Using NGS-enabled genetics to improve marker selection and design in hexaploid wheat (Yr15)

Ricardo H. Ramírez-González
Wheat Yellow Rust

- *Puccinia striiformis.*
- Fungus.
- Traditionally controlled by resistance genes (for example, Yr15).
- Yr15 locus is an introgression from *T. dicocoides*
The Genome Analysis Centre

**Progenitors**

- Yr15
- Avs

**F2 population**

- 4 lanes Illumina HiSeq 2000
- RNA-Seq 100 bp, read pair

**Sequencing**

- Library No. of reads
  - Lane 1: 4x10^6
  - Lane 2: 3x10^6
  - Lane 3: 2x10^6
  - Lane 4: 3x10^6

**Bioinformatics**

- BWA-0.5.9
- bioruby-1.4.3
- bioruby-samtools 0.6.1
- blat-0.3.4
- exonerate-2.2.0
- MAFFT-7.055
- primer3-2.3.4

**SNP Markers**

- KASP Assays
- Genetic map
- Validation by breeders

**Genotyping**

- Homoeologous in IWGSC scaffolds
- Genome specific primer

**d) Sequence Alignments**

- NCBI Unigenes v60
  - 56,954 genes
- Krasileva’s gene models
  - 94,177 genes

**Bulk Frequency Ratios**

- SNPs between progenitors
- Ratio of alleles between bulks

**e) SNP Markers**

- Genetic map
- Validation by breeders

**Resistant**

- R1: 70 ind.
- R2: 67 ind.
- R3: 50 ind.

**Susceptible**

- S1: 15 ind.
- S2: 17 ind.
- S3: 13 ind.
a) Progenitors

Yr15  Avs

b) F2 population

Resistant  Susceptible
R1: 70 ind.  S1: 15 ind.
R2: 67 ind.  S2: 17 ind.
R3: 50 ind.  S3: 13 ind.

c) Sequencing

4 lanes Illumina HiSeq 2000
100 bp, read pair

No. of reads
Lib 1 2 3 4

No. of reads
1x10^8 2x10^8 3x10^8 4x10^8

d) Sequence Alignments

- NCBI Unigenes v60
  56,954 genes
- Krasileva’s gene models
  94,177 genes

Bulk Frequency Ratios

- SNPs between
  progenitors
- Ratio of alleles between
  bulks

- Homoeologous in
  IWGSC scaffolds
- Genome specific
  primer

e) SNP Markers

KASP Assays
Genetic map
Validation by breeders

- Primer design
  blat-0.5.4
  exonerate-2.2.0
  MAFFF-7.055
  primer3-2.3.4
Parental Plants

Avocet+Yr15  *  Avocet

*Isogenic line developed by the University of Sydney
The Genome Analysis Centre

**a)** Progenitors

- Yr15
- Avs

**b)** F₂ population

- Resistant:
  - R1: 70 ind.
  - R2: 67 ind.
  - R3: 50 ind.
- Susceptible:
  - S1: 15 ind.
  - S2: 17 ind.
  - S3: 13 ind.

**c)** Sequencing

- 4 lanes Illumina HiSeq 2000
- RNA-Seq
- 100 bp, read pair

<table>
<thead>
<tr>
<th>Library</th>
<th>No. of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3x10⁸</td>
</tr>
<tr>
<td>2</td>
<td>2x10⁸</td>
</tr>
<tr>
<td>3</td>
<td>1x10⁸</td>
</tr>
<tr>
<td>4</td>
<td>4x10⁹</td>
</tr>
</tbody>
</table>

**d)** Sequence Alignments

- NCBI Unigenes v60
  - 56,954 genes
- Krasileva’s gene models
  - 94,177 genes

- SNPs between progenitors
- Ratio of alleles between bulks

**e)** SNP Markers

- KASP Assays
- Genetic map
- Validation by breeders

**f)** Bioinformatics

- Primer design
  - Homoeologous in IWGSC scaffolds
  - Genome specific primer

**g)** Genotyping

- SNP Markers
  - KASP Assays
  - Genetic map
  - Validation by breeders

- Sequence Alignments
  - NCBI Unigenes v60
    - 56,954 genes
  - Krasileva’s gene models
    - 94,177 genes

- SNPs between progenitors
  - Ratio of alleles between bulks

- Primer design
  - Homoeologous in IWGSC scaffolds
  - Genome specific primer

- Resistant
  - R1: 70 ind.
  - R2: 67 ind.
  - R3: 50 ind.

- Susceptible
  - S1: 15 ind.
  - S2: 17 ind.
  - S3: 13 ind.
**F₂ population**

**Avocet 'S' + Yr15**

- **Resistant**
  - R1: 70 ind.
  - R2: 67 ind.
  - R3: 50 ind.

- **Susceptible**
  - S1: 15 ind.
  - S2: 17 ind.
  - S3: 13 ind.

**Expected segregation:** 3 resistant : 1 susceptible

χ² P = 0.049; 187 resistant and 45 susceptible F₂ plants
The Genome Analysis Centre

Progenitors

a) Yr15 Avs

F2 population

b) Resistant Susceptible
   R1: 70 ind. S1: 15 ind.
   R2: 67 ind. S2: 17 ind.
   R3: 50 ind. S3: 13 ind.

Sequencing

c) 4 lanes Illumina HiSeq 2000
   RNA-Seq
   100 bp, read pair

No. of reads
   4x10^6 3x10^6 2x10^6 1x10^6
   Lane 1 2 3 4

Genotyping

e) SNP Markers
   KASP Assays
   Genetic map
   Validation by breeders

d) Sequence Alignments
   - NCBI Unigene v60 56,954 genes
   - Krasileva’s gene models 94,177 genes

   Bulk Frequency Ratios
   - SNPs between progenitors bioruby-1.4.3
   - Ratio of alleles between bulks bioruby-samtools 0.6.1

   Primer design
   - Homoeologous in blat-0.3.4
   - IWGSC scaffolds exonerate-2.2.0
   - Genome specific MAFFT-7.055
   - Primer primer3-2.3.4

Resistance

b) R1: 70 ind. S1: 15 ind.
   R2: 67 ind. S2: 17 ind.
   R3: 50 ind. S3: 13 ind.

The Genome Analysis Centre
The Genome Analysis Centre

Transcriptome size

Wheat genome:
- ~17 Gbp
- Hexaploid AABBDD
- Coverage: ~2X

Wheat transcriptome:
- ~76 Mnt
- Prone to gene expression bias
- Coverage: ~440x

Coverage per Illumina HiSeq 2000 lane per manufacturer specification
RNA-Seq

Genomic DNA

Exon  Intron  Exon  Intron  Exon  Intron

mRNA
RNA-Seq

4 lanes Illumina HiSeq 2000
100 bp, read pair

<table>
<thead>
<tr>
<th>Library</th>
<th>Lane</th>
<th>No. of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>1</td>
<td>1x10^8</td>
</tr>
<tr>
<td>R2</td>
<td>2</td>
<td>2x10^8</td>
</tr>
<tr>
<td>S3</td>
<td>3</td>
<td>3x10^8</td>
</tr>
<tr>
<td>R3</td>
<td>4</td>
<td>4x10^8</td>
</tr>
<tr>
<td>S1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVS</td>
<td></td>
<td>4x10^8</td>
</tr>
<tr>
<td>Yr15</td>
<td></td>
<td>3x10^8</td>
</tr>
</tbody>
</table>
a) Yr15, Avs

b) F₂ population

- Resistant
  - R1: 70 ind.
  - R2: 67 ind.
  - R3: 50 ind.
- Susceptible
  - S1: 15 ind.
  - S2: 17 ind.
  - S3: 13 ind.

b) SNP Markers
- KASP Assays
- Genetic map
- Validation by breeders

c) Sequence Alignments
- NCBI Unigenes v60
  - 56,954 genes
- Krasileva’s gene models
  - 94,177 genes

- Primer design
  - Homoeologous in IWGSC scaffolds
  - Genome specific primer

- BWA-0.5.9
- biorep-1.4.3
- biorep-samtools 0.6.1
- blat-0.3.4
- exonerate-2.2.0
- MAFFT-7.055
- primer3-2.3.4

d) Sequence Alignments
- SNPs between progenitors
- Ratio of alleles between bulks

- Homoeologous in IWGSC scaffolds
- Genome specific primer
Gene coverage per sample

AVS

Yr15

UCW

NCBI UniGene
a) Progenitors

b) F2 population

F1 population

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers

SNP Markers
Bulk Frequency Ratios

Genomic Sequence

Avocet S
- 1AS: c t G t g G g a
- 1BS: c t T t g G g a
- 1DS: c t G t g G g a

Avocet S + Yr15
- 1AS: c t G t g G g a
- 1BS: c t T t g A g a
- 1DS: c t G t g G g a

✓ ✗
### Bulk Frequency Ratios

#### Genomic Sequence

<table>
<thead>
<tr>
<th>Genomic Region</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocet S</td>
<td>c t G t g G g a</td>
</tr>
<tr>
<td>Avocet S + Yr15</td>
<td>c t G t g G g a</td>
</tr>
</tbody>
</table>

#### Consensus from parental

- **Homoeologous**
  - 1AS: c t K t g G g a
  - 1BS: c t K t g A g a
  - 1DS: c t G t g G g a

- **Allelic**
  - 1AS: c t K t g G g a
  - 1BS: c t K t g R g a
  - 1DS: c t G t g G g a
Bulk Frequency Ratios

Genomic Sequence

Avocet S
1AS: c t G t g G g a
1BS: c t T t g G g a
1DS: c t G t g G g a

Avocet S + Yr15
1AS: c t G t g G g a
1BS: c t T t g A g a
1DS: c t G t g G g a

Consensus from parental

c t K t g

Homoeologous Allelic

c t K t g

Susceptible bulk

Position Reference 181 184

c t G t g G g a
. . . . . .
T . . . . .
T . . . . .
A . . . . .

SNP Index 184A:

184:

\[ \frac{1}{8} = 0.125 \]

Resistant Bulk

Position Reference 181 184

c t G t g G g a
. . . . . .
T . . . . .
T . . . . .
T . . . . .

6:

\[ \frac{6}{8} = 0.75 \]

Bulk Frequency Ratio:

\[ \frac{0.75}{0.125} = 6 \]
BFRs near the 1B centromere

BFRs of SNPs mapping to chromosome 1B
Selection criteria

- **Origin (Yr15)**

- **Short arm Chromosome group 1**
  - 1A: 161 cM
  - 1B: 174 cM
  - 1D: 209 cM

- **BFR > 6**
Candidate selection

UCW gene models

From *Yr15* (11,230)

Putative genes with SNP: 16,022 (17.01%)
UCW gene models

Group 1S (554)

169
385
10,845

From Yr15 (11,230)

Putative genes with SNP: 16,022 (17.01%)
Candidate selection

UCW gene models

Group 1S (554)

147

287

98

22

10,364

481

42

From Yr15 (11,230)

BFR > 6 (643)

Putative genes with SNP: 16,022 (17.01%)
The Genome Analysis Centre

**a)** Progenitors

<table>
<thead>
<tr>
<th>Progenitors</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avs</td>
<td></td>
</tr>
<tr>
<td>Yr15</td>
<td></td>
</tr>
</tbody>
</table>

**b)** F₂ population

<table>
<thead>
<tr>
<th>Population</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>70</td>
<td>67</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

**c)** Sequencing

<table>
<thead>
<tr>
<th>Library Lane</th>
<th>No. of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4x10⁸</td>
</tr>
<tr>
<td>2</td>
<td>3x10⁸</td>
</tr>
<tr>
<td>3</td>
<td>2x10⁸</td>
</tr>
<tr>
<td>4</td>
<td>1x10⁸</td>
</tr>
</tbody>
</table>

**d)** Sequence Alignments

- NCBI Unigene v60: 56,954 genes
- Krasileva's gene models: 94,117 genes

**Bulk Frequency Ratios**

- SNPs between progenitors
- Ratio of alleles between bulks

**e)** SNP Markers

- KASP Assays
- Genetic map
- Validation by breeders

**Bioinformatics**

- Primer design
  - Homoeologous in IWGSC scaffolds: blat-0.3.4
  - Genome specific: exonerate-2.2.0
  - Primer
    - primer3-2.3.4
Target SNP in 1B
SNP-1 A  cgcat\texttt{t}tg\texttt{c}gc\texttt{y}gc\texttt{a}t\texttt{a}c\texttt{c}\texttt{g}gc\texttt{c}ct\texttt{K}tgGgaat\texttt{a}tt\texttt{t}\texttt{t}\texttt{g}\texttt{cag}\texttt{c}\texttt{g}\texttt{a}agg\texttt{g}ctg

SNP-1 B  cgcat\texttt{t}tg\texttt{A}gc\texttt{g}gc\texttt{y}gc\texttt{a}t\texttt{a}c\texttt{c}\texttt{g}gc\texttt{c}ct\texttt{K}tgAgaat\texttt{a}tt\texttt{t}\texttt{t}\texttt{g}\texttt{cag}\texttt{c}\texttt{g}\texttt{a}agg\texttt{g}ctg
SNP-1 A  cgcat\texttt{tG}gcgc\texttt{Y}gcgataccggcgcc\texttt{K}tgGgaatatttgcagcgaaggcgtg
SNP-1 B  cgcat\texttt{tA}gcgc\texttt{Y}gcgataccggcgcc\texttt{A}tgAgaatat\texttt{t}ttgcagcgaaggcgtg
IWGSC-1A cgcat\texttt{tG}gcgcgcgcgcgcgcgcgcctGtgGgaatatttgcagcgaaggcgtg
IWGSC-1B cgcat\texttt{tA}gcgcgcgcgcgcgcgcgcctTtgGgaatat\texttt{t}ttgc---gaagggcgtg
IWGSC-1D cgcat\texttt{tA}gcgcTgcgataccggcgccTtgGgaatatttgcagcgaaggcgtg
SNP-1 A  cgcat\textit{t}GcgYggtaccggcctKtgGaatatttcagcgaagggcgtg
SNP-1 B  cgcat\textit{t}A cgYgtaccggcctKtgAaatatttcagcgaagggcgtg
IWGSC-1A gc\textit{c}atttGcgYgtacccgcccGaatatttcagcgaagggcgtg
IWGSC-1B gc\textit{c}atttA gcYgtacccgcccGaatattttgc---gaagggcgtg
IWGSC-1D ca\textit{t}tttGcgTgtaccggcctGaatattttgcagcgaagggcgtg

&

SNP

non-homoeologous
PolyMarker: Candidate SNP

SNP-1 A  cgcatttGcggcYgczgataccg czgcctKtgGgaatatgnt gcagc gaaggcgtg
SNP-1 B  cgcatttAcgcgygcgataccg czgcctKtgAgaatatttg cagcgaaggcgtg
IWGSC-1A  cgcatttGcggcgcgcgataccg czgcctGtgGgaatatattgcagc gaaggcgtg
IWGSC-1B  cgcatttAcgcgcgcgataccg czgcctTtgGgaatatgattgc---gagggcgtg
IWGSC-1D  c--atttgGgcgcTgcgataccg czgcctGtgGgaatatttg cagcgaaggcgtg

SNP homoeologous
PolyMarker: Genome Semi-Specific

SNP-1 A  cgcat\texttt{t}tGgcgcGgcataccggcgcctKtg\texttt{G}gaatattgcagcgaaggcgtg

SNP-1 B  cgcat\texttt{t}tAgcgcGgcataccggcgcctKtgAgaatattgcagcgaaggcgtg

IWGSC-1A  cgcat\texttt{t}tGgcgcGgcataccggcgcctGtgGgaatattgcagcgaaggcgtg

IWGSC-1B  cgcat\texttt{t}tAgcgcGgcataccggcgcctTtgGgaatattgc\texttt{A}---gaaggcgtg

IWGSC-1D  c\texttt{A}--atttGgcgcGgcataccggcgcctGtgGgaatattgcagcgaaggcgtg

semi-specific
PolyMarker: Genome specific

SNP-1 A  cgcat\text{ttG}cgYgcgataccggcgcctKtgG\text{gaatat}tg\text{tgcagcgaaggcgtg}
SNP-1 B  cgcat\text{ttA}cgYgcgataccggcgcctKtgA\text{gaatatttgcagcagggcgtg}
IWGSC-1A cgcat\text{ttG}cgcgcgcgcgcgcgtcKtgG\text{gaatat}ttg\text{cagcagggcgtg}
IWGSC-1B cgcat\text{ttA}gcgcgcgcgcgcgcctTtgG\text{gaatat}ttgc---\text{cagcagggcgtg}
IWGSC-1D  c--\text{ttG}gcgcTgcgcgcgcgcgcctTtgG\text{gaatat}ttgcagcagggcgtg

-------:-----c-------------T--&----------------------

specific
PolyMarker: Selected primer

Tested with Primer3

SNP-1 A  cgctttGcgctYgcgcctKtgGgaatatttcagccagggctg
SNP-1 B  cgctttAgcgcYgcgcctKtgAgatatttgcagggctg
IWGSC-1A  cgctttGcgcgcgcctKtgGgaatatttcagccagggctg
IWGSC-1B  cgctttAgcgcYgcgcctKtgGgaatatttcagccagggctg
IWGSC-1D  cgctttGcgcTgcgcctKtgGgaatatttcagccagggctg

Request primers

PolyMarker is an automated bioinformatics pipeline for SNP assay development which increases the probability of generating homoeologue-specific assays for polyploid wheat. PolyMarker generates a multiple alignment between the target SNP sequence and the IWGSC chromosome survey sequences \cite{IWGSC2014} for each of the three wheat genomes. It then generates a mask with informative positions which are highlighted with respect to the target genome.

See \url{About} to know how to prepare your input.

We have \url{designed primers} for the iSelect 90K chip.

Upload File:  

Email:  

Upload
The Genome Analysis Centre

Progenitors

- Yr15
- Avs

F2 population

- Resistant
- Susceptible
- R1: 70 ind.
- R2: 67 ind.
- R3: 50 ind.
- S1: 15 ind.
- S2: 17 ind.
- S3: 13 ind.

Sequencing

- 4 lanes Illumina HiSeq 2000 RNA-Seq
- 100 bp, read pair
- Library No. of reads
- Lane

Bioinformatics

- BLAST v.0.8.5
- Bioruby-1.4.3
- BioRuby-Samtools 0.6.1
- Blat-0.3.4
- Exonerate-2.2.0
- Mafft-7.055
- Primer3-2.3.4

Genotyping

- SNP Markers
- KASP Assays
- Genetic map
- Validation by breeders

- SNPs between progenitors
- Ratio of alleles between bulks

- Homoeologous in IWGSC scaffolds
- Genome specific primer

SNP Markers

- NCBI Unigenes v68 56,954 genes
- Krasileva’s gene models 94,177 genes

Sequence Alignments

- BWA-0.5.9
- BioRuby-1.4.3
- BioRuby-Samtools 0.6.1

Bulk Frequency Ratios

- SNPs between progenitors
- Ratio of alleles between bulks

- Homoeologous in IWGSC scaffolds
- Genome specific primer

The Genome Analysis Centre
**Validation on breeding germplasm**

<table>
<thead>
<tr>
<th>SNP haplotype</th>
<th>Reaction to <em>P. striiformis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>R11</td>
<td>R5</td>
</tr>
<tr>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>T</td>
<td>G</td>
</tr>
</tbody>
</table>

Validation on 113 UK varieties
a) Progenitors

b) F_2 population

- Yr15
- Avs

R1: 70 ind.
R2: 67 ind.
R3: 50 ind.
S1: 15 ind.
S2: 17 ind.
S3: 13 ind.

SNP Markers
- KASP Assays
- Genetic map validation by breeders

c) Sequencing
- 4 lanes Illumina HiSeq 2000
- RNA-Seq
- 100 bp, read pair

Lane
- 1
- 2
- 3
- 4

No. of reads
- 4x10^8
- 3x10^8
- 2x10^8
- 1x10^8

Bulk Frequency Ratios
- SNPs between progenitors
- Ratio of alleles between bulks

d) Sequence Alignments
- NCBI Unigenes v60
- 56,954 genes
- Krasileva's gene models
- 94,177 genes

- BWA-0.5.9
- bioruby-1.4.3
- bioruby-samtools 0.6.1
- blat-0.3.4
- exonerate-2.2.0
- MAFFT-7.055
- primer3-2.3.4

- Homoeologous in IWGSC scaffolds
- Genome specific primer

PolyMarker

The Genome Analysis Centre
Acknowledgments

• TGAC
  – Mario Caccamo
  – Sarah Ayling
  – Paul Bailey
  – Jon Wright

• JIC
  – Cristobal Uauy
  – Nick Bird
  – Vanesa Segovia
  – Martin Trick

• Limagrain
  – Paul Fenwick
  – Simon Berry

• RAGT Seeds
  – Sarah Holdgate
  – Peter Jack

• University of Sidney
  – Robert McIntosh
Thank you for listening.