

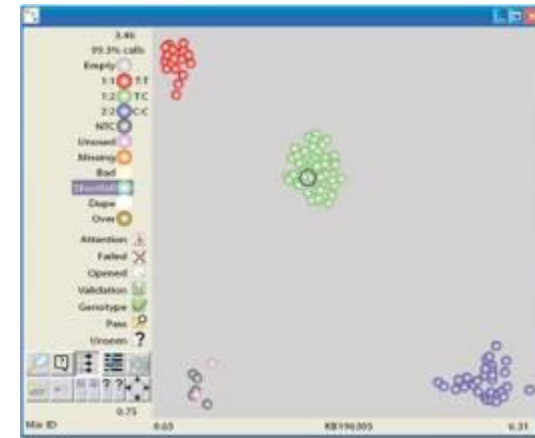
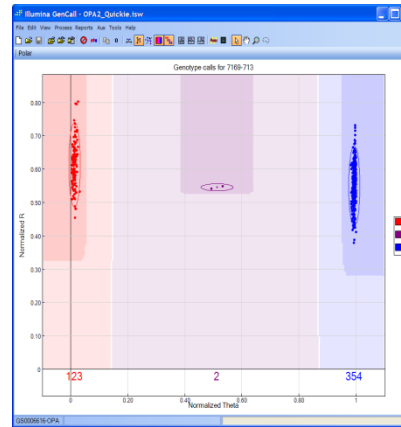
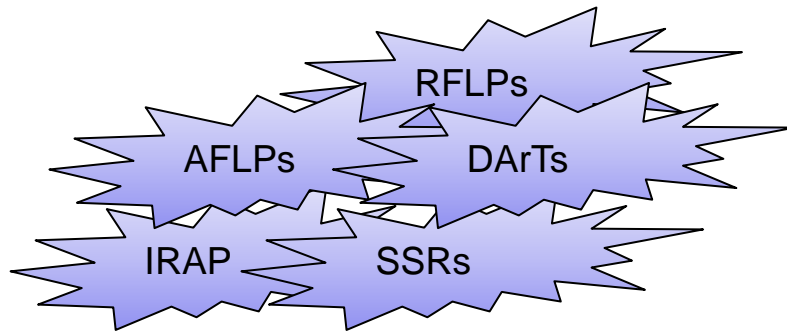
Genotypic Screening



Application of tools & resources



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Anonymous Markers

Functional Markers

Explore germplasm collections for useful variation



Tilling



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- Reverse Genetics
- Convert individual mutants into DNA
- Form pools 12, 24, 48?
- Use cleavage test to select mutant sites
- Deconvolute pools
- Phenotype mutant line

Optic, M₀ seed



Mutagenic treatment



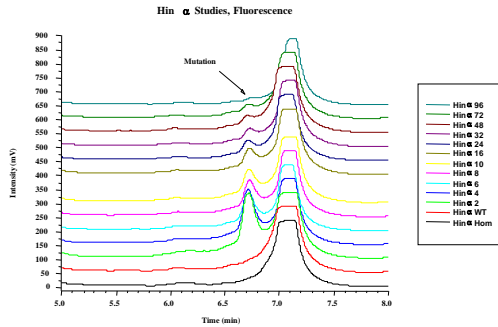
M₁ Plants



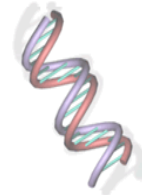
M₂ Plants



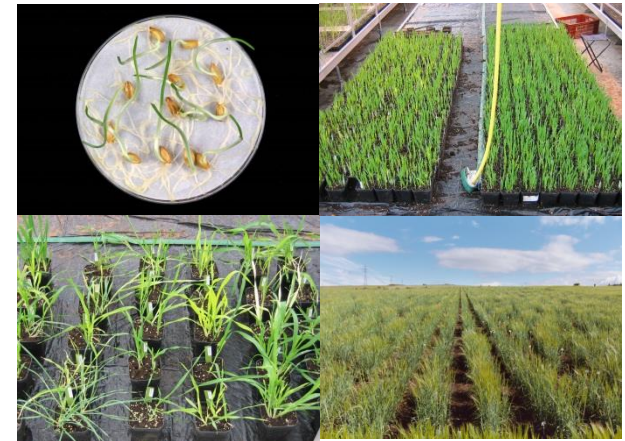
es
on
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Genotyping



DNA



Phenotyping
M₃ and M₄ families

Seed resource



Tilling



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- Demonstrated in all crop species
- Use Eco-Tilling for natural populations
- Efficient way to identify mutants
- Lacked sufficient sequence information
- Arguably biggest impact in forward genetics
- Many libraries now available, wheat and barley

New Sequencing Technologies



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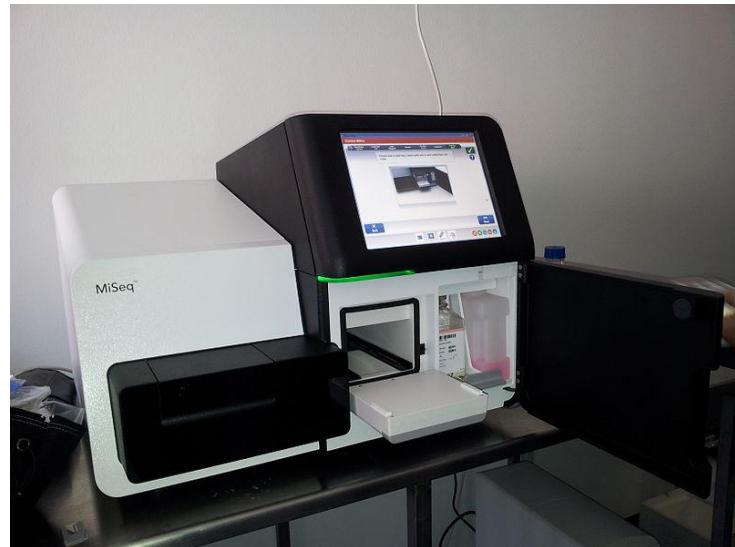
10 Years Ago

- Sanger sequencing
- Wellcome Trust Sanger Institute - approx 100 ABI sequencers
- 96 reads per machine per run



Illumina MiSeq

- Available on site
- 25 million paired end reads per run



Why Genome Sequencing?



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- Knowledge of genome structure – precise location of markers & genes.
- Links genetic/trait maps directly to the genome.
- Advances in sequencing technology.

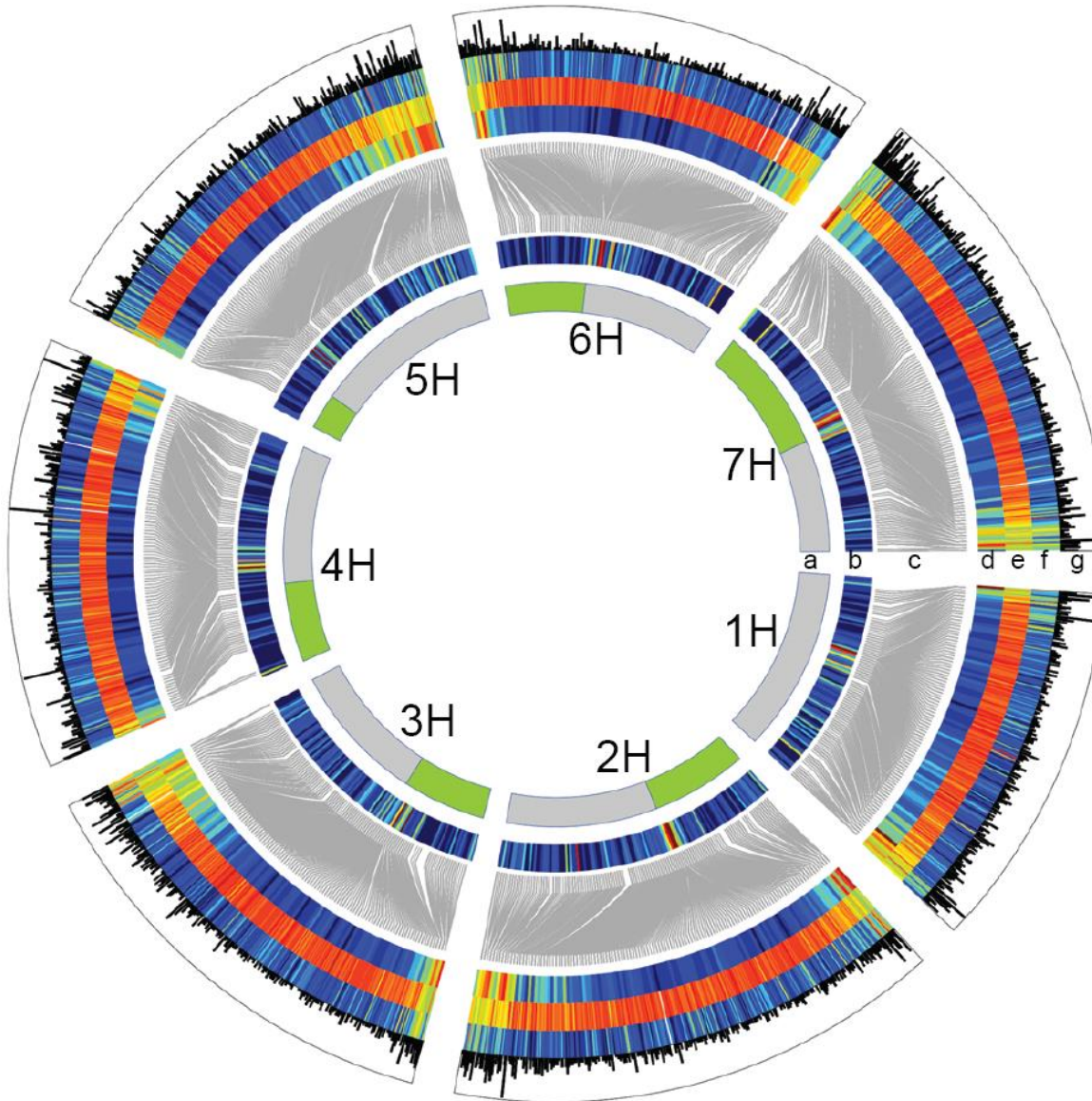
Challenges

- Barley
 - Very large genome (~5.3Gb), highly repetitive
 - Focus on sequencing the gene space
- Wheat
 - Exceptionally large genome (~17Gb), also highly repetitive
 - Allohexaploid; deconvoluting three ancestral genomes
 - Relative lack of variation in D genome
 - Again, focus on sequencing the gene space

Barley gene space



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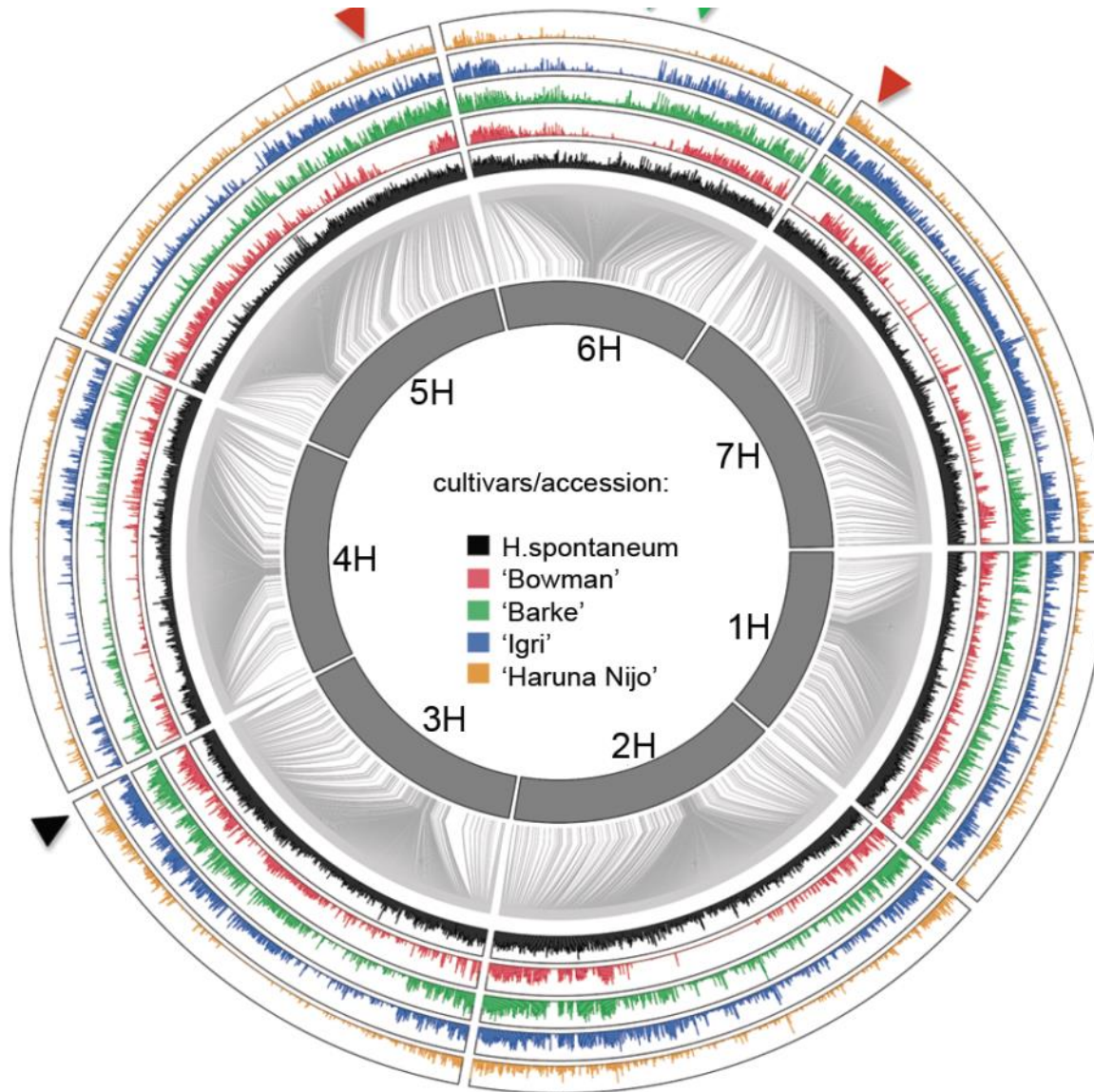


- a. Chr arms
- b. HC genes (genetic)
- c. Links
- d. HC genes (physical)
- e. Class I LTR – retro's
- f. Class II transposons
- g. Sequenced BACs

Barley Single Nucleotide Variation



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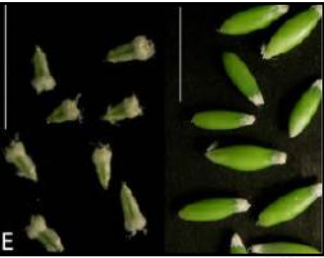


360 Barke x Morex RILs

Gene expression atlas



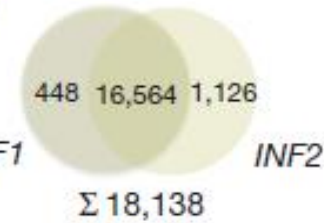
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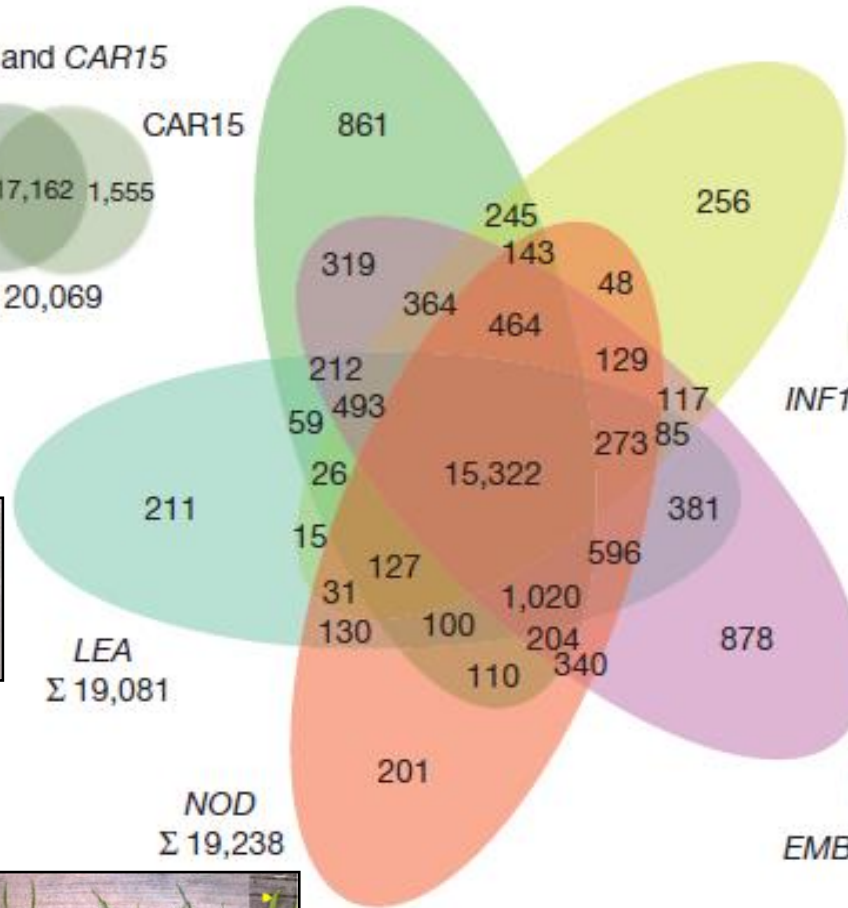
CAR5 and *CAR15*



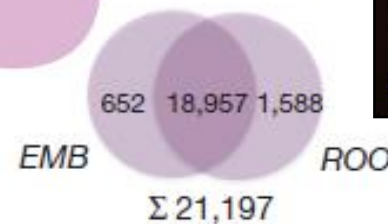
INF1 and *INF2*



LEA
Σ 19,081

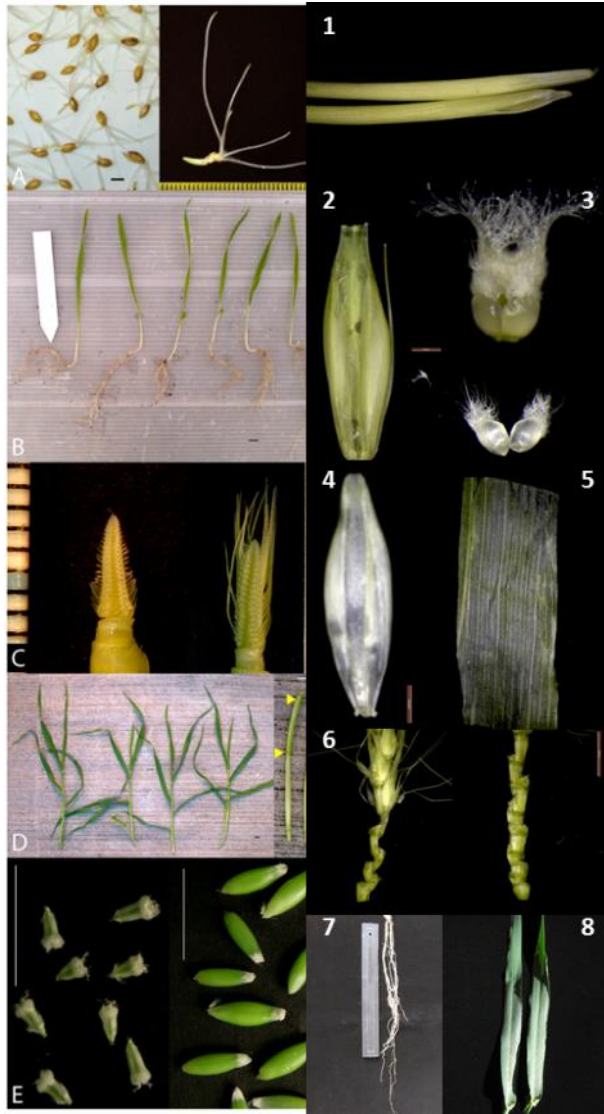


EMB and *ROO*



NOD
Σ 19,238

Expansion of barley RNAseq



Dissected tissues & treatments (16 samples, 3 reps)



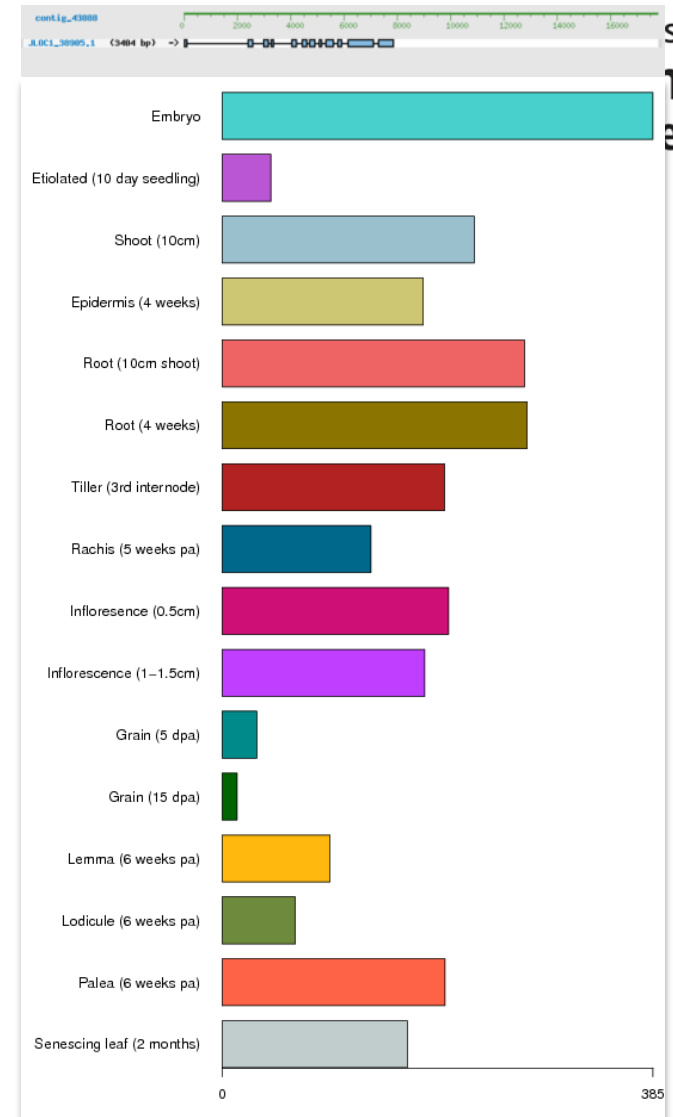
New gene model predictions



Transcript isoform analysis



Development of 'barleyGenes' database

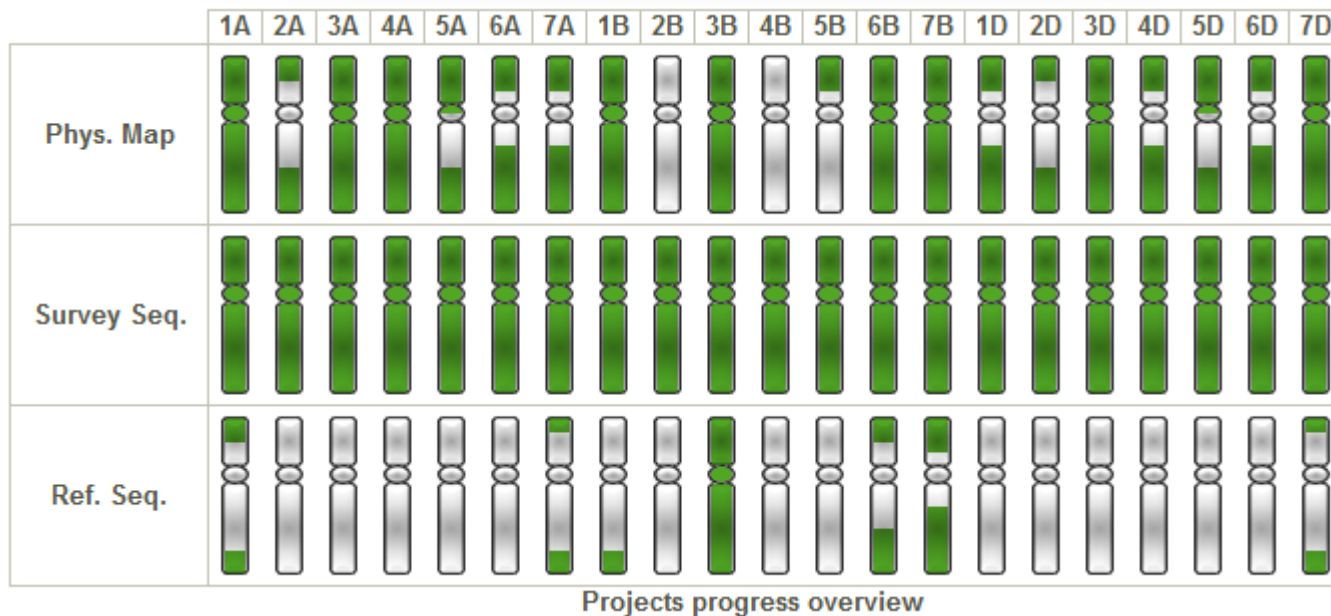


Wheat Genome



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- Over 90,000 genes identified
- Some gene fragments
- IWGSC (<http://www.wheatgenome.org/>)



Sequencing to date



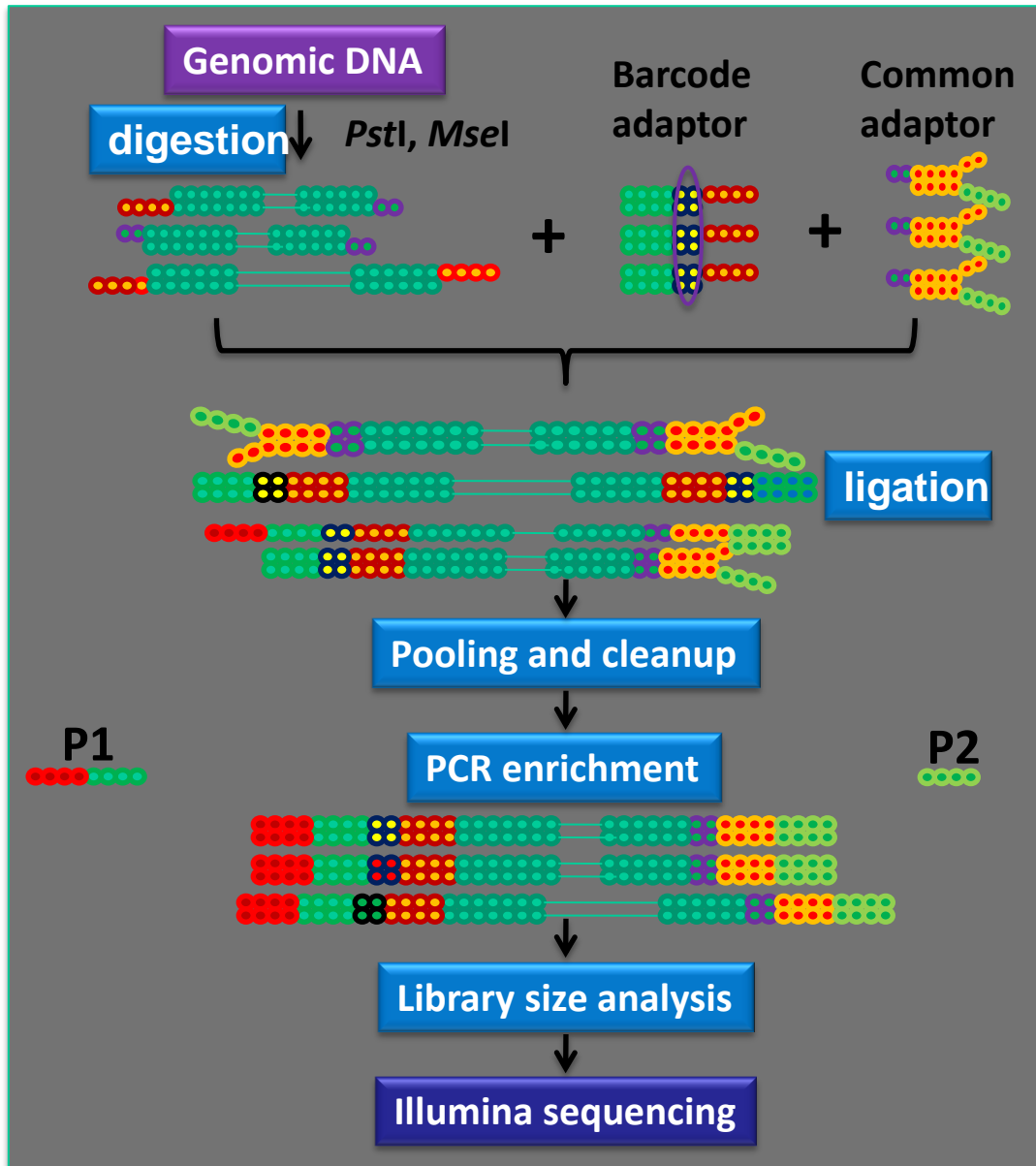
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- Number of varieties now with gene space sequenced
- Compare same sequence across varieties
- Or re-sequence genotype panels for target genes
- Align sequences and identify SNPs

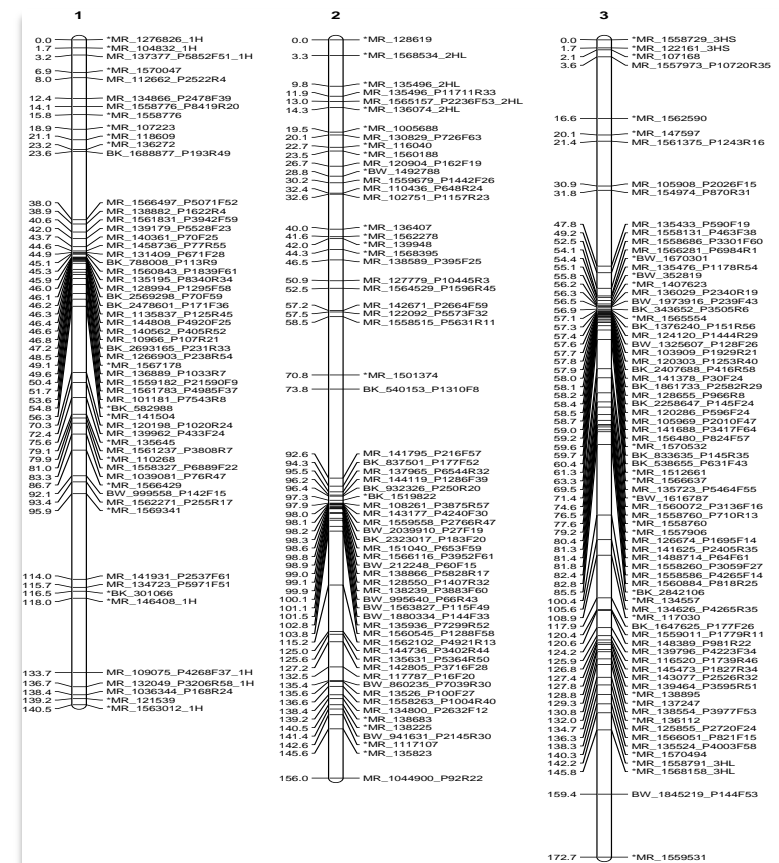
Genotyping by Sequencing



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GP x Morex map



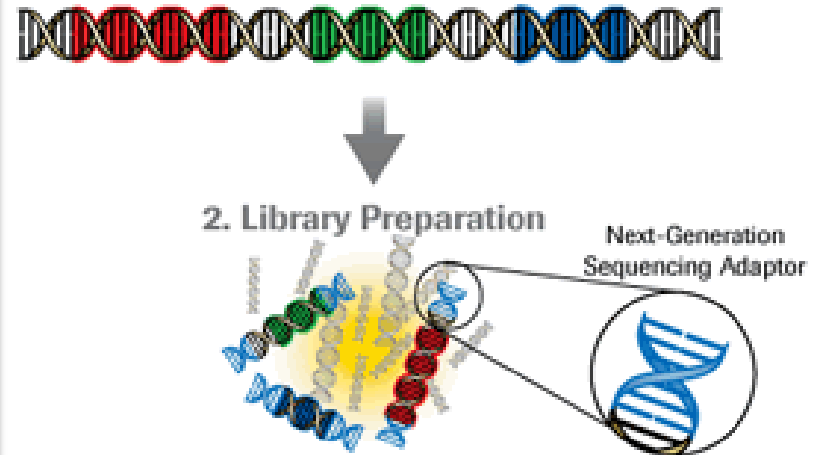
Exome capture



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Nimblegen Roche

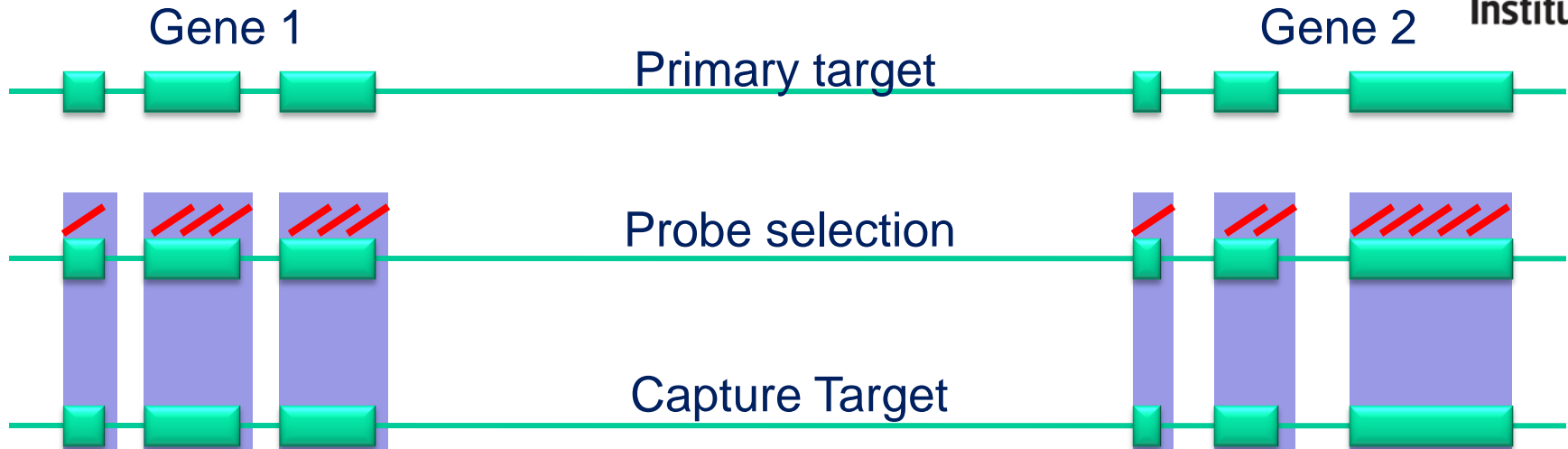
- Collaborative IBGS barley project
- Whole gene-coding complement
 - **1% of whole genome**
- 62 Mb barley exome sequence as long oligo baits
- TGAC library preparation and sequencing



Sequencing: Exome Capture



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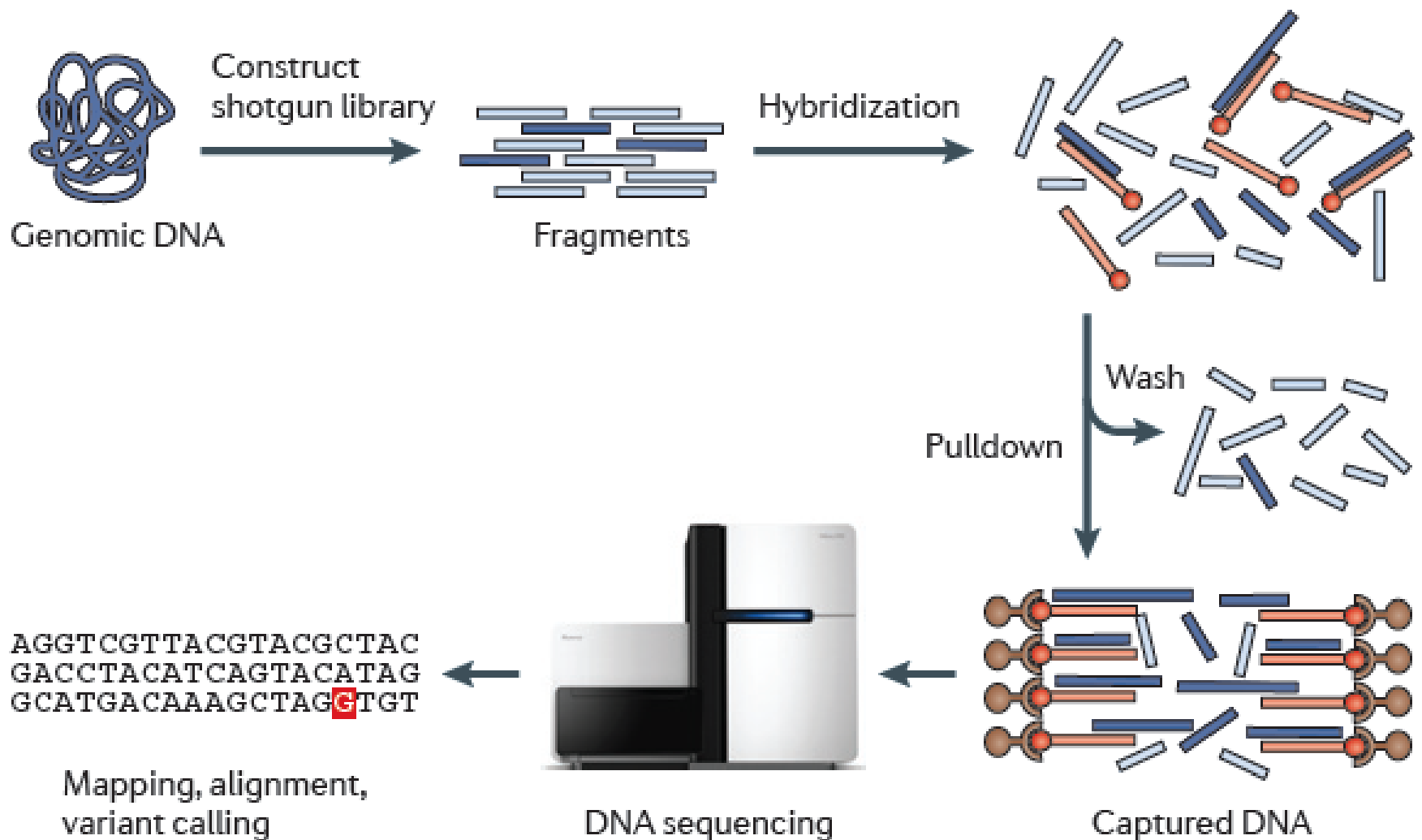


- Exome refers to all of the exons in the genome (~1% barley genome)
- Effectively reduces genome from 5300Mb to 60Mb
- Design based on Morex genome assembly v3, flcDNA and RNAseq data
- 1 Lane HiSeq2000 – 40Gb = ~600X coverage

Exome capture: processing



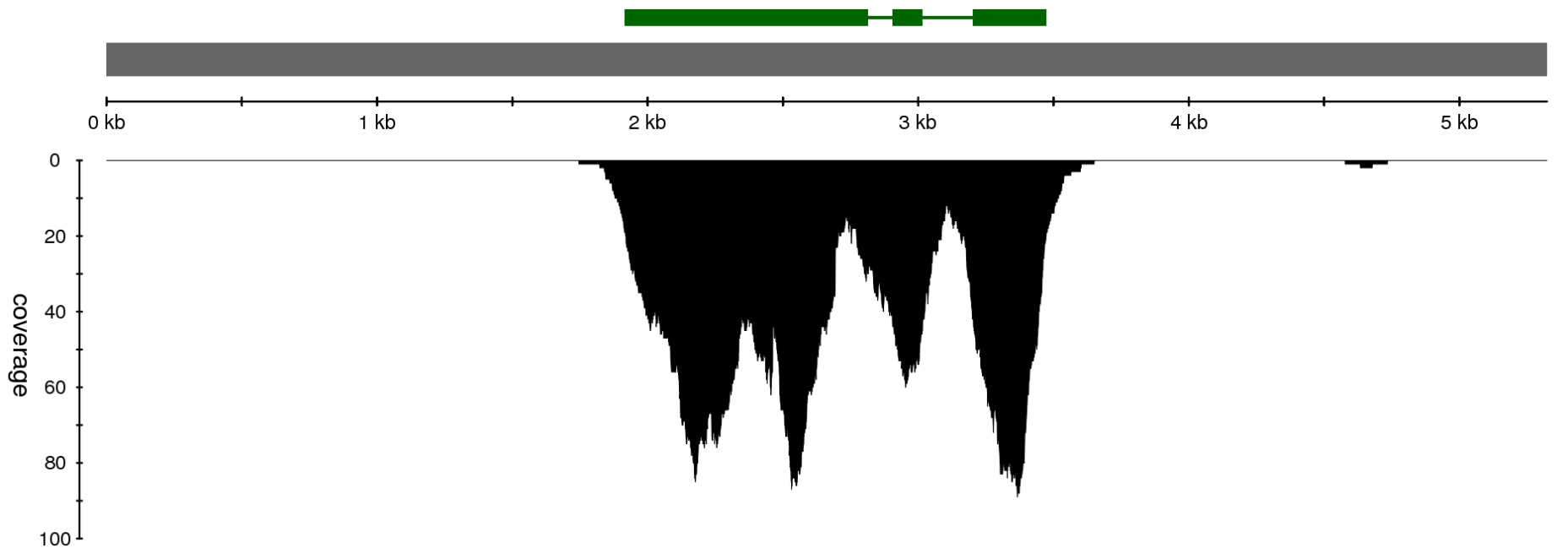
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Exome capture: pilot testing



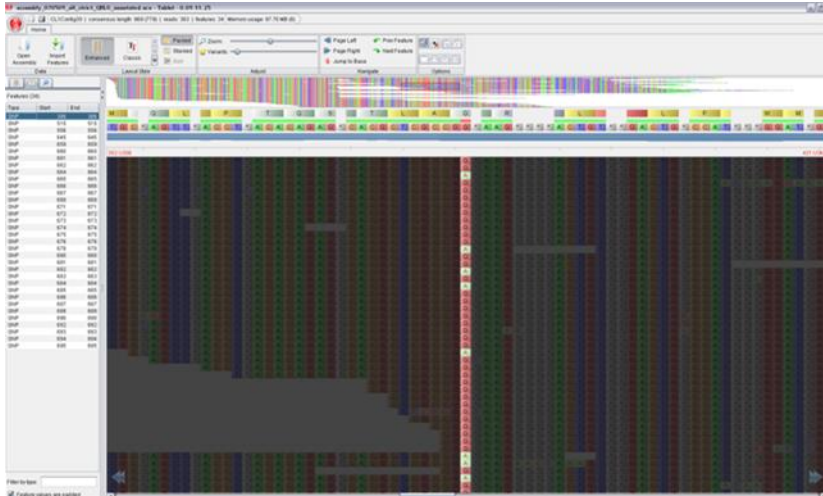
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Variant discovery



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- EC from 300 barley lines
- Variant discovery: FreeBayes
- Design new SNP chip
 - 675 k
 - 50 k




Affymetrix[®]
Revolutionize life



Bulk Segregant Analysis



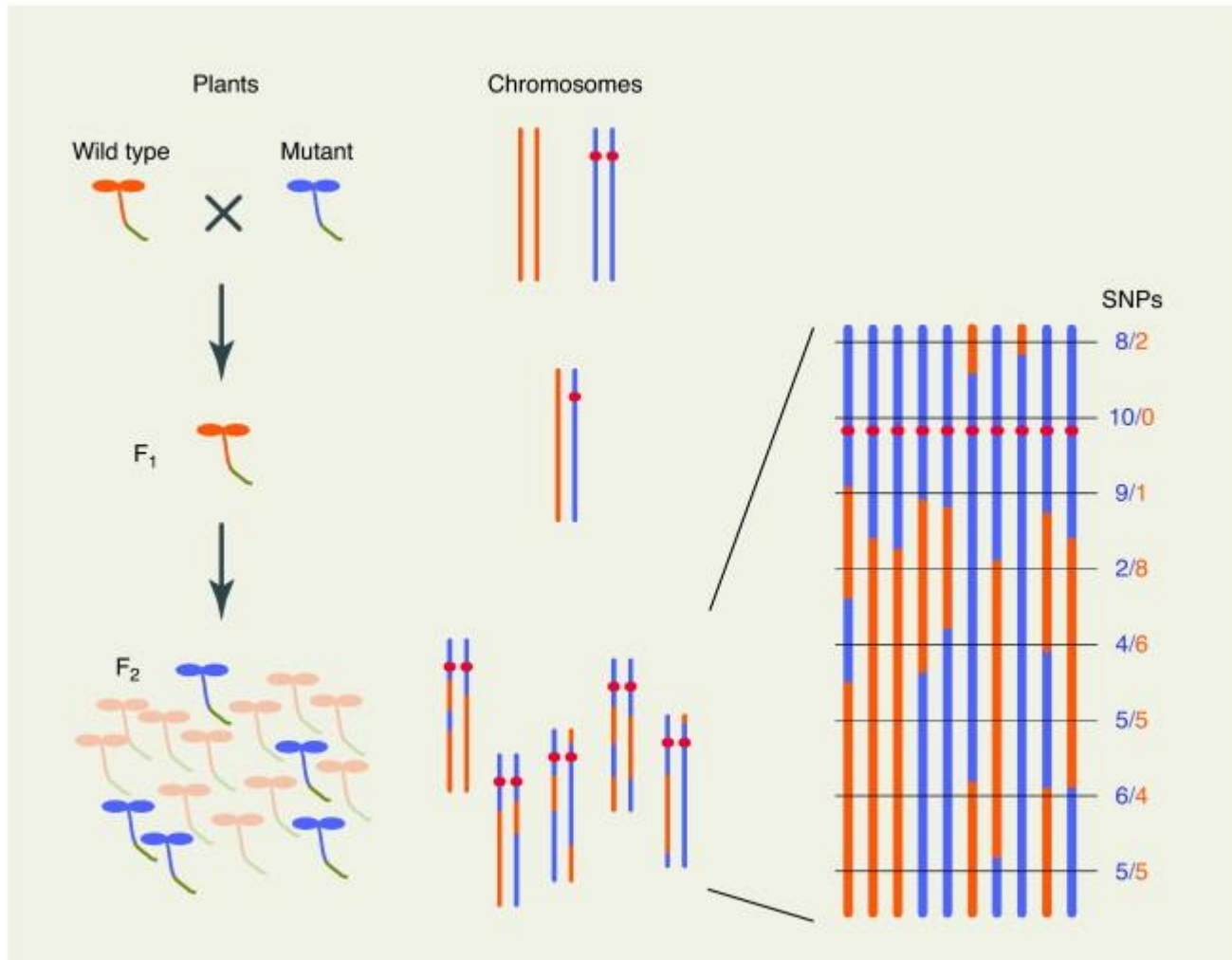
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- Originally developed by Michelmore & colleagues for simple marker systems
- Now been extended for sequencing analyses
- Form pools of lines with mutant character and wild type
- Extract DNA or RNA and sequence
- Align sequences
- Identify DNA variants & compare frequencies

SHOREMAP



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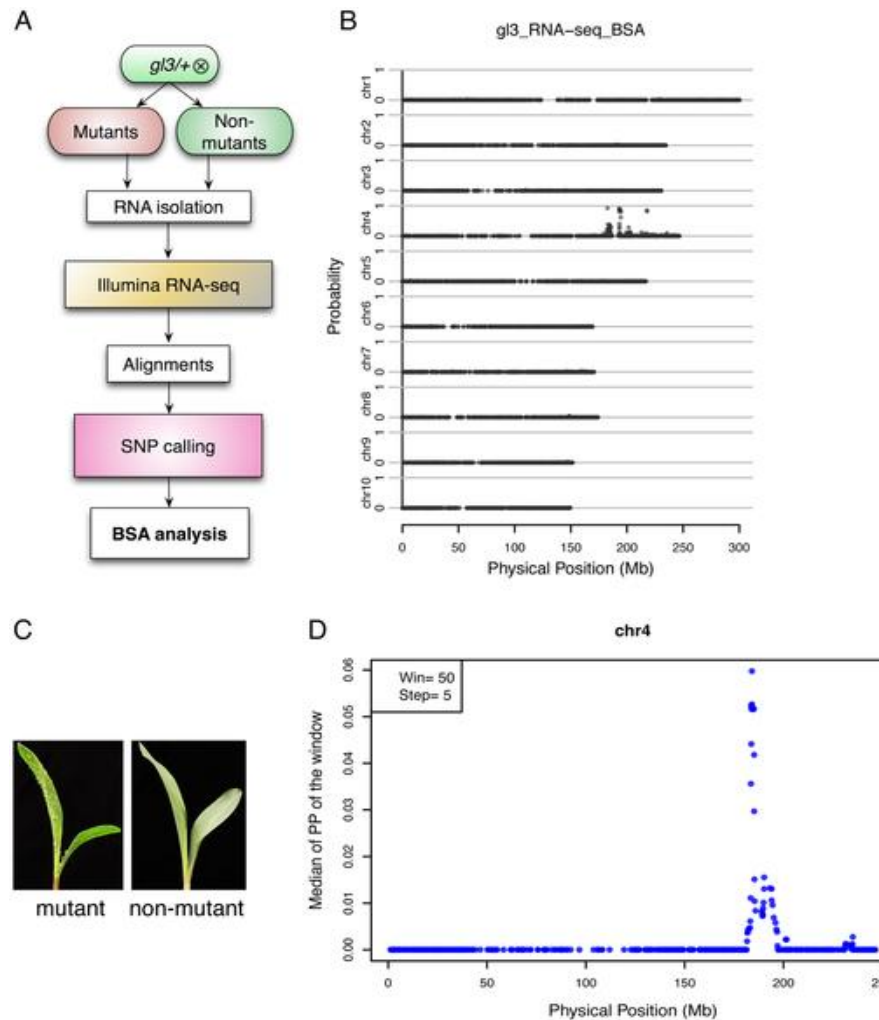


TRENDS in Plant Science

BSA & Sequencing



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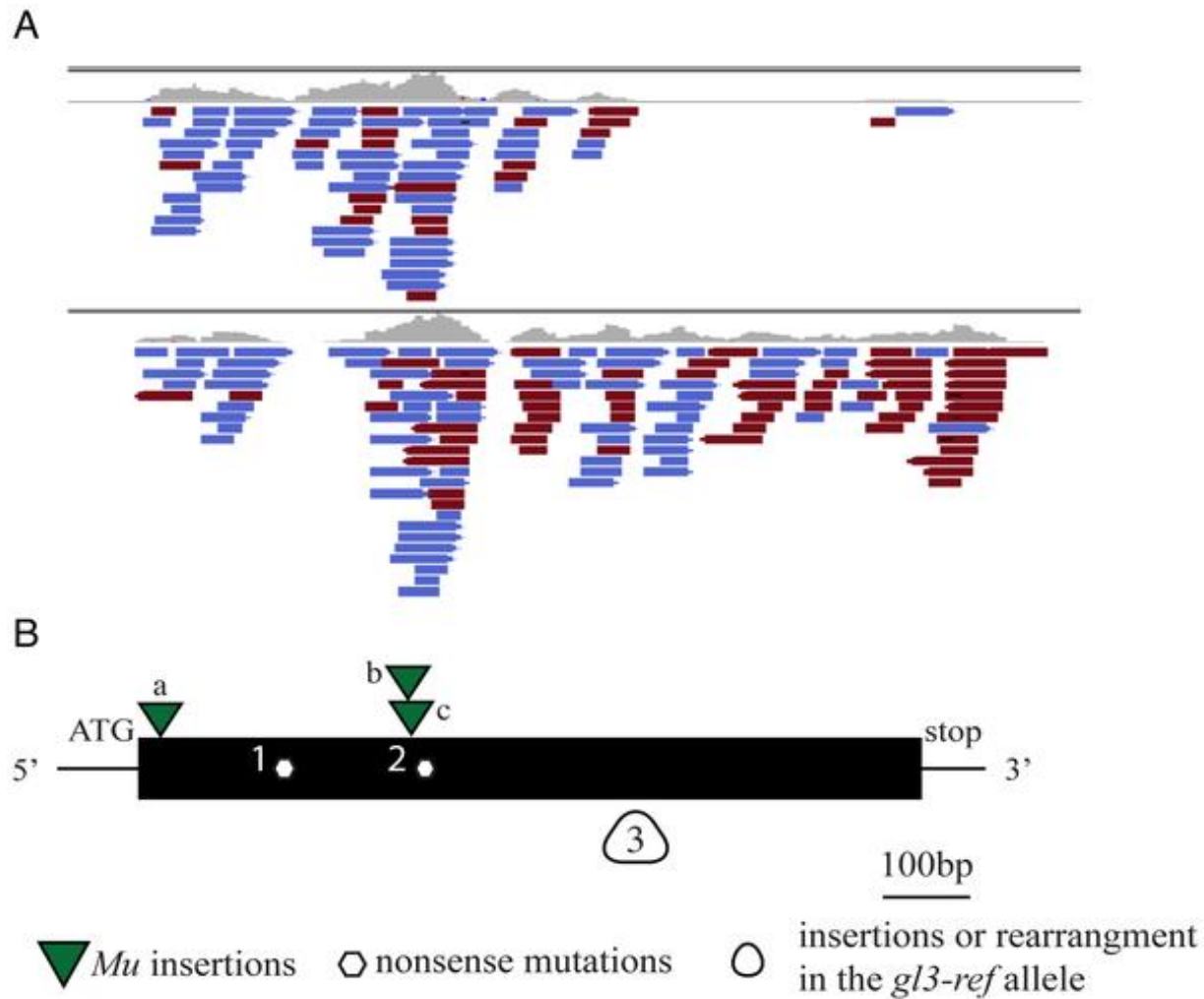
Liu S, Yeh C-T, Tang HM, Nettleton D, et al. (2012) Gene Mapping via Bulk Segregant RNA-Seq (BSR-Seq). PLoS ONE 7(5): e36406. doi:10.1371/journal.pone.0036406

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0036406>

BSA Alignments



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Liu S, Yeh C-T, Tang HM, Nettleton D, et al. (2012) Gene Mapping via Bulk Segregant RNA-Seq (BSR-Seq). PLoS ONE 7(5): e36406. doi:10.1371/journal.pone.0036406
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0036406>

Locating the gene



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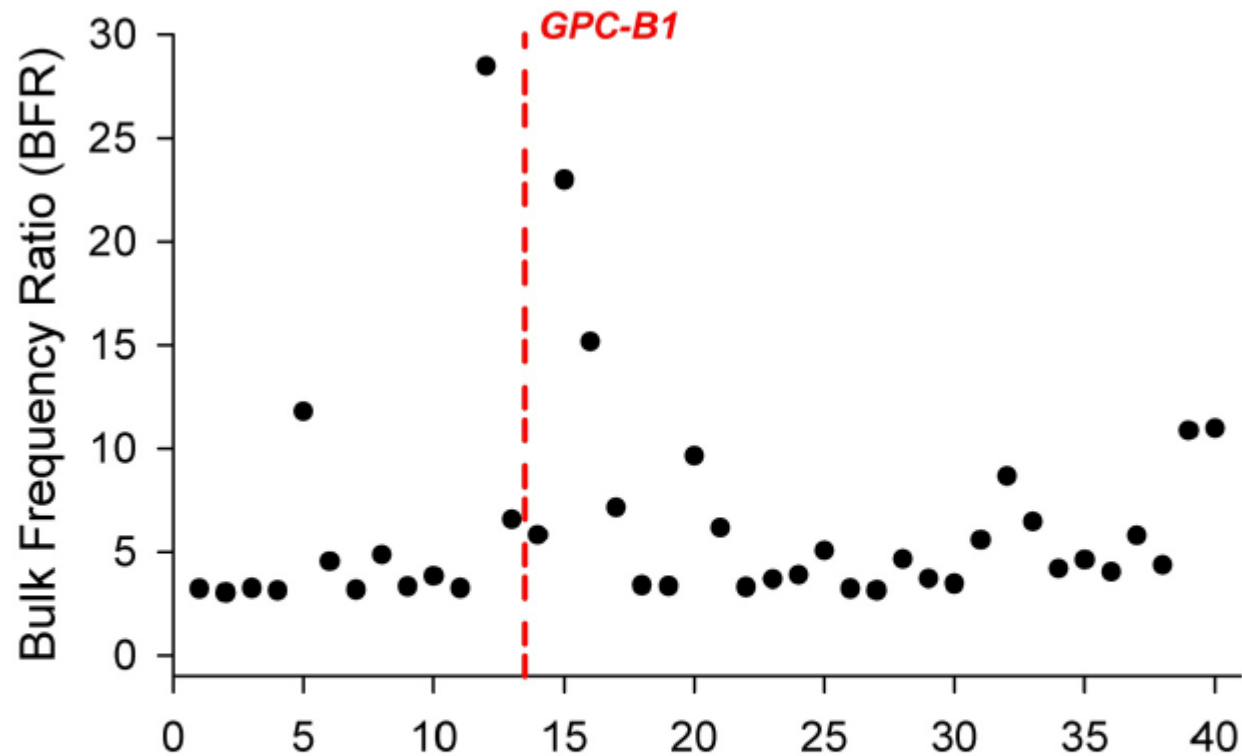


Figure S3: BFR of validated and mapped SNPs across the *GPC-B1* interval. SNP markers are plotted along the X-axis according to their map position. The broken red line indicates the position of the *GPC-B1* gene.

Barley: 2.865 kbp from contig_135563:650..3,514

Browser Upload and Share Tracks Preferences

Search

Landmark or Region:

contig_135563:650..3514 Search

Examples: HC-MLOC_54721.2, HC-MLOC_10482.5, HC-MLOC_68408, HC-MLOC_54947, HC-MLOC_55378, HC-MLOC_52876, nonHC-MLOC_44669, nonHC-MLOC_61014, or-MLOC_25409.1, contig_136630:5472..9180, contig_6440:2456..4258, contig_47818:700..4599, exonuclease, contig_100937:1..7397, contig_131638:1..6774, contig_66958, contig_37907, contig_1557936, contig_1563311, contig_41812, contig_1560609, contig_46037, contig_2521747, contig_48918, contig_46726.

Data Source

Barley

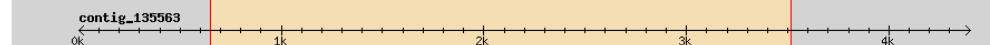
Annotate Blast Against Displayed Sequence

Configure... Go

Scroll Zoom: << < < Show 2.865 kbp > > >>

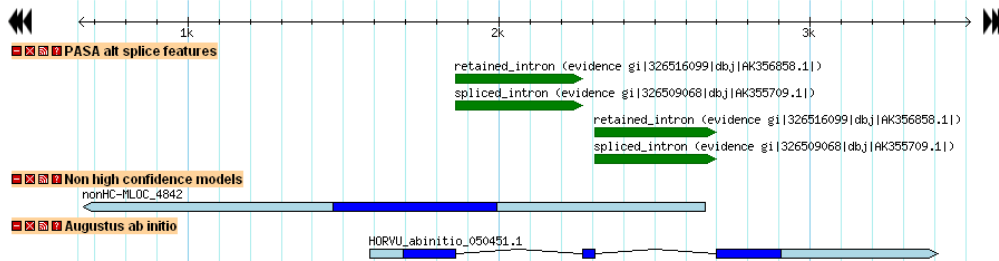
Flip

Overview



Region

Details



Clear highlighting

Tracks

1. Repeats All on All off

- Interspersed repeats (MIPS)
- Low complexity repeats (Repeatmasker)
- Low complexity repeats (Tantan)

2. PASA All on All off

- PASA alt splice features
- PASA training set models
- PASA assemblies (Run5)
- PASA validated transcripts
- PASA MIPS original models post PASA
- PASA Gmap alignments (Run5)
- PASA assemblies
- PASA_run3
- PASA Alternative Splice Features (Run5)
- PASA Gmap alignments

3. Gene prediction All on All off

- Augustus ab initio
- Augustus with evidence 2
- cuffmerge_noref.exon.cds.utr
- Augustus with evidence
- Jasmbl filtered
- cuffmerge_ref.exon.cds.utr

4. MIPS Annotation All on All off

- High confidence models
- Non high confidence models
- Original models

Ensembl genome browser: *Hordeum vulgare* - Windows Internet Explorer
http://plants.ensembl.org/Hordeum_vulgare/Info/Index

File Edit View Favorites Tools Help
Search
Facebook Listen to music Amazon YouTube 7 Aberdeen, United Ki... BBC News BBC Sports Fun Games Financial Times E-mail Options
Google Search Share More Sign In

Ensembl genome browser... Barley: 2.865 kbp from con...
Ensembl Plants BLAST | Sequence Search | BioMart | Tools | Downloads | Help & Documentation
Search Ensembl Plants species... Login Register

Hordeum vulgare

About this species


- Genome Statistics
 - Assembly and Genebuild
 - Top 500 InterPro hits
- Sample entry points
 - Karyotype
 - Location (5:1680000-1760000)
 - Gene (MLOC_81761)
 - Transcript (MLOC_81761.1)

Configure this page
Manage your data
Export data
Bookmark this page

[Ensembl Plants is produced in collaboration with Gramene](#)

Search *Hordeum vulgare*

Search *Hordeum vulgare*... Go
e.g. [MLOC_81761](#) or [5:1680000-1760000](#) or [Hordoidndoline](#)



Hordeum vulgare (barley) is the world's fourth most important cereal crop, and is an important model for ecological adaption, having been cultivated in all temperate regions from the Arctic Circle to the tropics [1]. It was one of the first domesticated cereal grains, originating in the Fertile Crescent over 10,000 years ago.

Barley is a member of the grass family and a true diploid, making it a natural model for the genetics and genomics of the Triticeae tribe, including polyploid wheat and rye. With a haploid genome size of 5.3 Gbp in 7 chromosomes, it is one of the largest diploid genome sequenced to date [2].

About two-thirds of the global barley crop is used for animal feed, while the remaining third underpins the malting, brewing, and distilling industries. Although the human diet is not a primary use, barley offers potential health benefits, and is still the major calorie source in several parts of the world [3].

Genome Sequencing and Gene Prediction


The assembly featured in Ensembl Plants is, specifically, a "gene space" assembly, covering 35% of the estimated total genome size but containing 80% of the expected genes. A physical map has been used to locate 22% of contig sequence within a chromosomal framework. Where multiple contigs have been mapped to the same location, but resolution is insufficient to determine contig order and orientation, contigs have been assigned successive locations in decreasing order of size, with nominal gaps of 100 bp between them, in accordance with the standard recommendations of the ENA/GenBank/DBJ databases. The assembly is described by publically available data, [downloadable here](#).

For more details about genome sequencing and gene prediction see [4].

- Gene-ome sequence: [ERP001435](#)
- Gene-ome assembly: [CAJW010000001](#)

References

- [At the Threshold of Efficient Access to the Barley Genome](#). Schulte D et al. Plant Physiology. 2009.
- [Nuclear dna amounts in angiosperms](#). Bennett MD, Smith JB. Philos Trans R Soc Lond B Biol Sci. 1976.
- [Barley in Wikipedia](#).
- [A physical, genetical and functional sequence assembly of the barley genome](#). The International Barley Genome Sequencing Consortium (IBSC). Nature 2012.



[Download *Hordeum vulgare* genome sequence](#) (FASTA)

Ensembl Plants release 16 - October 2012 © EBI

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Genotypic Screening



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- Now feasible and cost effective
- Can apply to unmapped phenotypic mutants
- Or can use it to search for allelic variants in known genes

Barley Mutants



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- First barley mutant (Jutta) released in 1955
- Pallas (1960) dominated European barley in 1960s
- Golden Promise sown on up to 75% of Scottish barley area in 1970s to 1980s
- Most European spring barleys have at least one induced

- Gene reduction (Green Revolution Gene)

