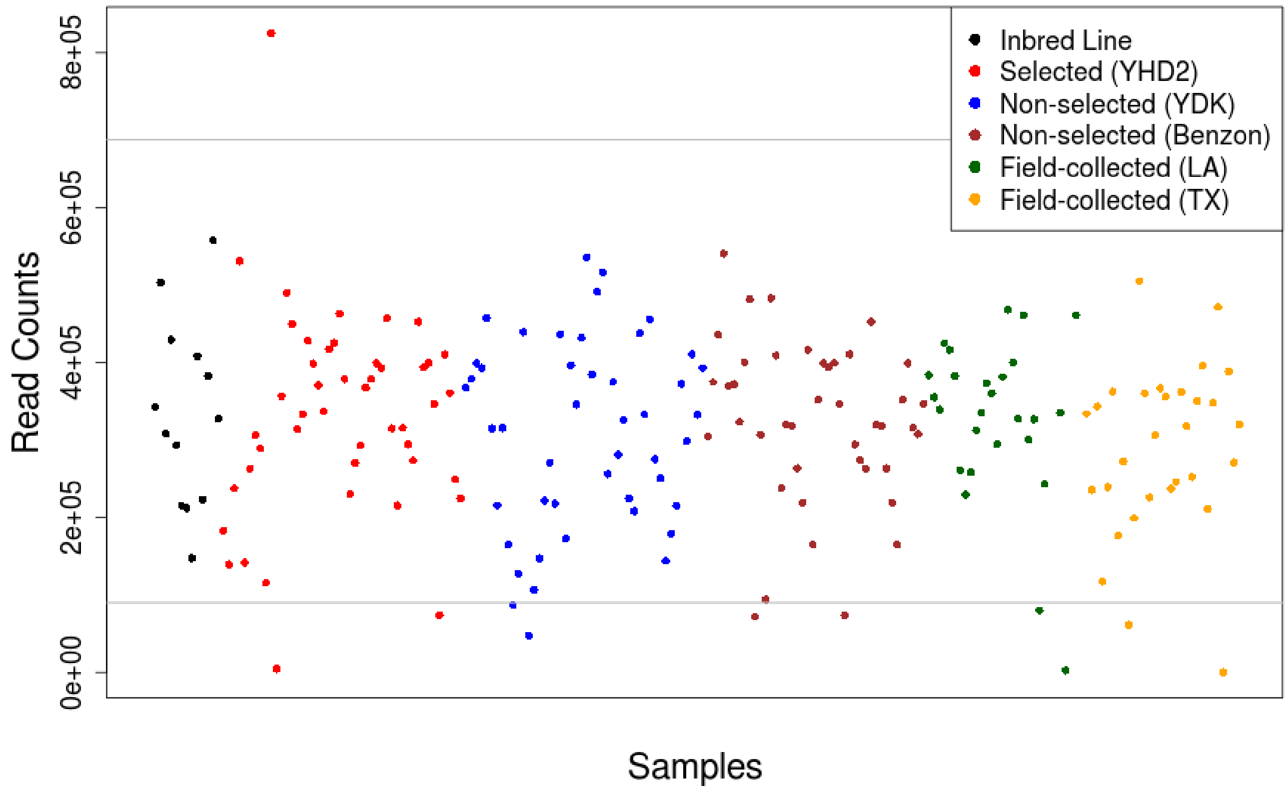
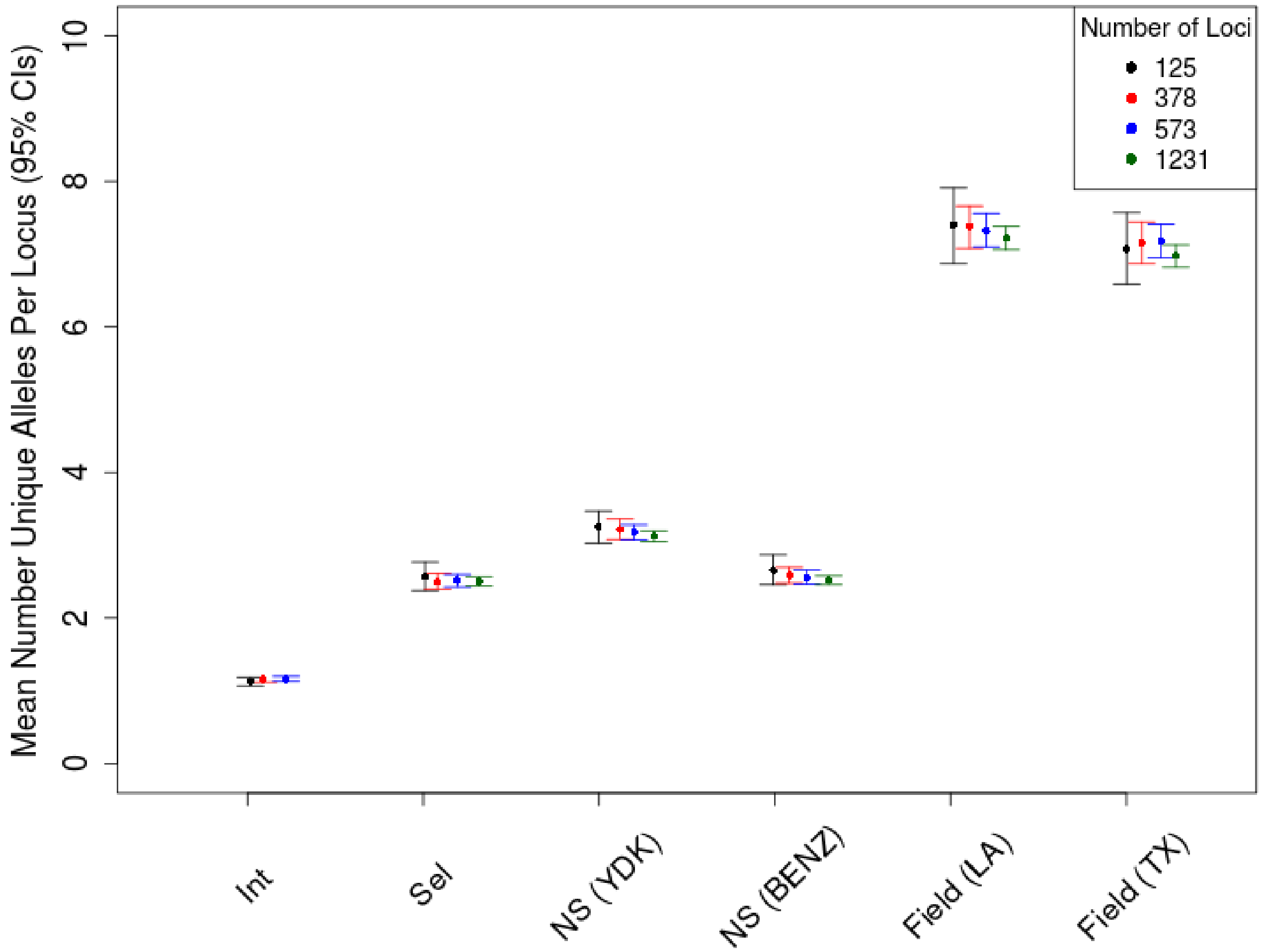
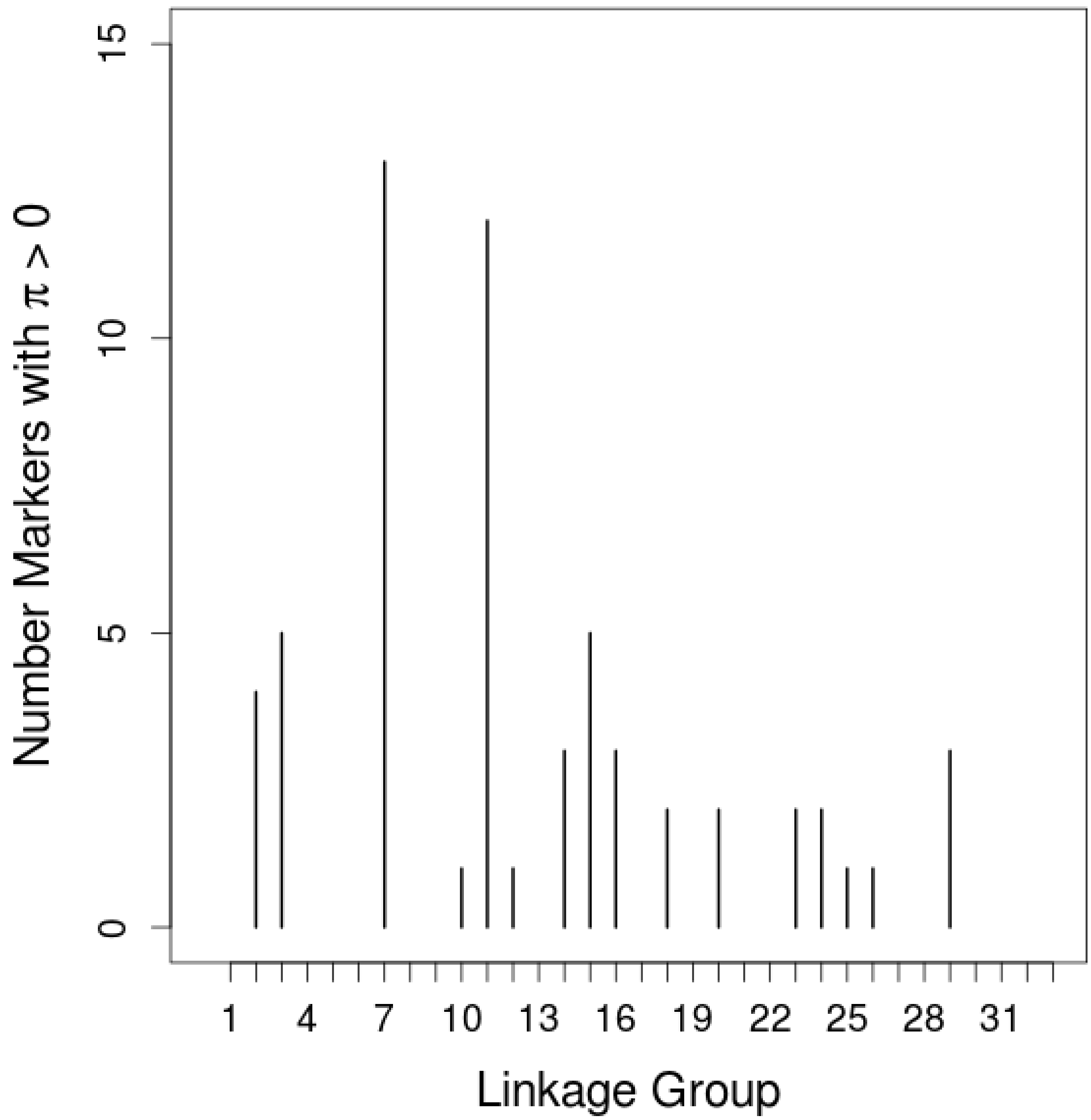


Supplementary Figure 1 - Read counts per individual, color-coded by population. Individuals with read counts above (3×) and below (1/3 ×) the grey lines were excluded from downstream analyses.

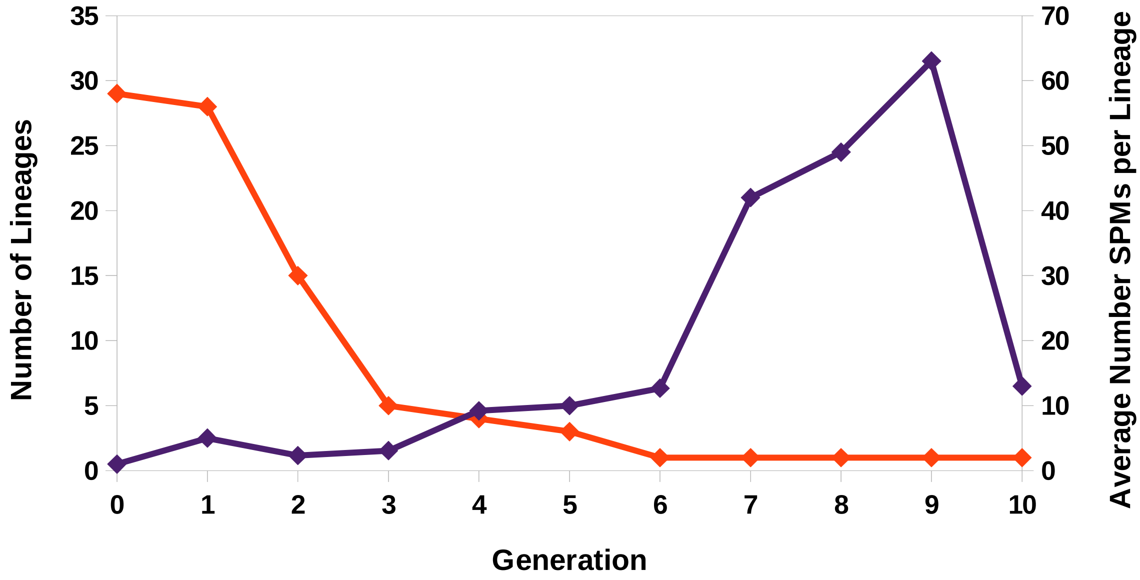


Supplementary Figure 2 – Plot of genome-wide average number of unique alleles (± 95% CIs) across populations. Each dataset, represented by a unique color, contained different numbers of consensus loci. Haplotype sampling was set to either a depth of 6 (data not shown) or 12. Mean numbers of unique loci were not different across datasets within a population, as indicated by overlapping 95% confidence intervals.

Supplementary Figure 3 - Number of markers per linkage group with a nucleotide diversity value (π) of greater than zero in the inbred line.



Supplementary Figure 4 – Number of lineages that remained following each generation of full-sibling mating (orange) during production of the inbred line. The blue line represents the average number of SPMs set up per generation across remaining lineages. Only a single lineage existed following generation 5, at which the blue line represents the number of SPMs set up for the existing line.



Supplementary Table 1 – HPSF-purified oligonucleotide sequences (Eurofins MWG Operon LLC, Huntsville, AL, USA) used to create uniquely barcoded double-stranded adapters with an EcoRI overhang site. Two oligonucleotides (p1.1 and p1.2) corresponding to the same barcode were annealed according to Poland *et al.* (2012) to generate each adapter.

Oligonucleotide Name	Barcode	Nucleotide Sequence 5'-3'
p1.1_EcoRI_CGCGGT	CGCGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCGGT
p1.1_EcoRI_CTATTA	CTATTA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATTA
p1.1_EcoRI_GCCAGT	GCCAGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCAGT
p1.1_EcoRI_GGAAGA	GGAAGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAAGA
p1.1_EcoRI_GTACTT	GTACTT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACTT
p1.1_EcoRI_GTTGAA	GTTGAA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTGAA
p1.1_EcoRI_TAACGA	TAACGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAACGA
p1.1_EcoRI_TGGCTA	TGGCTA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGCTA
p1.1_EcoRI_TATTTTT	TATTTTT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATTTTT
p1.1_EcoRI_CTTGCTT	CTTGCTT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGCTT
p1.1_EcoRI_ATGAAAC	ATGAAAC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAAAC
p1.1_EcoRI_AAAAGTT	AAAAGTT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAAGTT
p1.1_EcoRI_GAATTCA	GAATTCA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAATTCA
p1.1_EcoRI_GAACCTC	GAACCTC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAACCTC
p1.1_EcoRI_GTCGATT	GTCGATT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCGATT
p1.1_EcoRI_AACGCCT	AACGCCT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACGCCT
p1.1_EcoRI_AATATGC	AATATGC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATATGC
p1.1_EcoRI_ACGTGTT	ACGTGTT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTGTT
p1.1_EcoRI_ATTAATT	ATTAATT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTAATT
p1.1_EcoRI_ATTGGAT	ATTGGAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGGAT
p1.1_EcoRI_CATAAGT	CATAAGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAAGT
p1.1_EcoRI_CGCTGAT	CGCTGAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCTGAT
p1.1_EcoRI_CGGTAGA	CGGTAGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGTAGA
p1.1_EcoRI_CTACGGA	CTACGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTACGGA
p1.1_EcoRI_GCGGAAT	GCGGAAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGGAAT
p1.1_EcoRI_TAGCGGA	TAGCGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCGGA
p1.1_EcoRI_TCGAAGA	TCGAAGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAAGA
p1.1_EcoRI_TCTGTGA	TCTGTGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGTGA
p1.1_EcoRI_TGCTGGA	TGCTGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCTGGA
p1.1_EcoRI_ACGACTAC	ACGACTAC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGACTAC
p1.1_EcoRI_TAGCATGC	TAGCATGC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCATGC
p1.1_EcoRI_TAGGCCAT	TAGGCCAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGGCCAT
p1.1_EcoRI_TGCAAGGA	TGCAAGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAAGGA
p1.1_EcoRI_TGGTACGT	TGGTACGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGTACGT
p1.1_EcoRI_TCTCAGTC	TCTCAGTC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCAGTC
p1.1_EcoRI_CCGGATAT	CCGGATAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCGGATAT
p1.1_EcoRI_CGCCTTAT	CGCCTTAT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCCTTAT
p1.1_EcoRI_AACCGAGA	AACCGAGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACCGAGA
p1.1_EcoRI_ACAGGGAA	ACAGGGAA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGGGAA
p1.1_EcoRI_ACGTGGTA	ACGTGGTA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTGGTA
p1.1_EcoRI_CCATGGGT	CCATGGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATGGGT
p1.1_EcoRI_CGCGGAGA	CGCGGAGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCGGAGA
p1.1_EcoRI_CGTGTGGT	CGTGTGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTGTGGT
p1.1_EcoRI_GCTGTGGA	GCTGTGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGTGGA
p1.1_EcoRI_GGATTGGT	GGATTGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATTGGT
p1.1_EcoRI_GTGAGGGT	GTGAGGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAGGGT
p1.1_EcoRI_TATCGGGA	TATCGGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATCGGGA
p1.1_EcoRI_TTCCTGGA	TTCCTGGA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCCTGGA

Supplementary Table 1 continued.

Oligonucleotide Name	Barcode	Nucleotide Sequence 5'-3'
p1.2_EcoRI_CGCGGT	CGCGGT	[Phos]AATTACCGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CTATTA	CTATTA	[Phos]AATTTAATAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GCCAGT	GCCAGT	[Phos]AATTACTGGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GGAAGA	GGAAGA	[Phos]AATTTCTTCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GTACTT	GTACTT	[Phos]AATTAAGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GTTGAA	GTTGAA	[Phos]AATTTTCAACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TAACGA	TAACGA	[Phos]AATTTCGTTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TGGCTA	TGGCTA	[Phos]AATTTAGCCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TATTTTT	TATTTTT	[Phos]AATTAAAAATAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CTTGCTT	CTTGCTT	[Phos]AATTAAGCAAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ATGAAAC	ATGAAAC	[Phos]AATTGTTTCATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_AAAAGTT	AAAAGTT	[Phos]AATTAACTTTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GAATTCA	GAATTCA	[Phos]AATTTGAATTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GAACCTC	GAACCTC	[Phos]AATTGAGGTTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GTCGATT	GTCGATT	[Phos]AATTAATCGACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_AACGCCT	AACGCCT	[Phos]AATTAGGCGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_AATATGC	AATATGC	[Phos]AATTGCATATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ACGTGTT	ACGTGTT	[Phos]AATTAACACGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ATTAATT	ATTAATT	[Phos]AATTAATTAATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ATTGGAT	ATTGGAT	[Phos]AATTATCCAATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CATAAGT	CATAAGT	[Phos]AATTACTTATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CGCTGAT	CGCTGAT	[Phos]AATTATCAGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CGGTAGA	CGGTAGA	[Phos]AATTTCTACCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CTACGGA	CTACGGA	[Phos]AATTTCCGTAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GCGGAAT	GCGGAAT	[Phos]AATTATTCCGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TAGCGGA	TAGCGGA	[Phos]AATTTCCGCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TCGAAGA	TCGAAGA	[Phos]AATTTCTTCGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TCTGTGA	TCTGTGA	[Phos]AATTTCACAGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TGCTGGA	TGCTGGA	[Phos]AATTTCCAGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ACGACTAC	ACGACTAC	[Phos]AATTGTAGTCGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TAGCATGC	TAGCATGC	[Phos]AATTGCATGCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TAGGCCAT	TAGGCCAT	[Phos]AATTATGGCCTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TGCAAGGA	TGCAAGGA	[Phos]AATTTCCTTGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TGGTACGT	TGGTACGT	[Phos]AATTACGTACCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TCTCAGTC	TCTCAGTC	[Phos]AATTGACTGAGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CCGGATAT	CCGGATAT	[Phos]AATTATATCCGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CGCCTTAT	CGCCTTAT	[Phos]AATTATAAGGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_AACCGAGA	AACCGAGA	[Phos]AATTTCTCGGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ACAGGGAA	ACAGGGAA	[Phos]AATTTTCCCTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_ACGTGGTA	ACGTGGTA	[Phos]AATTTACCACGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CCATGGGT	CCATGGGT	[Phos]AATTACCCATGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CGCGGAGA	CGCGGAGA	[Phos]AATTTCTCCGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_CGTGTGGT	CGTGTGGT	[Phos]AATTACCACACGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GCTGTGGA	GCTGTGGA	[Phos]AATTTCCACAGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GGATTGGT	GGATTGGT	[Phos]AATTACCAATCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_GTGAGGGT	GTGAGGGT	[Phos]AATTACCCTCACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TATCGGGA	TATCGGGA	[Phos]AATTTCCCGATAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
p1.2_EcoRI_TTCCTGGA	TTCCTGGA	[Phos]AATTTCCAGGAAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Supplementary Table 2 - HPSF-purified oligonucleotide sequences (Eurofins MWG Operon LLC, Huntsville, AL, USA) used to create MSP overhang, and primers used to add Illumina indices to each gDNA library.

Oligo Name	Oligo Type	Index	Oligonucleotide Sequence 5'-3'
MSPI_P2.1	Adapter – MSP overhang	-	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
MSPI_P2.2	Adapter – MSP overhang	-	[Phos]CGAGATCGGAAGAGCGAGAACAA
ddRAD_PCR1	Primer – Forward	-	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
PCR2_Idx1	Primer – Reverse	ATCACG	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx2	Primer – Reverse	CGATGT	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx3	Primer - Reverse	TTAGGC	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx4	Primer - Reverse	TGACCA	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx6	Primer – Reverse	GCCAAT	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx12	Primer – Reverse	CTTGTA	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGC