Supplementary Table ST1

NGS datasets and mapping interval correlation

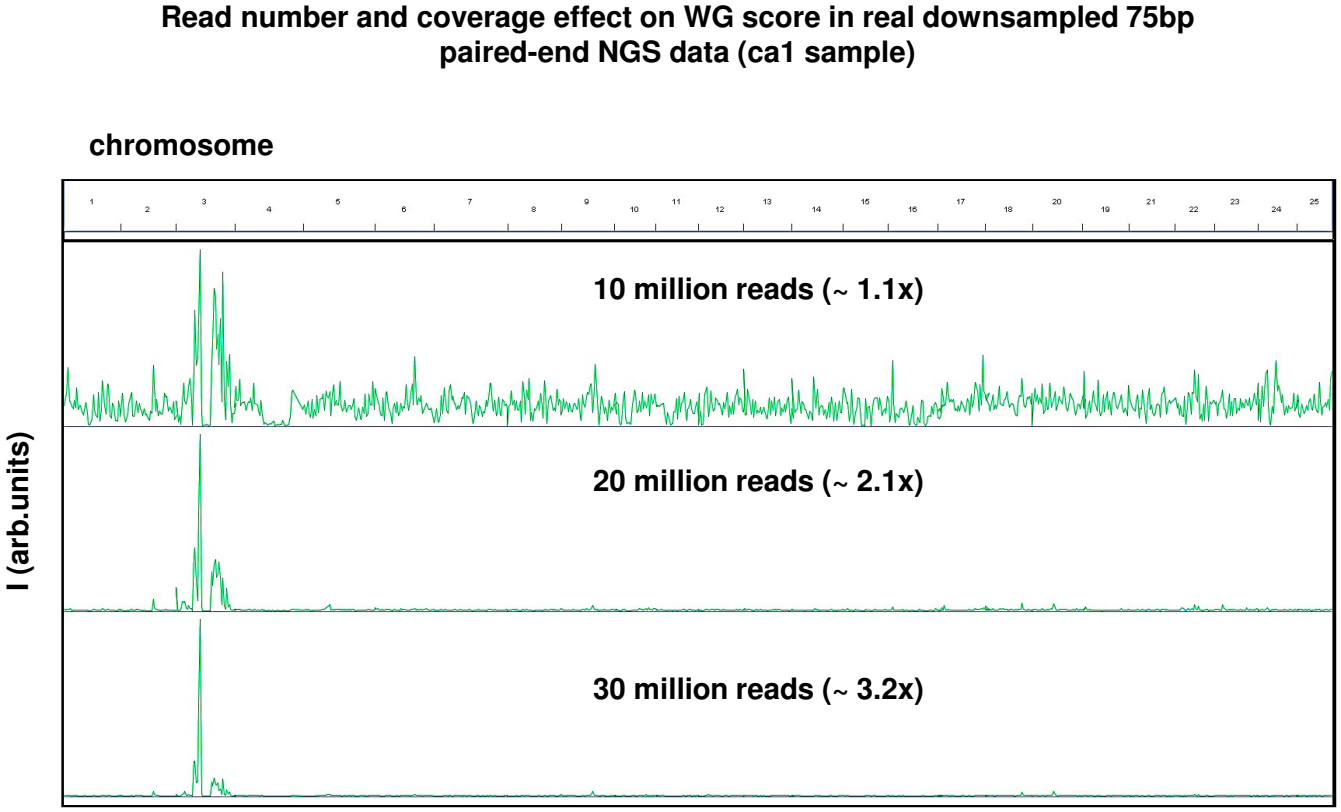
Sample name	Individuals in the pool	Read type	Obtained number of reads	Average genomic coverage	Approximate mapped interval
clo s5 wt	20	100bp se	94,846,364	6.8x	800kb*
clo s5 mt	20	100bp se	99,737,453	7.1x	800kb*
clo m39 wt	160	100bp se	104,967,597	7.5x	800kb*
clo m39 mt	160	100bp se	102,756,452	7.3x	800kb*
ca1 wt	20	75bp pe	89,609,570	9.6x	7Mb
ca1 mt	20	75bp pe	94,626,189	10.1x	7Mb
mlb wt	5	75bp pe	73,585,494	7.9x	20Mb
mlb mt	20	75bp pe	82,549,416	8.8x	20Mb
Line 27SH	9	75bp pe	131,573,054	7.6x	5.5Mb

***likely missing contigs in the genome assembly**

Supplementary Table ST1

Summary of all Next Generation Sequencing runs on HiSeq2000 used in the work, displaying: the number of individuals per pool used for mapping, read length and type used, number of reads returned from the sequencer, average genomic coverage and corresponding mapping interval.

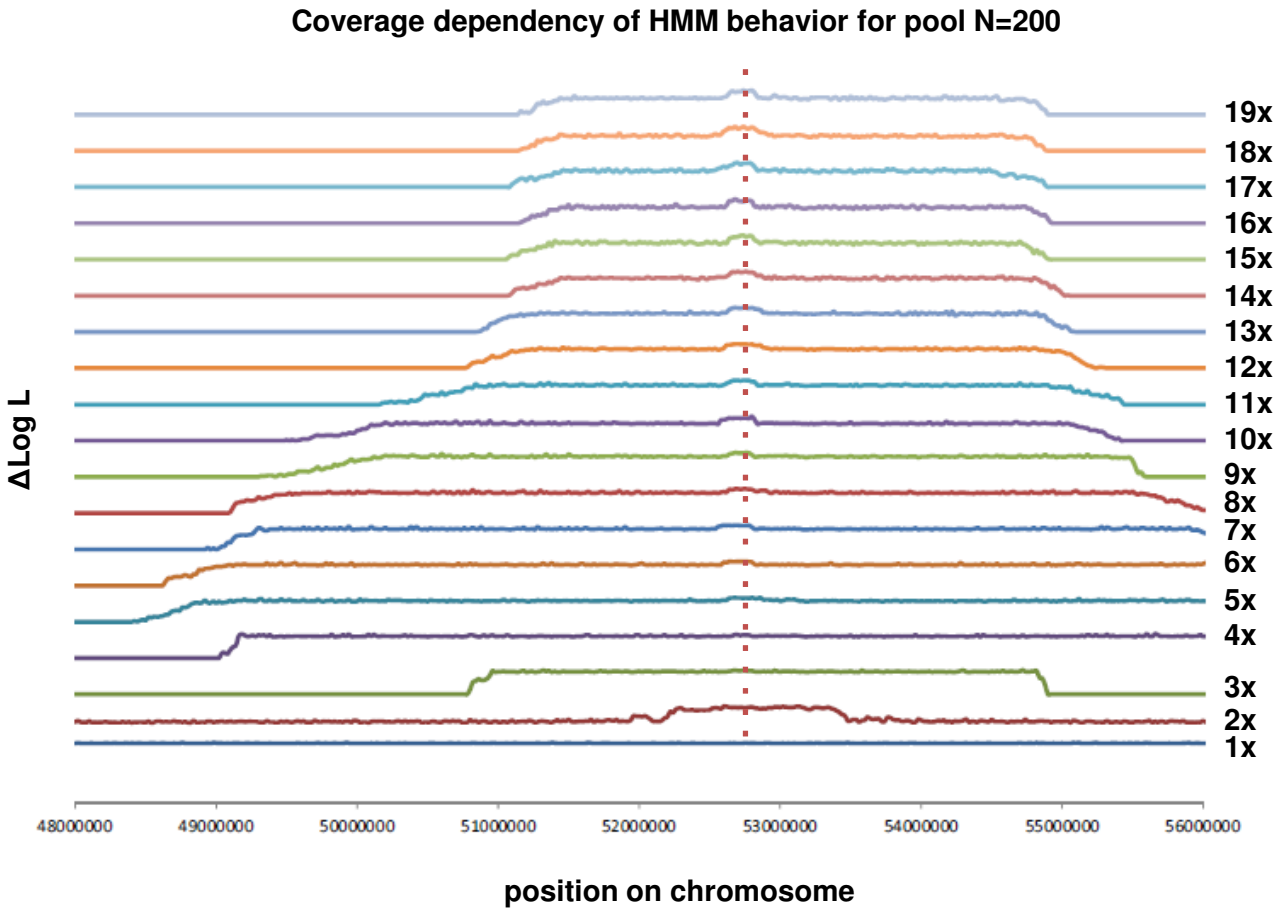
Supplementary Figure S1



Supplementary Figure S1

Model experiment to analyze the required minimum coverage to roughly map the mutation region. The initial ~90 million reads received for both pools of ca1 were downsampled to 10, 20 and 30 million reads accordingly. The homozygosity score still picks up the correct region amid having increased noise level on the whole genome plot.

Supplementary Figure S2

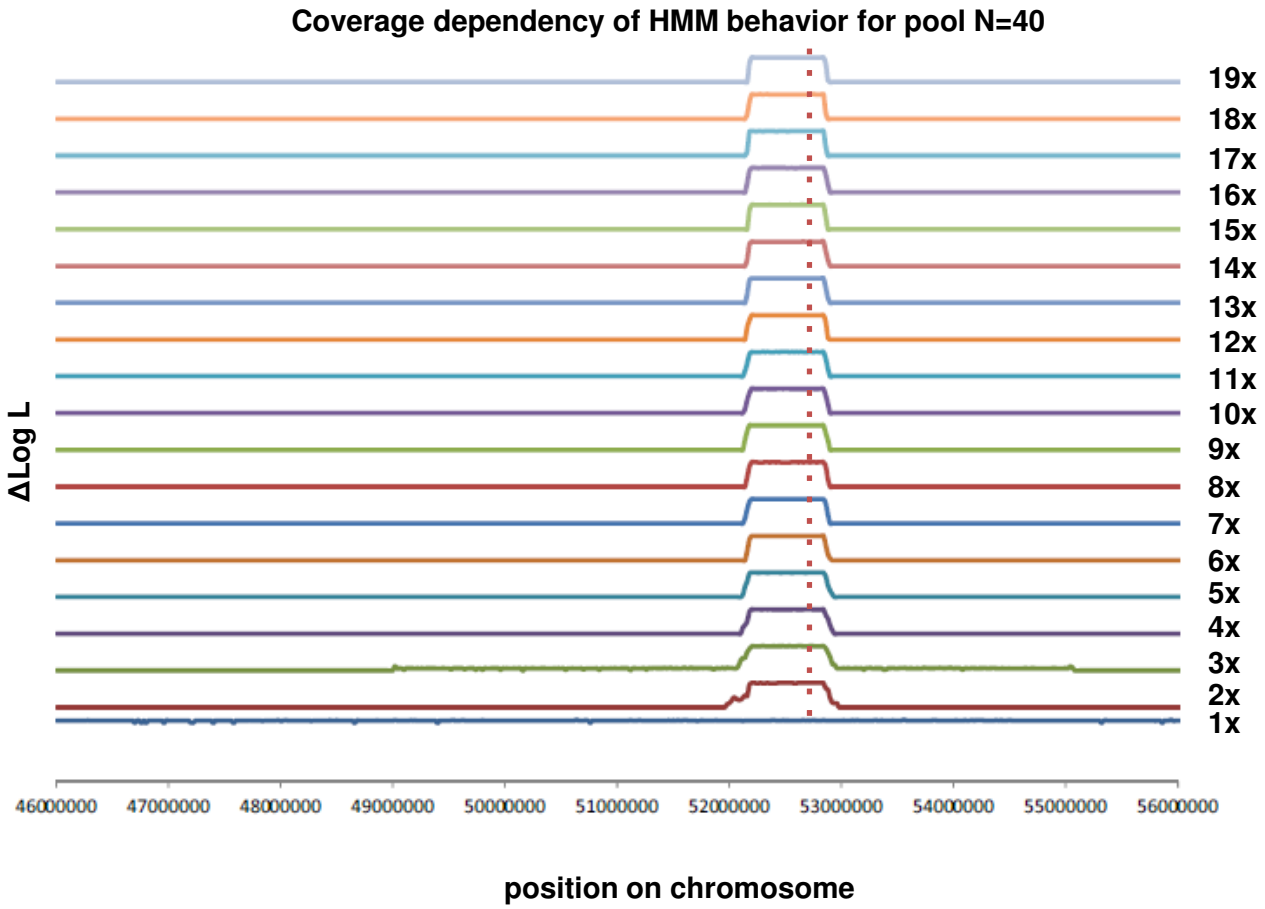


Coverage	Mapped area start	Mapped area end	Mapped Interval
1x	-	-	-
2x	52600000	53120000	520kb
5x	52680000	52860000	180kb
7x	52600000	52700000	100kb
11x	52680000	52760000	80kb
18x	52680000	52740000	60kb

Supplementary Figure S2

Simulation experiment to analyze the effect of sequencing depth and pool size on the HMM accuracy and behavior. Results for a pool of DNA from 200 individuals.

Supplementary Figure S3

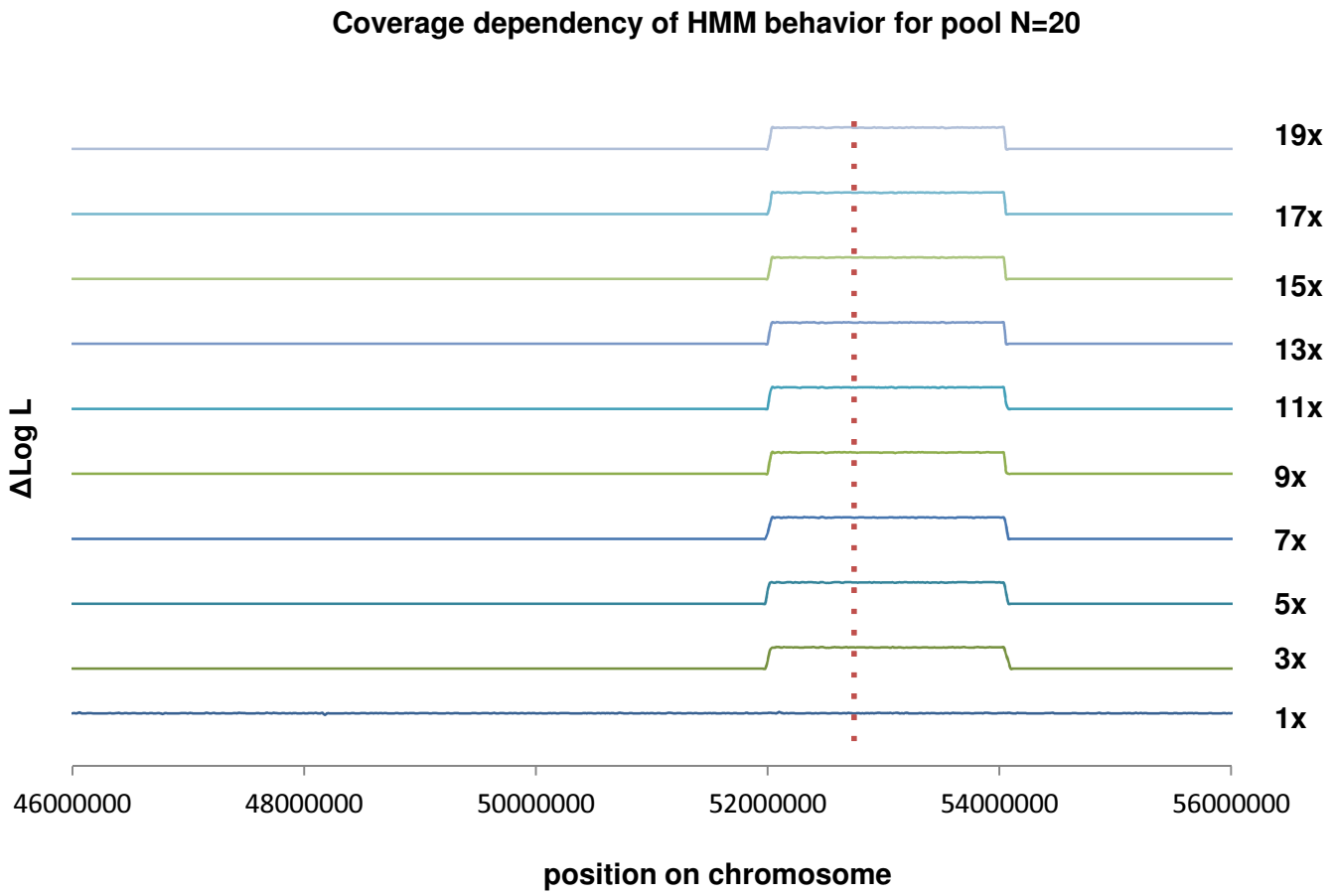


Coverage	Mapped area start	Mapped area end	Mapped Interval
1x	50640000	53560000	-
2x	52200000	52840000	640kb
3x	52280000	52840000	560kb
7x	52200000	52800000	600kb
11x	52300000	52780000	480kb
15x	52360000	52740000	380kb

Supplementary Figure S3

Simulation experiment to analyze the effect of sequencing depth and pool size on the HMM accuracy and behavior. Results for a pool of DNA from 40 individuals.

Supplementary Figure S4

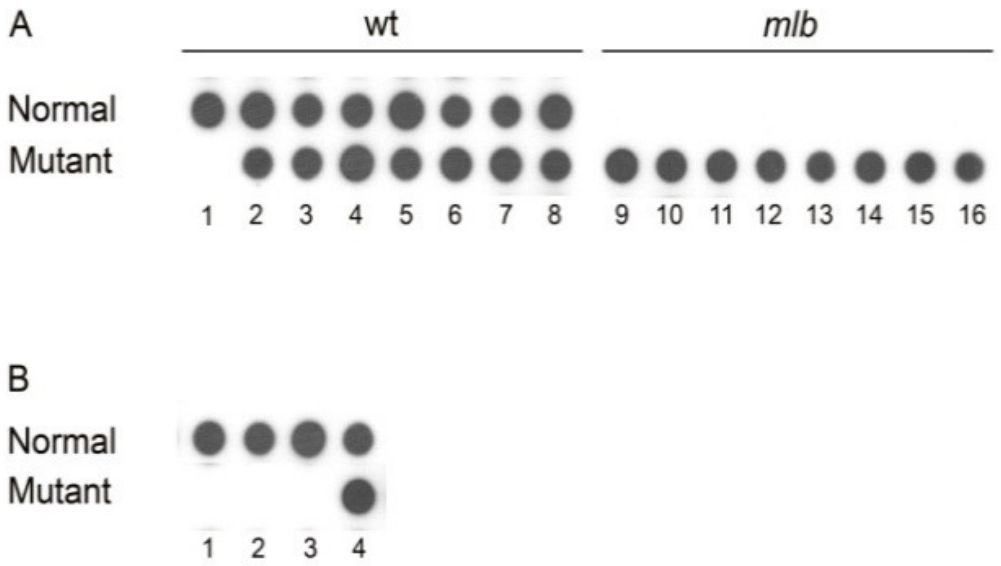


Coverage	Mapped area start	Mapped area end	Mapped Interval
1x	-	-	-
3x	52080000	53920000	1840kb
9x	52040000	53900000	1860kb
13x	52080000	53880000	1800kb

Supplementary Figure S4

Simulation experiment to analyze the effect of sequencing depth and pool size on the HMM accuracy and behavior. Results for a pool of DNA from 20 individuals.

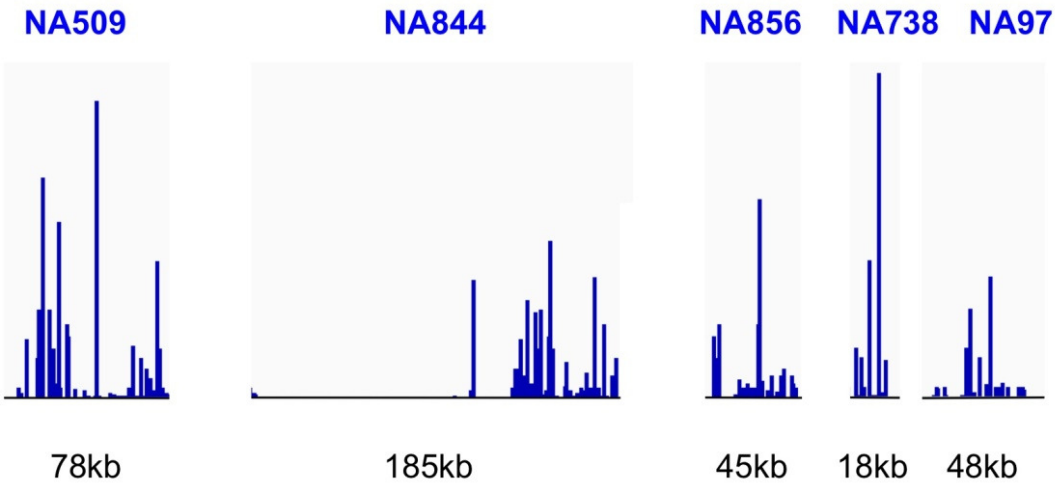
Supplementary Figure S5



Supplementary Figure S5

Allele-specific oligonucleotide (ASO) hybridization assay was employed to show complete co-segregation between the mutant phenotype and genotype from sorted progenies of a *mlb* heterozygous mating. A - Wild type siblings were either +/+ or +/- (lanes 1-8), while phenotypic *mlb* embryos were all homozygous for mutant non-sense allele (lanes 9-16). B - DNA from a mis-identified *mlb* heterozygote individual (lane 4) revealed the presence of a mutant allele by ASO hybridization.

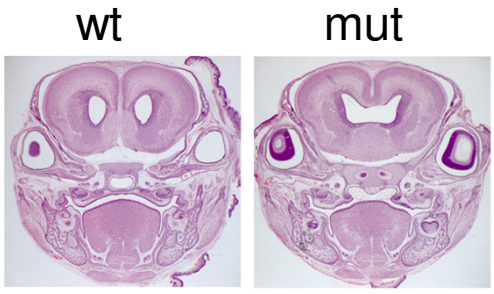
Supplementary Figure S6



Supplementary Figure S6

Example of utilizing NGS-based SNP analysis to place previously unassociated scaffolds into the genome assembly. Sequence from *cloche* ^{s5} mutants aligned to Zv9scaffolds NA509, NA844, NA856, NA738 and NA 97 all demonstrated high homozygosity scores indicating likely association with the mutation region.

Supplementary Figure S7



Supplementary Figure S7

Histological feature of the Line 27H mutant (mut) compared to wild-type (wt) mice.