Genotype-by-Sequencing; breaking the bottleneck

Robert Brueggeman

North Dakota State University

NDSU NORTH DAKOTA STATE UNIVERSITY

Utilizing marker assisted selection

- Complex resistance mechanisms requires the development and characterization of multiple populations
- Development of molecular markers
- Genome wide selection requires efficient and affordable genotyping

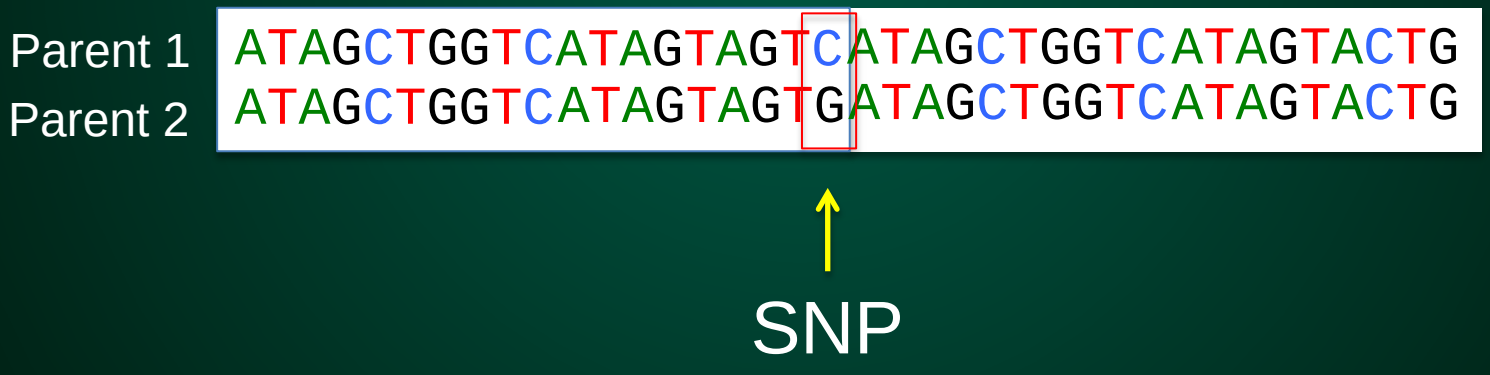
Current genotyping technology

- Illumina GoldenGate Assay
- 9K barley SNP chip cost approximately $60/line
- Relatively inexpensive for the time but still cost prohibitive
- Very few resources available for pathogen genotyping

Genotype-by-Sequencing

- What is GBS?

  Utilizing sequence to identify SNP markers



GBS has been utilized in barley

- Restriction site-associated genomic DNA (RAD) using Illumina sequencing technology
  - Bioinformatics problems
    - short reads (64-84 bases)
    - quality
  - Reproducibility

Elshire *et al.*, (2011) *PLoS One*

Baird *et al.*, (2008) *PLoS One*

Adapting Ion Torrent Sequencing technology for GBS

- Low cost
- Small footprint
- Data analysis pipeline can be simplified

Adapting Ion Torrent Sequencing technology for GBS

- Low cost

  Ion torrent personal genomics machine costs

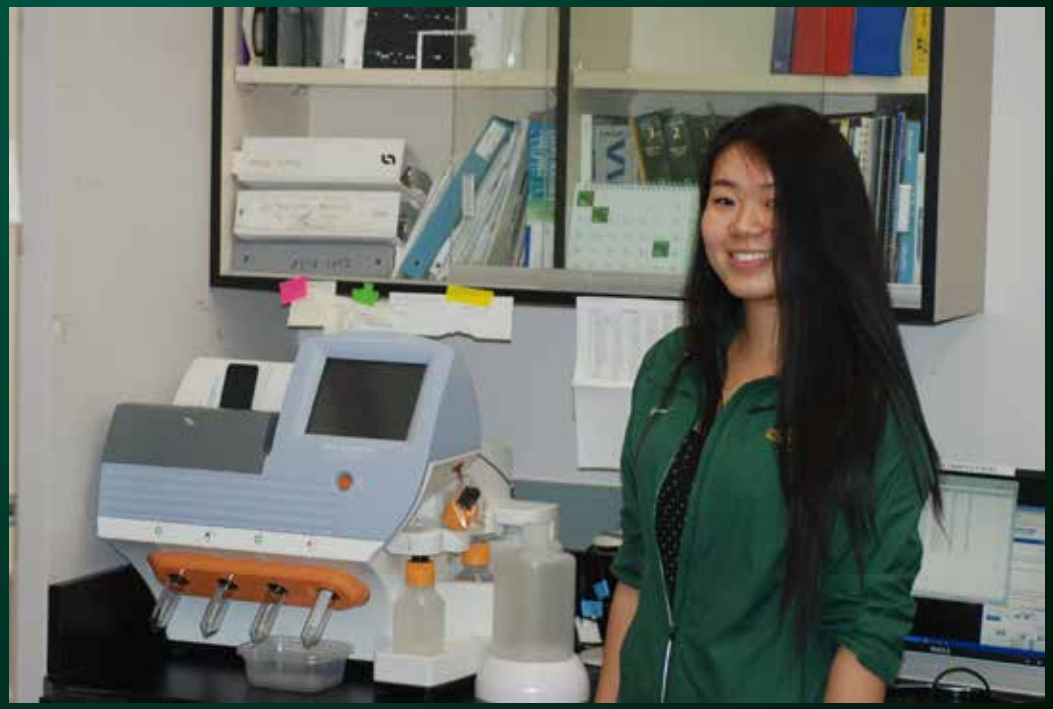
  $44,000 for the unit but an additional $45,000 on a server, equipment for library prep and emulsion PCR.

  Grand total ~$90,000

Adapting Ion Torrent Sequencing technology for GBS

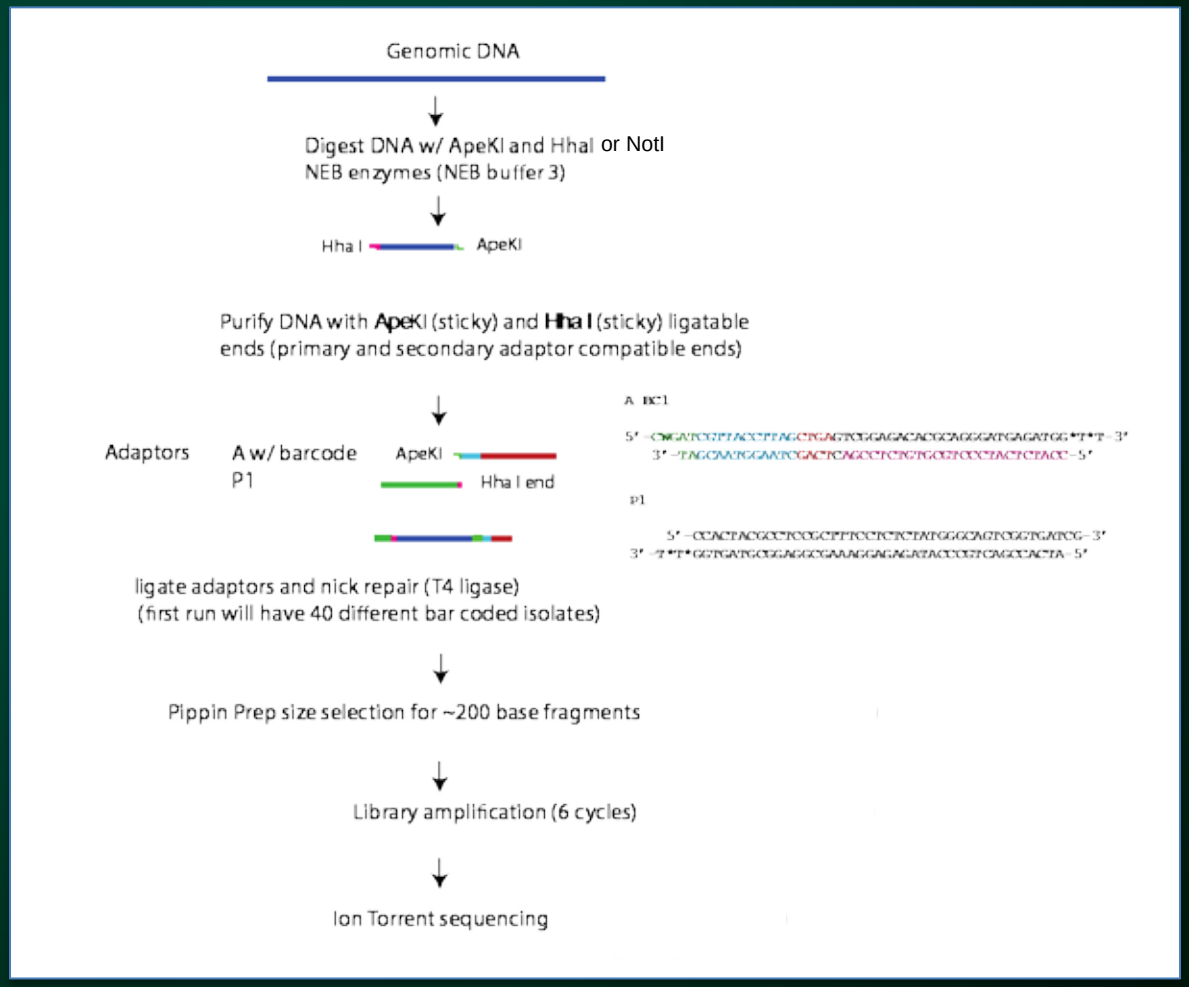
- Small footprint

  Takes up a very limited amount of space for the amount of sequencing data it is capable of generating



Adapting Ion Torrent Sequencing technology for GBS

- Data analysis pipeline can be simplified



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Data analysis is relatively

90 minute run generates
6-10 million 200 or 400 bp
reads (up to 4 billion bases/run).
Sequences come of the server
sequestered by barcodes into
separate files organized by
individual isolate or line.

DNASTAR Lasergene® 10.1

5 minutes to set up sequence assembly
and ~4-7 hours to run assembly with
~ 5-10 sequences from each line assembling
Into the unique SNP contigs.

Bam output files converted to excel files
ready to be analyzed by mapping software

Development of RAD GBS utilizing Ion Torrent Sequencing Technology

- *Pyrenophora teres* f. *teres* bi parental population (~40 Mb genome) 14,143 unique RAD loci, 843 quality SNPs
- *Septoria musiva* natural population of 20 diverse isolates (~30 Mb genome) 10,418 unique RAD loci, ~20,000 quality SNPs
- Barley bi-parental population, Harrington x Sw645 (5 Gb genome) 18,243 unique RAD loci, ~ 900 quality SNPs

The goal of our research

- Develop user friendly GBS technology for barley and its fungal pathogens

Next generation genotyping will be a game changer for genotyping but there is still the the requirement of accurate phenotyping.

Conclusions

- Next generation sequencing will break the genotyping bottleneck
- Facilitate identification, characterization and deployment of genetic resistant
- This GBS technology only requires routine bioinformatics so we believe that it will not be a limiting factor

Continue the pursuit of high yielding, high quality barley varieties with FHB resistance

A bad day fishing is better than a good day genotyping or spraying

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