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Genotype-by-sequence in oat

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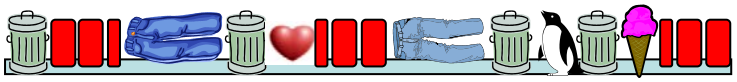
USDA

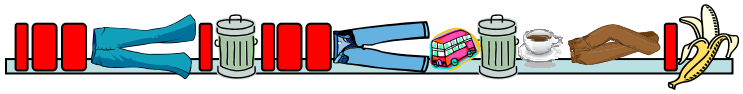
North American Millers' Association NAMA

Prairie Oat Growers Association

Agriculture and Agri-Food Canada

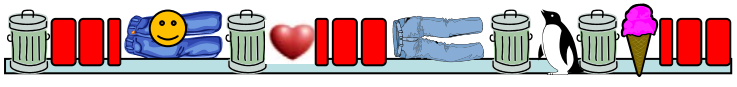
Imagine ….. the complete oat genome!

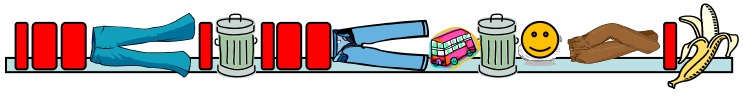


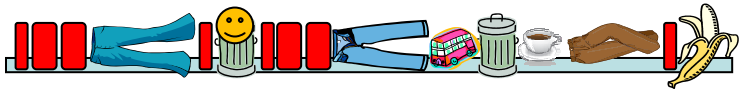


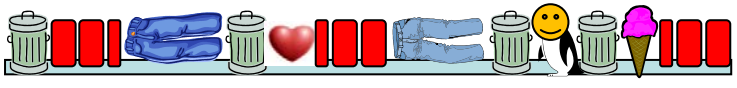
← (this genome stretches across China) →

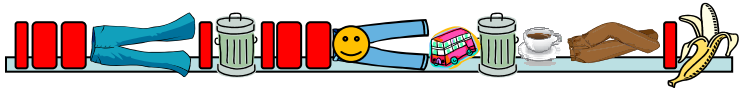
Imagine ….. every complete oat genome !







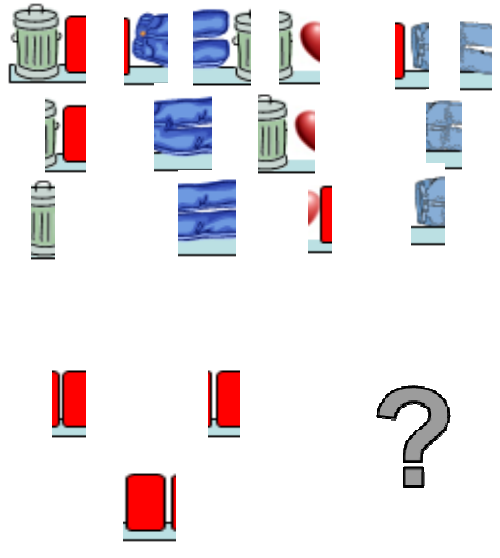




State-of-the-art DNA sequencing

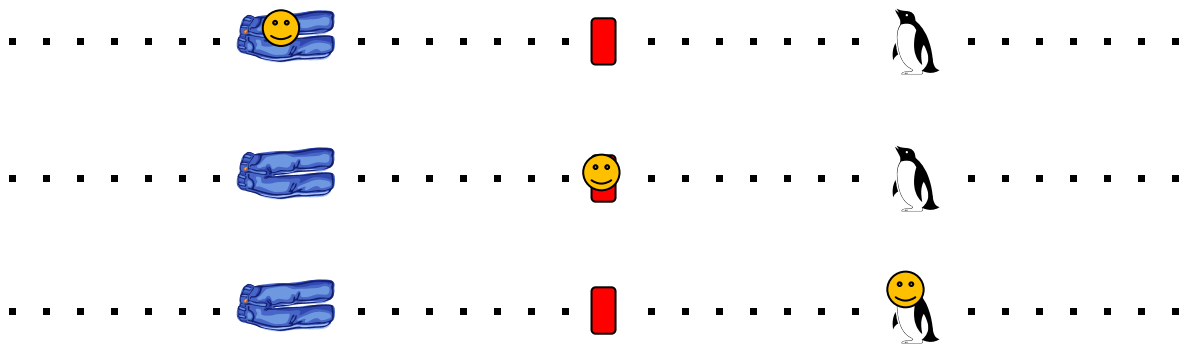
- Make 100's of copies
- Put them through a shredder
- Try to put them back together

e.g. 150x coverage
= 20 billion pieces
x 100 bp
= $100 K



Until then….

- Focus on differences among varieties
  - That's what we care about the most
- Order differences by linkage (count recombinations)
- Associate with phenotype (also by linkage)
  - Mapping populations (2-parents, lots of kids)
  - 'Natural' populations (unknown family structure)



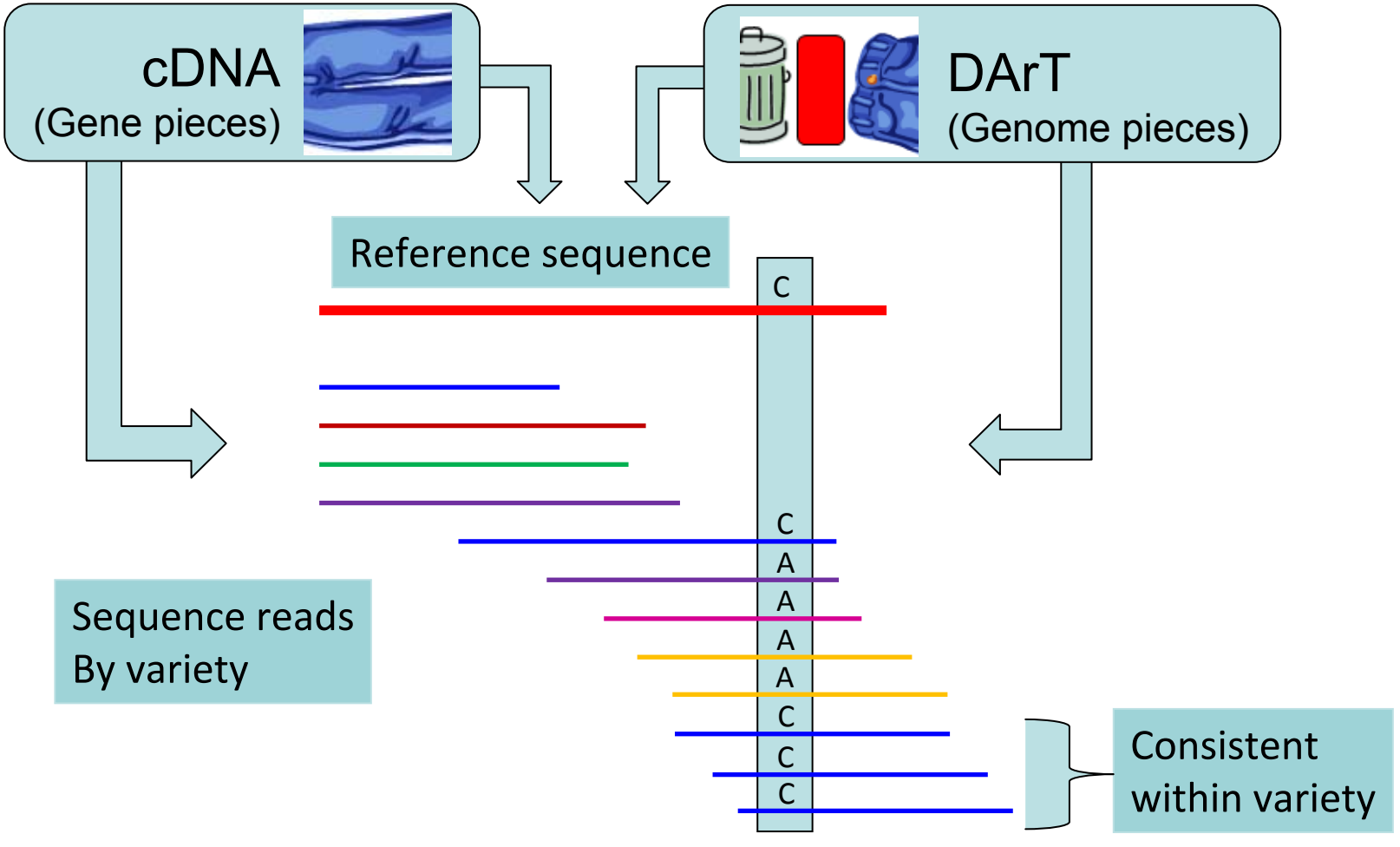
Single Nucleotide Polymorphism (SNP)

- The most common genetic difference

```
    GTACCATGATCGCTAACTGGCATGGCTTACGGCTTGAC
(A) .......T..........G....................
(B) .......G..........G....................
(C) .......G..........A....................
(D) .......T..........A....................
(E) .......G..........G....................
```

- SNP = SNP …. no matter how you find it !
  - "Old" non-sequence-based methods (AFLP, DArT)
  - Discover by sequence / assay by design
  - Discover and assay by sequencing (GBS)

SNP – discover by sequence, assay by design



CORE – Illumina SNP array

cDNA
(Gene pieces)

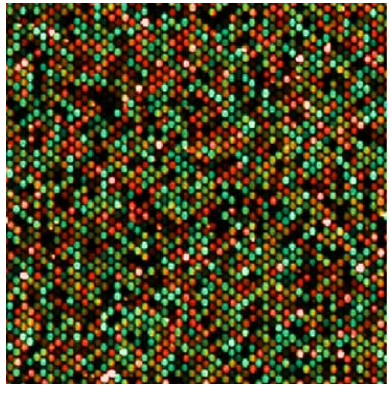
- 20 varieties
- 9 million reads
- 18,000 templates

DArT
(Genome pieces)

- 25 varieties
- 4 million reads
- 12,000 templates

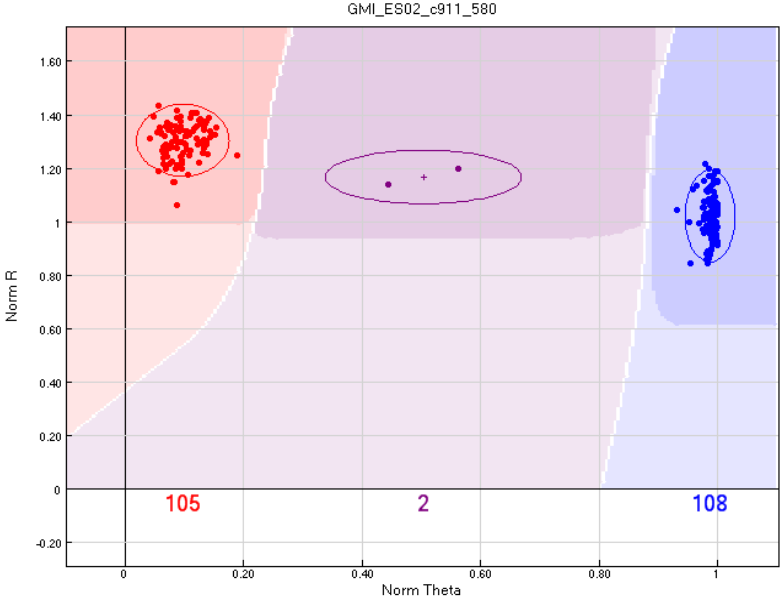
…**TGATCGCTA[G/T]CTGGCATGGCT**……

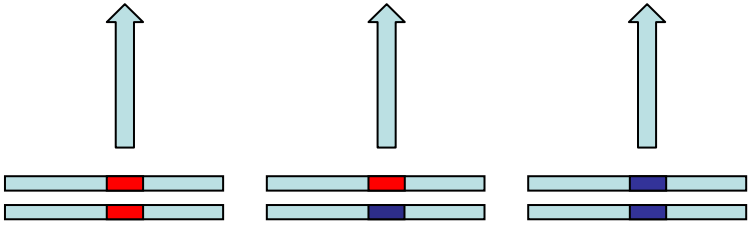
- 80,000 predicted SNPs
- 4600 tested SNPs
- 2300 validated SNPs (Golden Gate)

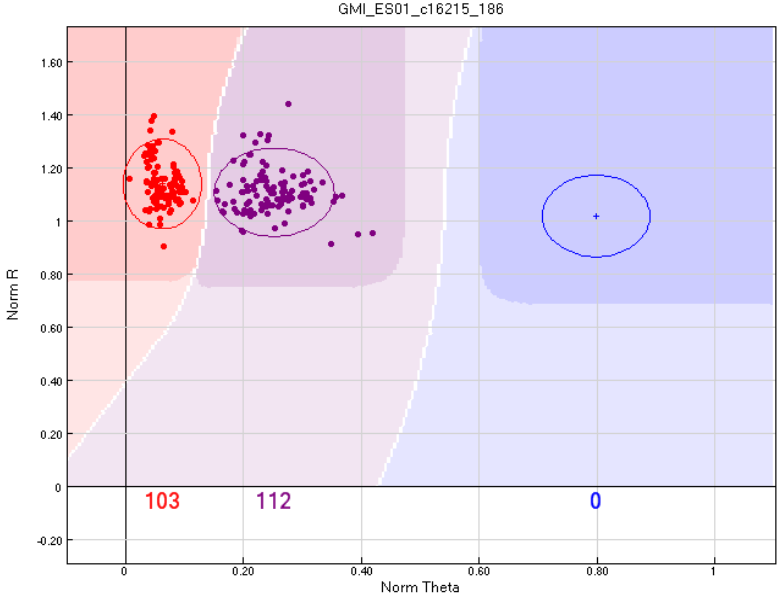


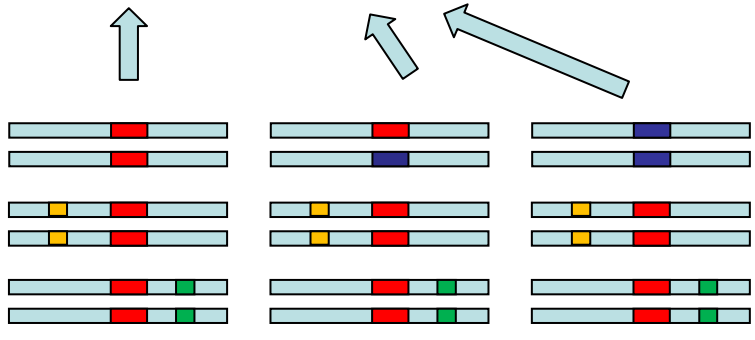
- 6000 SNPS in progress (Infinium)
- (we estimate 4000 will work)

Genome Studio Software (example SNPs)



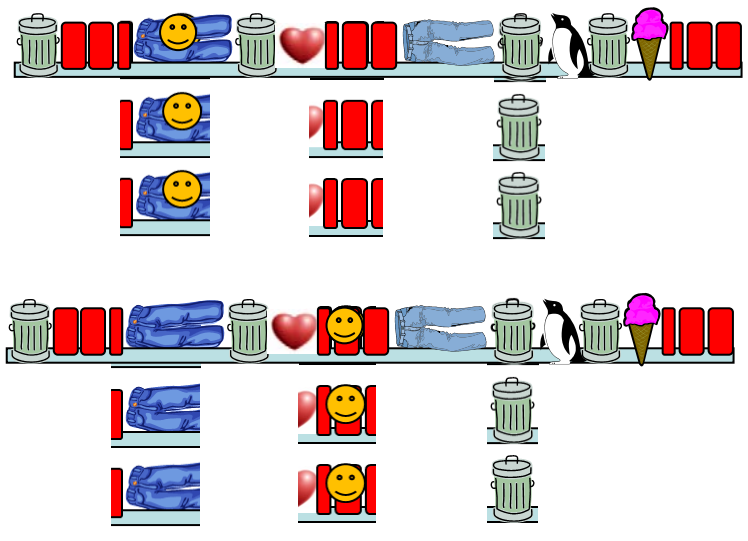






Genotype by sequence (GBS) - concept

- Discover and assay SNPs by direct sequencing
- Similar to SNP discovery for planned assay
  - But much larger numbers of and sequences
- Based on subset of genome (enzyme / amplify)



Variety A

Variety B

GBS details

OPEN ACCESS Freely available online

PLoS one

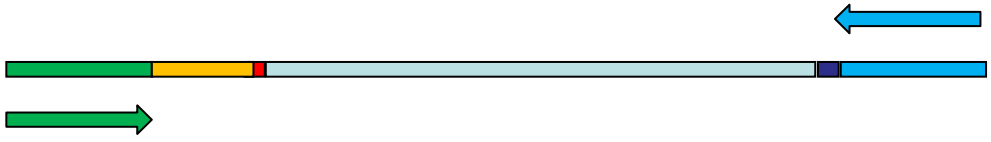
Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach

Jesse A. Poland[1,2*], Patrick J. Brown[3], Mark E. Sorrells[4], Jean-Luc Jannink[4,5]

1. Digest with *PstI* & *MspI*

2. Ligate sequencing adapters + variety-specific barcode

3. Mix together (multiplex), amplify



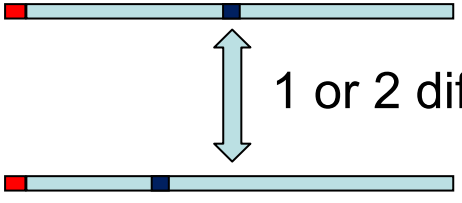
GBS - details

3 billion sequenced fragments

5. Trim barcode, trim to 64 bases, keep track of variety

6. Identify all unique tags, count in each variety (Tag x Taxa)

7. Match tag pairs, call SNPs (across full data set)

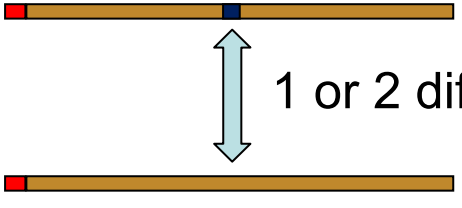


Varieties: A, C, E

Varieties: B, D, H

Is a SNP

100,000



Varieties: A, C, D, H

Varieties: B, C, D, E

Not a SNP

GBS - caveats

- Missing data 10% to 70%
  - Depends on sequencing depth (“plexity”)
  - Depends on how many SNPs you call
  - e.g. to get 95% complete, I could only call 2400 SNPs
- Bioinformatics is under development
  - Different methods give different SNP sets
- Massive storage and computing requirements
- Data sets too large for some software
  - More markers and more samples
  - Have not yet managed a consensus map

DArT *vs* SNP *vs* GBS

	DArT	SNP	GBS
Assay cost per sample	$50	$68	$20 *
Markers across all taxa	1500	4000 *	40 k → 100 k
Markers per population	300	800	4 k → 20 k
Missing data	>5 %	< 1%	10% → 50%
Co-dominant	0%	> 25%	100% *
Genes / orthology	20%	100%	5%
Duplicate loci (map inconsistently)	> 5%	< 3 %	?

Are they useful ?

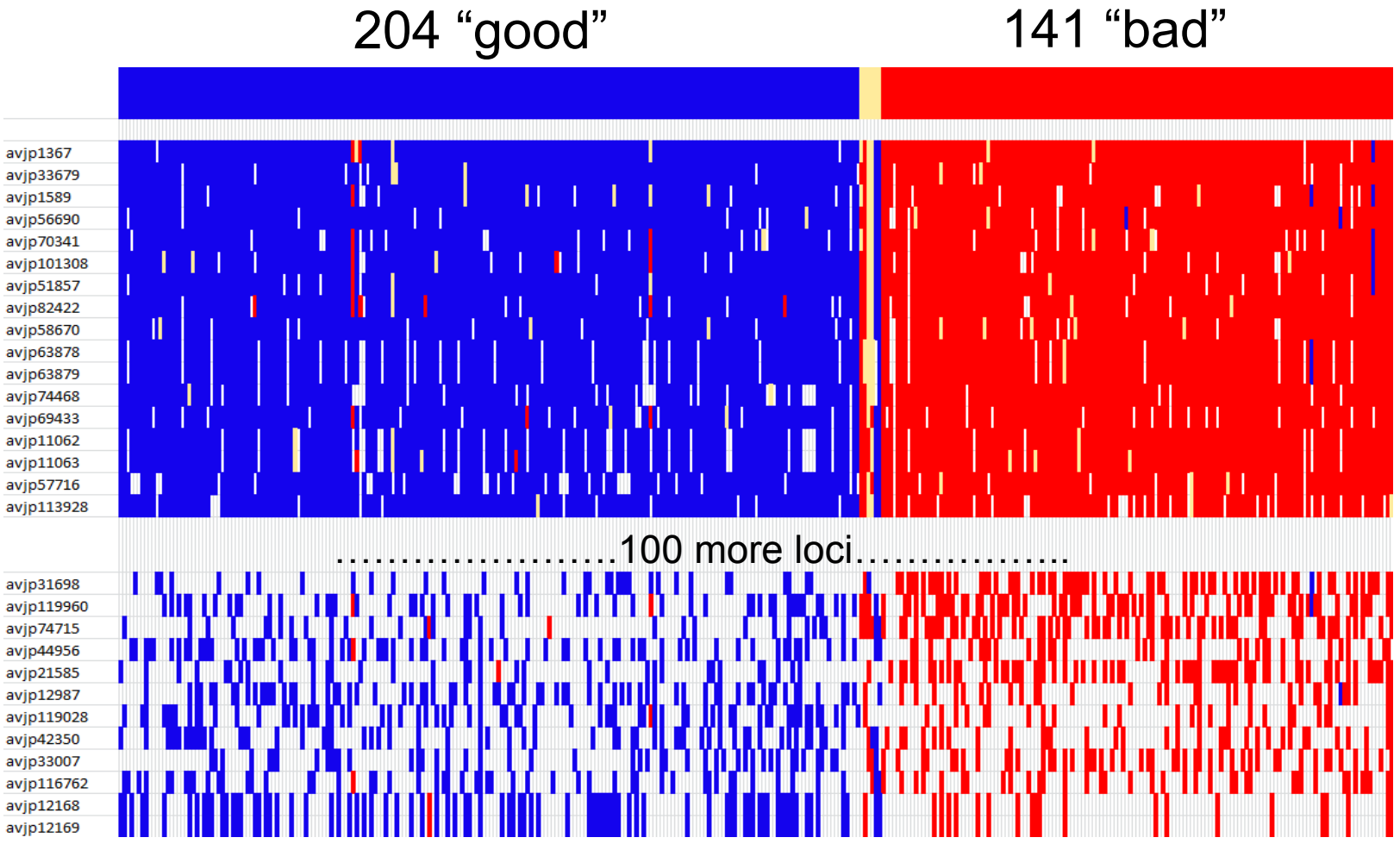
- Already have more GBS data than anything else:
  - Not just more loci..... more varieties too
  - DArT: 350 diversity + 4 bi-parental populations
  - SNP: 108 diversity + 6 bp-pop (400)
  - GBS: 738 diversity + 8 bp-pop (700) + 16 iso-lines
- 10 x more likely to find **[marker – QTL]** ?
- But missing data …
  - Mapping difficulty ?
  - Association artefacts ?
  - MAS predictions ?

Simulated MAS using GBS

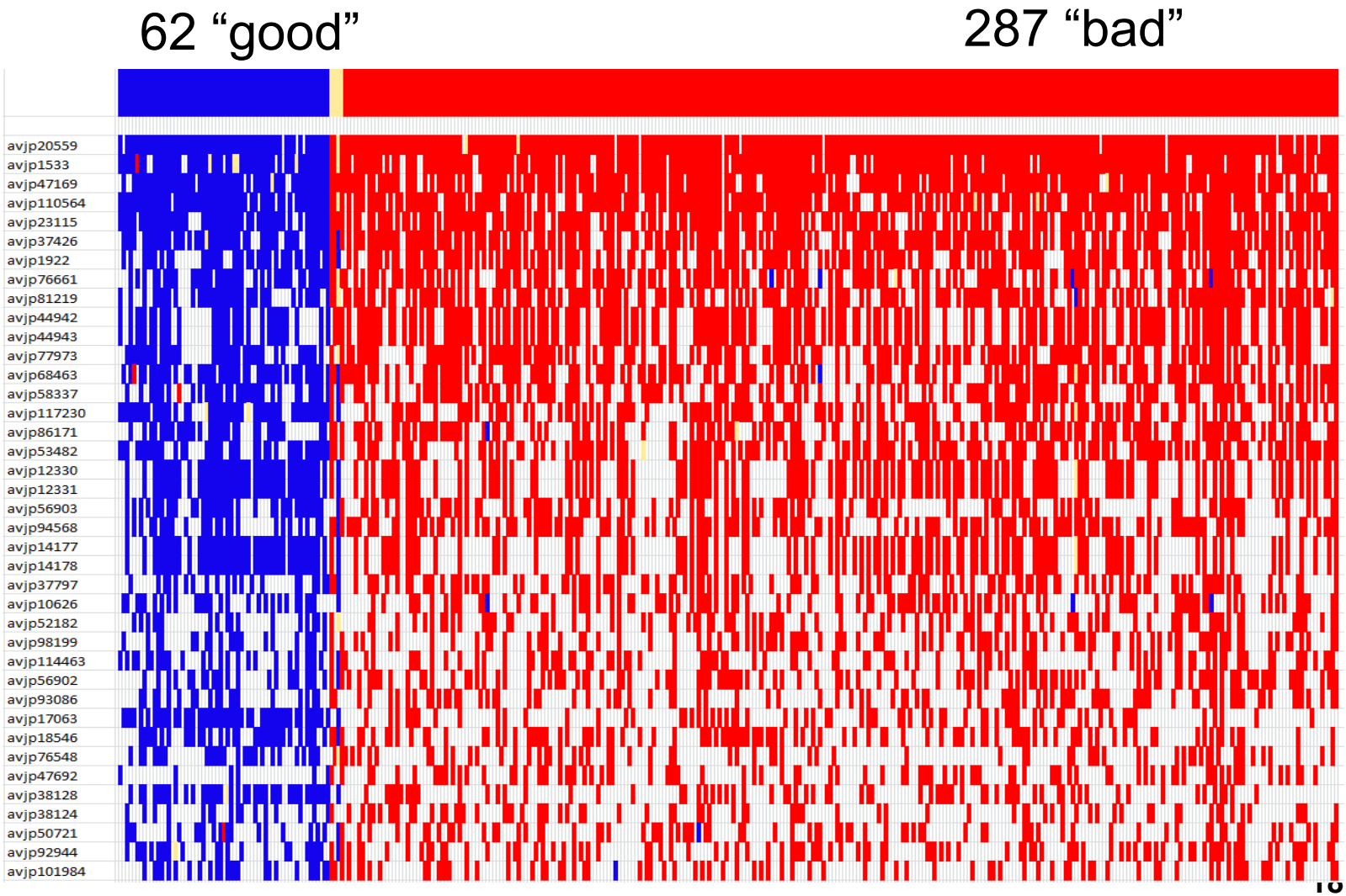
- Scenario:
  - 4 target loci, simulate with random GBS loci
  - Discover markers by association in odd # lines
  - Predict genotypes in even # lines

Marker	f(A)	f(B)	f(H)	Chr
avjp100014	568	782	20	1C
avjp100585	339	966	18	16A
avjp100261	150	1188	9	9D
avjp108144	1196	143	8	7C_17A

Predictive markers (target locus avjp100014)



Predictive markers (target locus avjp100261)



Simulated MAS using GBS

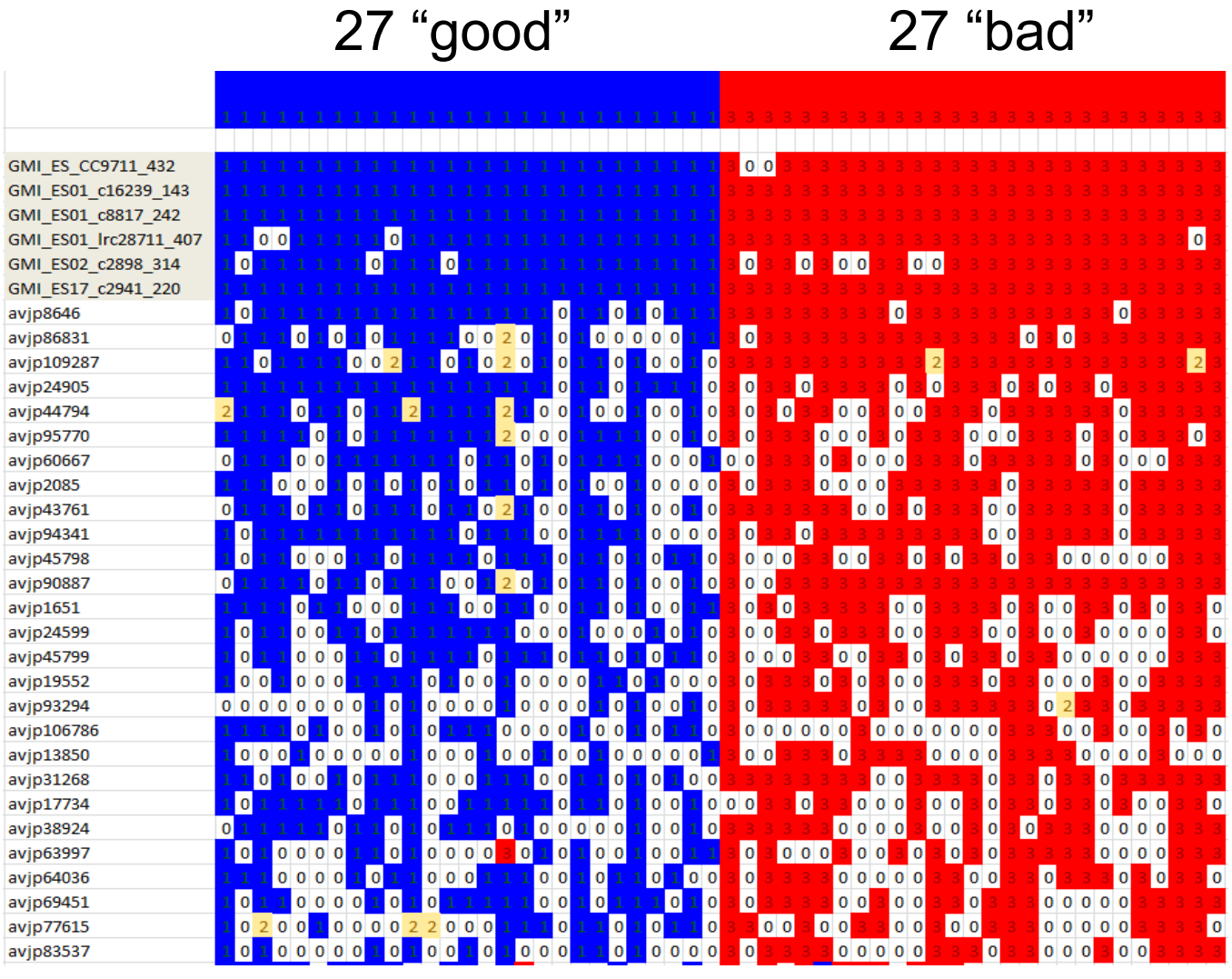
- Surprise!
  - Many loci in (near) perfect LD with a “QTL”
  - Bias in genome regions sampled by GBS ?
  - Too much good data ?
- Test predictions of array-based SNPs
  - Independently discovered
  - Most based on expressed genes
  - BUT…. SNP data only available for 108 varieties

Simulated QTLs based on Illumina SNPs

- 4 random Illumina cDNA SNPs (targets)
- Fit model with 54 odd # lines
- Test model with 54 even # lines

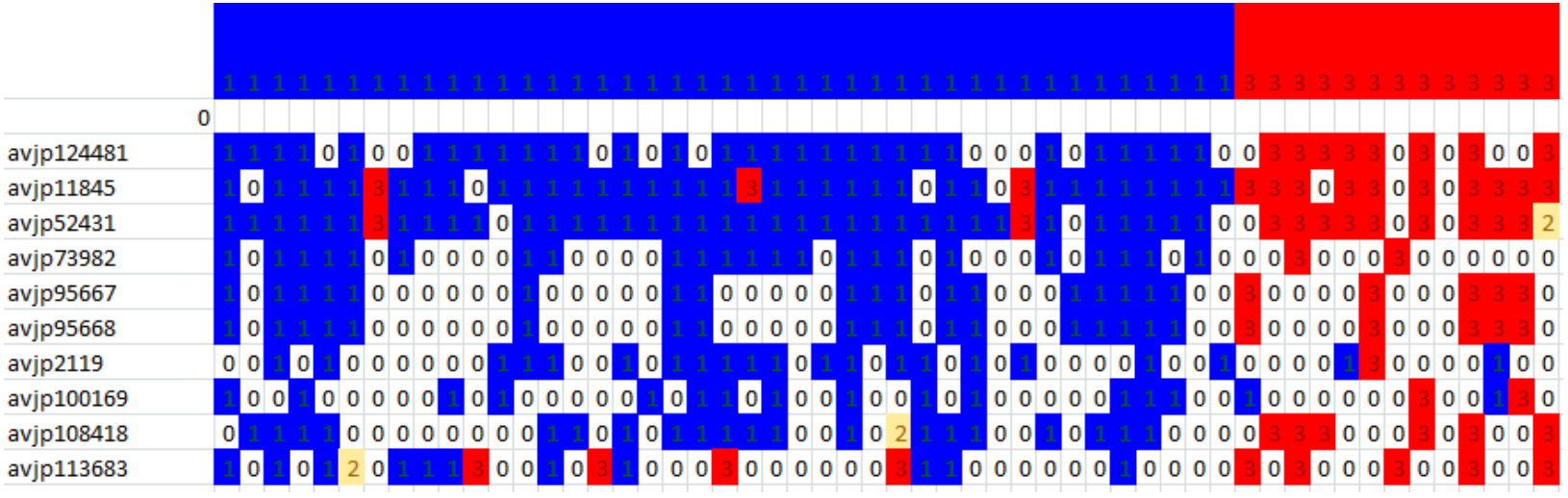
Marker	f(A)	f(B)	Chr
GMI_ES01_c10033_104	56	52	1C
GMI_ES15_c10388_464	57	51	18D
GMI_ES01_c796_180	84	24	19A
GMI_ES15_c8064_341	25	83	3C

Predictive markers (locus GMI_ES01_c10033_104)



Predictive markers (locus GMI_ES15_c8064_341)

41 “good” 13 “bad”



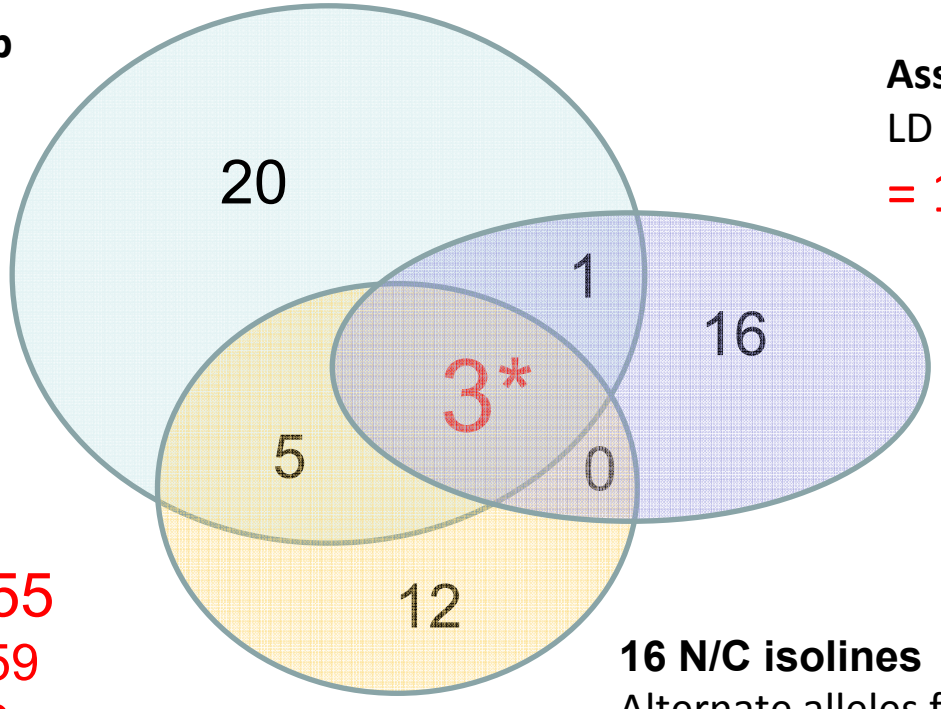
Case study with naked (hulless) oat

- Other genes (N2,N3,N4) or will we just find N1 ?
  - N1 was Mapped in Terra x Marion
  - Poorly placed by comparative mapping
- GBS data:
  - Diversity lines (*rare trait)
    - I only had phenotypes for 100 covered + 20 naked
  - 100 Terra x Marion progeny
  - 8 pairs of naked / covered iso-lines
    - OT253/Marion, from F6 heterozygotes
    - Developed by Solomon Kibite

Look at all pairwise linkages and LD's

N1 ←→ GBS-tag

TxM Map
R < 0.05
= 28



Association
LD R2 > 50%
= 18

*avjp23455
avjp123459
avjp17890

16 N/C isolines
Alternate alleles fixed
= 20

N1 predictions

- LD analysis based on partial data:
  - "training set" (the only ones I had data for at the time)
  - 20 naked / 100 covered
- Based on "avjp23455" we predicted:
  - 12 more naked lines (remaining 600 = covered)
  - All were correct!

Training set	
95Ab13050	OT253
Boudrias	Provena
Bullion	Racoon
Gehl	Salomon
IOI033	Shadow
IOI108	Terra
IOI114	VAO-44
IOI150	Vao-48
IOI191	VAO-51
Navaro	VAO--58

Predicted Naked Lines
98Ab7265
FL03184-FLID-B-S1
FL04178-FLID-B-S-2
HLA05AB1-34
IL02-10836
IL03-7936
LA02012-S-B-139-S2-B-S2-B-S2
LA0210SBSBSBSB-S1
LA03066SBS-L1
Lennon
Nudist
Zuton

Conclusions

- GBS will be exceptionally efficient for tagging genes
  - Bi-parental AND association mapping
- Excellent potential for MAS
- SNP array or custom assays are better for
  - Genome analysis, comparative genomics
  - Critical genotyping, well-characterized targets
- Work required
  - Streamline informatics
  - Build GBS/SNP based consensus map
  - Evaluate consistency of map position
  - Evaluate genomic selection

First Announcement: **web:** aowc.ca

American Oat Workers Conference

Ottawa Canada, 2014

Sun. July 13 to Wed. July 16, 2014

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