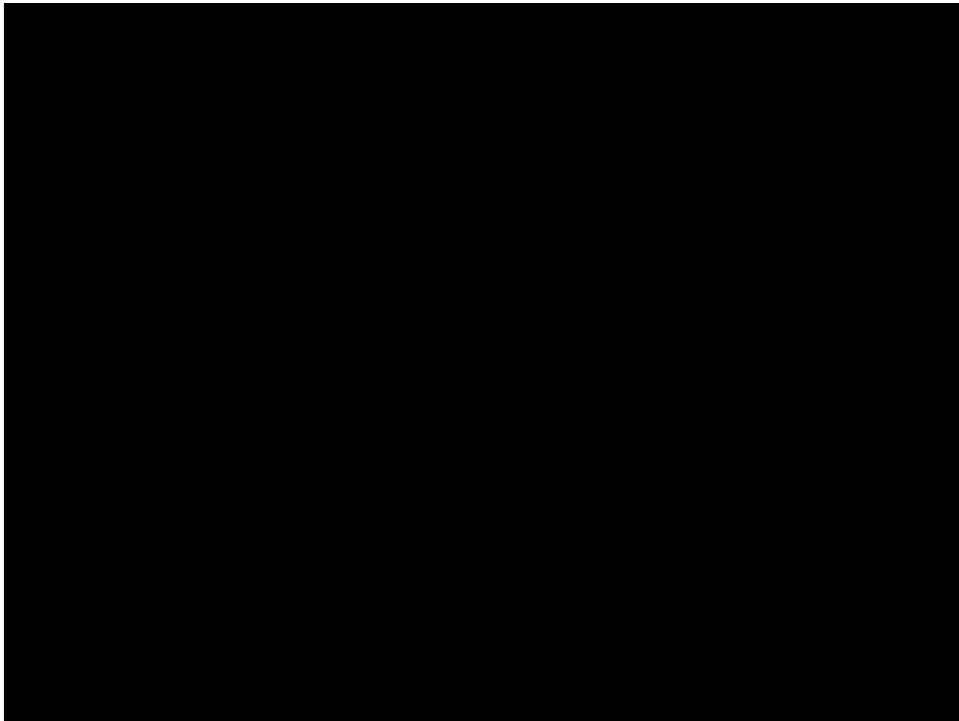
- Valuable EST resource for pea and *S. sclerotiorum*
- Marker development for pea and *S. sclerotiorum*
- Refine the genome annotation of *S. sclerotiorum*
- Expression profiling studies

## Acknowledgements

- Sclerotinia Initiative – Funding
- Amit Dhingra (WSU) – 454 sequencing
- Kawabe Masato (WSU) – genotyping WMA1
- Dongyan Zhao, Ed Kabara and Jie Deng (MSU) – bioinformatics



**National  
Sclerotinia  
Initiative**



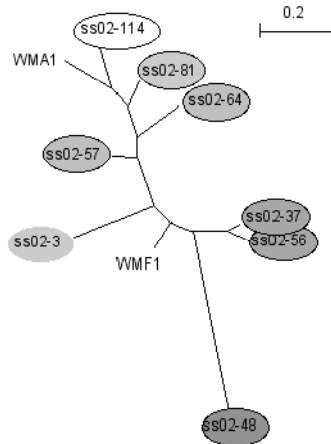


Fig. 1 Dendrogram of genetic distances among *Sclerotinia sclerotiorum* isolates. Isolate WMF1 was isolated from lentil and all other isolates are from pea. Dendrogram was constructed from genetic distances estimated with RAPD markers and clustered using neighbor joining.