GBS Usage Cases: Examples from Maize

Jeff Glaubitz (jcg233@cornell.edu)
Senior Research Associate, Buckler Lab, Cornell University
Panzea Project Manager

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Some potential applications of GBS Data

- Marker discovery
- Phylogeny/Kinship
- Linkage mapping of QTL in a biparental cross
- Fine-mapping QTL
- Genomic selection
- Genome Wide Association Studies (GWAS)
- NAM-GWAS
- Improving reference genome assembly
Marker Discovery

• GBS markers can be converted to SNPs or PCR assays of indels
• Develop SNP assays from polymorphic tags at same location
• Develop PCR primers from adjacent tags & hope for large indels

*ApeKI site (GCWGC)*

Sample1

Sample2

(→) 64-base sequence tag

< 450 bp

Loss of cut site
Phylogeny/Kinship

• Missing data not an issue for estimating pairwise genetic distance or kinship
  ▪ Each pair of individuals has large, “random” sample of markers in common

•Works really well even in non-model organisms
  ▪ Fei Lu’s previous talk on switchgrass

• Principle Coordinates Analysis better than Principle Components Analysis
  ▪ Uses distance matrix rather than every genotype
  ▪ Missing data not an issue for Prin. Coord. Analysis

• SNPs can be strongly affected by ascertainment bias
  ▪ Panel used to discover the SNPs can severely distort estimates of population genetic parameters (e.g., kinship, diversity)
  ▪ Industry SNPs on the Maize 55K SNP chip an extreme example
Less ascertainment bias than array-based SNPs?

Ram Sharma

Non stiff stalk, nss (106)
Stiff stalk, ss (28)
Tropical/sub tropical (66)
Popcorn (9)
Sweet corn (6)
Unclassified (67)
B73 (ss)
Mo17 (nss)
Less ascertainment bias than array-based SNPs?

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Linkage mapping of QTL in a biparental cross

• In maize, we use the reference genome to order markers
• With ApeKI, too many markers for traditional software (MapMaker, JoinMap, R-QTL etc.)
• Filter for a smaller set of markers with high coverage
• Use 6 base cutter (or combination of enzymes) for fewer markers with higher coverage
• JoinMap can handle at least 3,000 markers
• Newer software?
  ▪ MSTMap claims 10,000 – 100,000 markers
  ▪ Guided Evolutionary Strategy (Mester et al. 2004, Comp Biol Chem 28: 281) – currently being implemented in TASSEL
  ▪ Others?
HMM for calling hets/correcting errors in biparental RIL populations

\[ P(G \mid S) = \frac{P(S \mid G)P(G)}{P(S)} \propto P(S \mid G)P(G) \]

distance between nodes = \( \log[P(\text{obs}_i \mid g_i)P(g_i \mid g_{i-1})] \)
HMM for calling hets/correcting errors in biparental RIL populations

Low % het RIL

Med % het RIL

High % het RIL

Physical Position on Chr1 (bp)
GBS analysis of Teosinte/W22 BC$_2$S$_3$

• 868 RILs in total
• Framework map with 492 SNPs
• 51,238 bi-allelic GBS markers (ApeKI)

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TeoW22 BC2S3 – Chr9 – 4122 bi-allelic GBS markers
Reproducibility – 60 RILs – Chr9
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Parallel domestication of the *Shattering1* genes in cereals

Zhongwei Lin¹, Xianran Li¹, Laura M Shannon², Cheng-Ting Yeh³,⁴, Ming L Wang⁵, Guihua Bai¹,⁶, Zhao Peng⁷, Jiarui Li⁷, Harold N Trick⁷, Thomas E Clemente⁸, John Doebley², Patrick S Schnable³,⁴, Mitchell R Tuinstra⁹, Tesfaye T Tesso¹, Frank White⁷ & Jianming Yu¹

Maize *Sh1* orthologs are located at seed shattering QTLs
Fine mapping QTL

- Need to saturate interval containing QTL with markers
- GBS a good source of markers
- Also need to collect recombinants in the interval
- Near-isogenic lines (NILs) helpful (Mendelize)
- Good reference genome
Fine Mapping of Domestication QTL in Maize

7,756 GBS SNPs along Chr?

Trait Y

136 RC-NILS

W22  Teo  Het  Trait Z
Genomic Selection & GWAS

• Genomic Selection: very basic imputation approaches adequate?
  
  ▪ *e.g.*, code non-missing genotypes as 0, 0.5, 1 & missing data as the mean genotypic value
Accurate genomic prediction of height based on GBS data

2800 diverse maize breeding lines

![Graph showing observed vs predicted height with regression line R²=0.82]

USDA Ames Inbreds
Ridge-Regression BLUP

R²= 0.82

Jason Peiffer
Genomic Selection & GWAS

• Genomic Selection: very basic imputation approaches adequate?
  ▪ e.g., code non-missing genotypes as 0, 0.5, 1 & missing data as the mean genotypic value

• Missing data are more problematic for GWAS
  ▪ erroneous imputation might cause spurious results
  ▪ avoid false imputation of biologically missing regions
  ▪ area of active research
GWAS directly hits known Mendelian traits

GWAS of white vs. yellow kernels in 1,595 USDA Ames inbreds

The best hit for kernel color lies within Y1

Romay et al. (2013) Genome Biology 14: R55
GWAS of a more complex trait directly hits known flowering time genes

Even with ~660k SNPs we almost missed ZmCCT (only 1 significant SNP)

Romay et al. (2013) Genome Biology 14: R55
GWAS of a more complex trait directly hits known flowering time genes

GWAS of growing degree days to silking in 2,279 inbreds

Putative *CCT* (0.2 Mb)
*d8* (~1 Mb)
*PhyA1* (~1 Mb)

15th largest NAM QTL

5th largest NAM QTL
*ZmRap2.7* (2.6 Kb)
*ZCN8* (5 Kb)

Largest NAM QTL
*ZmCCT* (2.4 Kb)

27th largest NAM QTL
*DLF1* (2.3 Mb)
Putative *Frigida* (0.4 Mb)

Lipka *et al.* (2013) Bioinformatics 28: 2397-2399
Genomic Selection & GWAS

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• Missing data are more problematic for GWAS
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• In NAM-GWAS, imputation is much less of an issue
  ▪ NAM = “Nested Association Mapping” population
The maize NAM population was built for NAM-GWAS
liguleless1 and liguleless2 explain the two “biggest” leaf angle QTL.

Tian, Bradbury, et al 2011 Nature Genetics
We are using GBS to pinpoint the location of cross overs in the NAM RILs

- B73 is the reference genome: complete knowledge
- Remaining NAM parents whole genome sequenced via Illumina at 4x coverage (paired end random sheared)
  - 26 million high quality SNPs
- Precise knowledge of crossover locations in NAM RILs allows us to more accurately project sequences of parents onto RILs:
GBS data improves the resolution of joint linkage analysis in the NAM population.

Trait: Days to silk (flowering time)

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<th>Markers</th>
<th>Median QTL support interval</th>
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<td>1,106 array SNPs</td>
<td>6.2 cM</td>
</tr>
<tr>
<td>171,479 GBS SNPs</td>
<td>2.6 cM</td>
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Preliminary NAM-GWAS results also show improved resolution.
Recombination Rates for NAM from GBS Data

Peter Bradbury – USDA Scientist, Buckler lab, Cornell (unpublished)
The maize B73 reference genome: room for improvement?

1) The B73 reference genome accurate for B73 but less so for other maize lines (e.g., Mo17)

2) Even for B73, some regions of the genome are in the wrong place

3) Some large (multiple BAC) contigs could not be anchored
   - assigned to “chromosome 0”
   - 30 chr0 contigs in B73 RefGenV1
   - 17 chr0 contigs in B73 RefGenV2

4) Some regions of the genome are missing
   - ≈5% of B73 sequence is not in the B73 reference genome
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Most tags can be mapped as individual alleles

- In a biparental cross such as maize IBM (B73 x Mo17)
- Provided that they are polymorphic between the parents
Genetically mapping individual GBS alleles in IBM

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B73 reference genome highly accurate for B73...

- 0.4% of B73 tags genetically map to different chromosome than they align to
B73 reference genome highly accurate for B73...

• 0.4% of B73 tags genetically map to different chromosome than they align to

...but far less so for other maize lines

• 9.3% of Mo17 tags genetically map to different chromosome than they align to
Only 50% of the maize genome is shared between two varieties.

Fu & Dooner 2002, Morgante et al. 2005, Brunner et al 2005
Numerous PAVs and CNVs - Springer, Lai, Schnable in 2010
Some chunks of the B73 reference genome are in the wrong place

<table>
<thead>
<tr>
<th>Physical Chr</th>
<th>Start (Mb)</th>
<th>End (Mb)</th>
<th>Genetic Chr</th>
<th>Approx. Genetic Location (Mb)</th>
<th># Tags</th>
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<tbody>
<tr>
<td>10</td>
<td>139.3</td>
<td>139.8</td>
<td>2</td>
<td>16.5–16.8</td>
<td>49</td>
</tr>
<tr>
<td>9</td>
<td>102.5</td>
<td>106.9</td>
<td>9</td>
<td>15–32</td>
<td>49</td>
</tr>
<tr>
<td>7</td>
<td>150.1</td>
<td>161.8</td>
<td>5</td>
<td>192–214</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>0.4</td>
<td>4</td>
<td>83–151</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>48.4</td>
<td>50</td>
<td>2</td>
<td>61–127</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>0.07</td>
<td>0.2</td>
<td>7</td>
<td>47–100</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>231.2</td>
<td>231.2</td>
<td>7</td>
<td>18–26</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>228.1</td>
<td>230.5</td>
<td>5</td>
<td>194–212</td>
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