Haplotype reconstruction using low-pass whole-genome sequencing in genetically diverse mouse populations

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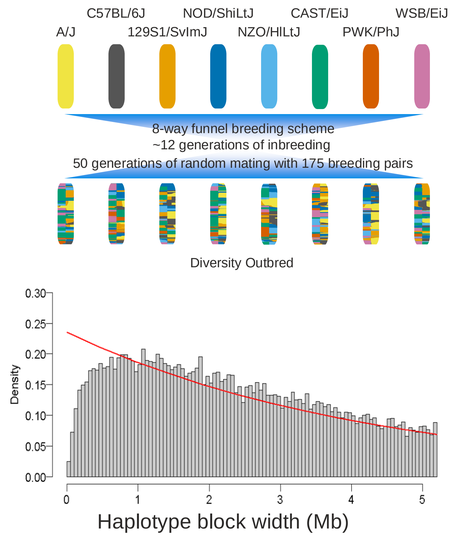
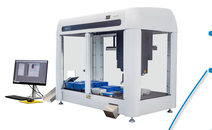
MOTIVATION

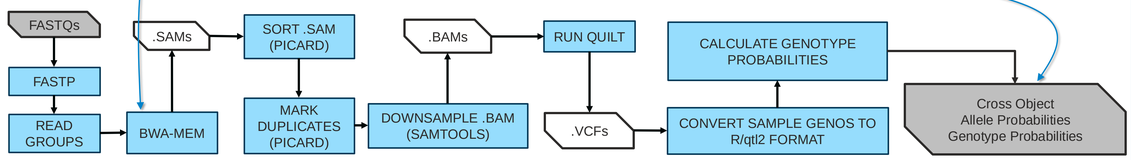
- The Diversity Outbred (DO) mouse population is a premier resource for systems genetics and quantitative trait locus (QTL) mapping.
- Eight founder strains were crossed in a funnel breeding scheme to produce recombinant inbred strains, followed by random mating, resulting in highly recombined outbred mice.
- Each DO mouse is unique and must be genotyped independently, usually with the Giga Mouse Universal Genotyping Array (GigaMUGA).
- Genotyping hundreds of samples on the GigaMUGA can be cost prohibitive; the cost per sample is roughly $100**.**
- With increasing random mating, recombination reduces the size of founder haplotype blocks, increasing mapping resolution.
- Compared to the fit of an exponential distribution (red), there is a depletion of blocks less than 1 Mb in generation 36 of the DO using the GigaMUGA**.**
- **To address these limitations, we have developed a low-coverage genotyping-by-sequencing (lcGBS) approach to genotyping DO individuals that is accurate and cost-effective.**



METHODOLOGY



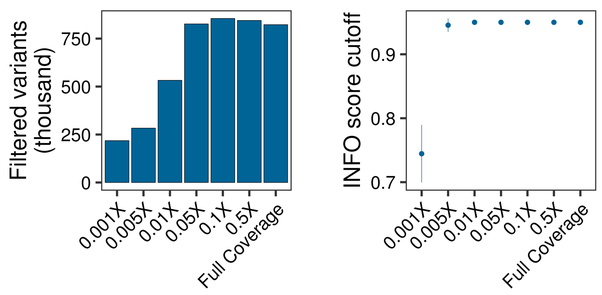
- We prepared Illumina sequencing libraries from 48 DO mice using the seqWell purePlex DNA Library Prep Kit (**Booth 684**) automated on a Revvity Sciclone G3 NGSx (*left*).
- We aligned Illumina short reads to the mouse reference genome build GRCm39.
- Reference haplotypes were constructed using 48 million biallelic SNPs segregating in the DO founders curated by the Sanger Mouse Genomes Project.
- The lcGBS pipeline uses QUILT to impute genotypes using the founder reference haplotypes.
- Imputed sample genotypes were used to reconstruct individual haplotypes with respect to founder alleles (“haplotype reconstructions”) using R/qtl2.



SEQUENCING METRICS

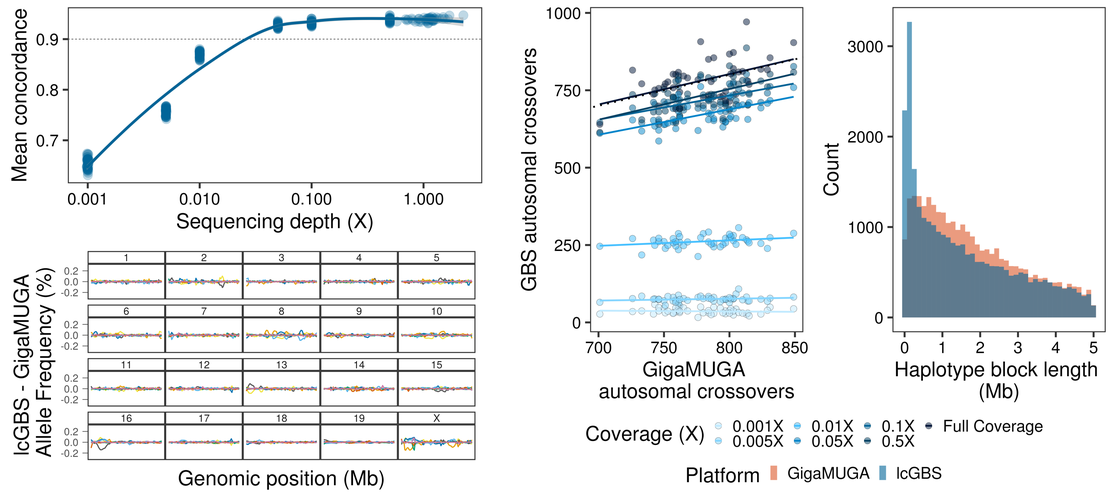
Pre-imputation → Filter to 1M Grid, HWE, INFO > 0.95 → Post-imputation

Mapped HQ reads (million)	19.0 ± 4.77
Sequencing depth	1.14X ± 0.29X
Genome coverage (>Q20)	0.79X ± 0.21X
Coverage at captured sites	1.91X ± 0.17X
Captured variants (million)	1.57 ± 0.38

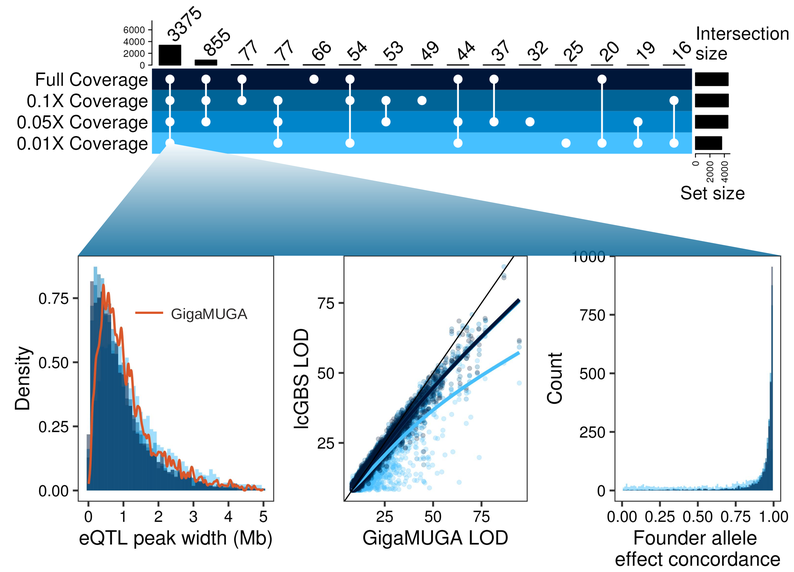


ACCURATE HAPLOTYPE RECONSTRUCTION AT A COMPETITIVE PRICE POINT

- GigaMUGA and lcGBS and haplotype reconstructions were 94% concordant at full coverage, and 93% concordant at 0.1X coverage.
- Crossover counts did not differ significantly between samples genotyped with full coverage lcGBS or GigaMUGA.
- Haplotype blocks shorter than 1 Mb were significantly more abundant in lcGBS samples than in GigaMUGA.
- Founder strain allele frequencies were faithfully preserved over most of the genome.



POWERFUL AND PRECISE SYSTEMS GENETICS



- We genotyped 183 embryoid bodies derived from DO embryonic stem cell lines using lcGBS and GigaMUGA.
- We mapped expression QTL using haplotype reconstructions from both methodologies.
- 3,375 local eQTL closest to their regulated gene were detected in GigaMUGA and lcGBS at all tested coverage levels.
- Low-coverage GBS produced mappings with greater resolution, similar power, and unbiased founder allele effects.
- **We recommend lcGBS as a viable replacement for genotyping DO mice for scaled systems genetics.**

ACKNOWLEDGEMENTS

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