New whole genome de novo assemblies of three divergent strains of rice (*O. sativa*) documents novel gene space of *aus* and *indica*

Supplementary Tables and Figures

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Supplementary Table 1. Maker input Sequences

Sequence evidence used for protein-coding gene annotation by MAKER-P

Evidence	Sequence Count
Oryza EST (NCBI)	1,319,326
Oryza FL_CDNA & mRNA (NCBI)	61,203
Nipponbare annotated CDS (non-redundant IRGSP-1.0 + MSU7.0: Kawahara et al. 2013)	73,621
93-11 annotated CDS (Gao et al. 2013)	37,573
PA64s annotated CDS (Gao et al. 2013)	34,690
Nipponbare annotated proteins, non-redundant IRGSP-1.0 + MSU7.0 (Kawahara et al. 2013)	67,725
93-11 annotated proteins (Gao et al. 2013)	37,573
PA64s annotated proteins (Gao et al. 2013)	34,690

Supplementary Table 2. Gene summary statistics of MAKER-P annotation and comparison to MSU and RAP-DB rice annotations of IRGSP1.0.

Assembler / Parameters	DJ123 MAKER	IR64 MAKER	Nipponbare MAKER	IRGSP1.0 MSU	IRGSP1.0 RAP-DB
Gene count (protein-coding)	37812	37758	39083	39049	35472
Median gene length (nt)	2285	2275	2224	2188	2458
Average transcripts/gene	1.36	1.35	1.34	1.26	1.18
Median coding length (nt)*	873	873	846	849	803
Median peptide length (aa)*	291	291	282	283	268
Median exon length (nt)*	157	158	157	165	177
Median intron length (nt)*	185	186	188	174	155
Average exons/transcript*	4.9	4.8	4.8	4.3	4.4
Percent genes single-exon	20.7	20.6	20.7	24.9	28.8
*Based on single representative transcript per gene having longest coding sequence					

Supplementary Table 3. Alternate Nipponbare assemblies

Note, the SOAPdenovo assemblies used Quake to error correct the reads before assembly (indicated with a *), while ALLPATHS-LG and SGA have integrated error correction packages so used the raw reads directly.

Assembler / Parameters	Libraries	Span (Mbp) Bases (Mbp)	Scaffold N50 (kbp)	Contig N50 (kbp)
ALLPATHS-LG				
+ MIN_CONTIG=300	180bp frag 2.1kbp jump 4.8kbp jump	355.6 322.5	213.7	21.5
+ MIN_CONTIG=300	180bp frag 1.8kbp jump 2.1kbp jump	357.6 317.2	98.7	20.8
SOAPdenovo				
+ K=33	180bp frag* 2.1kbp jump* 4.8kbp jump*	364.9 263.3	5.5	0.6
+ K=35	“	368.7 272.8	5.9	0.8
+ K=37	“	366.3 281.9	6.2	1.0
+ K=39	“	368.1 289.7	6.6	1.2
+ K=41	“	368.7 296.4	7.0	1.4
+ K=43	“	359.1 302.7	5.6	1.7
+ K=45	“	359.8 307.5	5.7	1.9

Assembler / Parameters	Libraries	Span (Mbp) Bases (Mbp)	Scaffold N50 (kbp)	Contig N50 (kbp)
SGA				
+ K=71	180bp frag 2.1kbp jump 4.8kbp jump	380.3 512.1	13.6	1.2
+ K=73	“	379.3 498.4	13.8	1.5
+ K=75	“	378.3 485.5	13.9	1.7
+ K=77	“	376.7 472.9	13.9	2.0

Supplementary Table 4a. Nipponbare-specific PCR Validated regions

Note the ordering and gene status has slightly changed since the analysis was finalized for publication.

#	Scaffold / Coordinates	Forward Primer / Reverse Primer	Notes
1	scaffold_45 198303 - 211765	AACATAGCGGGGAAGGCCTT GACGCCAGTCTAGGTCCCAC	
2	scaffold_450 64623 - 76993	TGTCCTCCACGTGCCTGTTC TTCACAAGTGGACCGCTCGT	
3	scaffold_849 41087 - 53325	TACGCCGCCGTCAATACCAG TGGACGAGAGGAAGAGGGGT	
4	scaffold_139 54757 - 66719	GGCACCTCGCATCTCAAGGT CGGGCACAATTCCGACTACAC	
5	scaffold_37 99481 - 111372	TTGGGCCGTAAGCTTGAACC CATCATGTGCCTTGTGTACGTG	
6	scaffold_995 40472 - 51224	TCTCGCTGGACGGTCTCTGA ACCTTCCCTTGTTGATGCCCT	Currently 8th longest
7	scaffold_18 671704 - 681347	GTGCCACACTTGCTGATGGC ACCATCAGTTTGTTTTCGGCCC	Currently 9th longest
8	scaffold_290 184750 - 192614	GGAACGCTGAGGCACACAAG GACGTTGTTGCCAGGCTCAG	No longer intersects an exon
9	scaffold_77 448149 - 458404	CTGAACCACATGACCGCTGC GATCGTCGGCTTGTCGGAGA	Currently 10th longest
10	scaffold_2377 14202 - 21834	TTTTTCCGCCGGCCAAAACG TGGGCCGGAGTAAAACAATCA	Currently 11th longest

Supplementary Table 4b. IR64-specific PCR validated regions

Note the ordering and gene status has slightly changed since the analysis was finalized for publication.

#	Scaffold / Coordinates	Forward Primer / Reverse Primer	Notes
1	scaffold_918 12095 - 27494	GGCATTAGCACAGGCAGCAG CAATTGAAAGGCCACTCACCCT	
2	scaffold_712 115731 - 129191	AGTATTCAGCTCTGTGGCAGCA TGGATCGACACAGCTCCGTG	
3	scaffold_1091 78493 - 90663	GGTGCGTGTTGGATGATGCT ACTGATTGGACAAGGGCGGC	
4	scaffold_408 2395 - 14990	GCGCTTGAGTTGGGATGCTA ACGGCATTAGCAGGGGACAA	
5	scaffold_299 284265 - 296829	CCCTCCTTTGTGACAGGCCA GCGGCAACAGACTCGTTATCG	
6	scaffold_479 190704 - 202637	CACGAGTTGCAACTGCCAGT ACATGTGCCGATCCCATGGA	Currently 7th longest
7	scaffold_382 149495 - 161037	ACAGGACGGGACTGTTCGTC TTGAGCCCTTCATGCACCCT	Currently 8th longest
8	scaffold_1261 47601 - 59036	GCCCATACACCGTCATGGGT GGACAGCGTGGTGTACAGAGA	Currently 9th longest
9	scaffold_201 190458 - 201884	CCATATGCCGGCCAGGATCT TAGTGTGGCACATCGCAACC	Currently 10th longest
10	scaffold_977 3058 - 14164	GTTGTCGTCGCATCCGTGTC CCATGGATCAACCCGGTGTG	Currently 11th longest

Supplementary Table 4c. DJ123-specific PCR validated regions

Note the ordering and gene status has slightly changed since the analysis was finalized for publication.

Num	Scaffold / Coordinates	Forward Primer / Reverse Primer	Notes
1	scaffold_1266 34254 - 47074	CTGCCACAAGCCTCCCAATT CCCAGGGCTCCTTATGCGAT	No longer intersects a gene
2	scafold_903 70491 - 82898	TCCGCAGCATAGAAGGCCC GCCCATAGATGCGCCATCCA	
3	scaffold_1392 10086 - 22243	ACAGCCACTGCCACATGTGA TGGGCTGATCGATCATGCGA	
4	scaffold_185 381192 - 292627	AACCGAGCGACTGTAGCCAC TGATGCTCTTGAGGGCGACA	
5	scaffold_318 70856 - 82242	TTGACCATGGGCAAGACTGG GCTCATTGGACAAGGCGGTT	
6	scafold_289 28477 - 49393	TATCAGCGTCGACCTGGTGG ACAAGCTGCCTCACCGATGT	Currently 7th longest
7	scaffold_328 55971 - 66840	TGGGCCCAATCAGATGCCAT GAGCGACCCCTTAGGCCTTC	Currently 8th longest
8	scaffold_92 124892 - 135721	TGACGGAGCTGCTGAAGGAG TTGACAAGGCAGCGACGGAT	Currently 9th longest
9	scaffold_82 177274 - 187967	TGGACATTGTGGTGCAGCCA ACCTGCTCCAAACCAGTCGA	Currently 10th longest
10	scaffold_132 88873 - 99280	CCACCCACCACTCGCACTAG AAGCAACACGGTGTCGGAGA	Currently 11th longest

Supplementary Table 5. Scaffold sizes containing the regions of interest

Region	Nipponbare	IR64	DJ123
S5	279.2 Kb	542.2 Kb	499.5 Kb
Sub1	49.6 Kb	520 Kb	299.5 Kb
LRK	96.4 Kb	210.2 Kb	299 Kb
Pup1	50.5 Kb 36.5 Kb	217.8 Kb	114.8 Kb 69.2 Kb

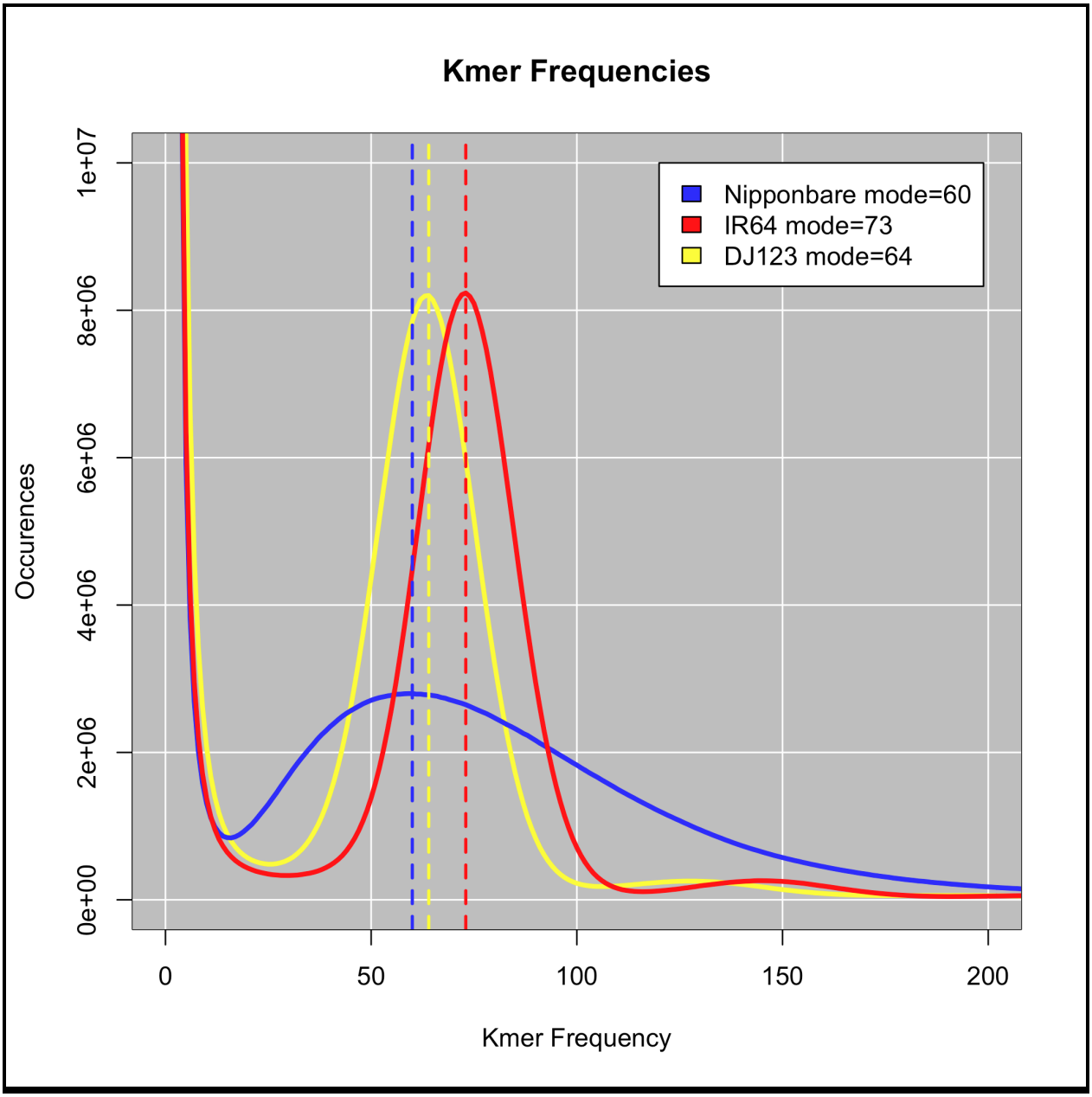
Supplementary Table 6. Primers for regions of interest

Primers for Sanger sequencing to confirm polymorphisms in S5 region

S5-Fwd1	TGCCCCTGAGCAAGCAAGAA
S5-Fwd2	CCTACGTTTGACTGCCTGCC
S5-Fwd3	GTTCGGGTGCAGCATGGATG
S5-Rev1	ACTACTACACGCGGCTTCGG
S5-Rev2	TGGCGCCTTGAGAGTTCACA
S5-Rev3	GTGTAGCGCGGGAGAAGACT

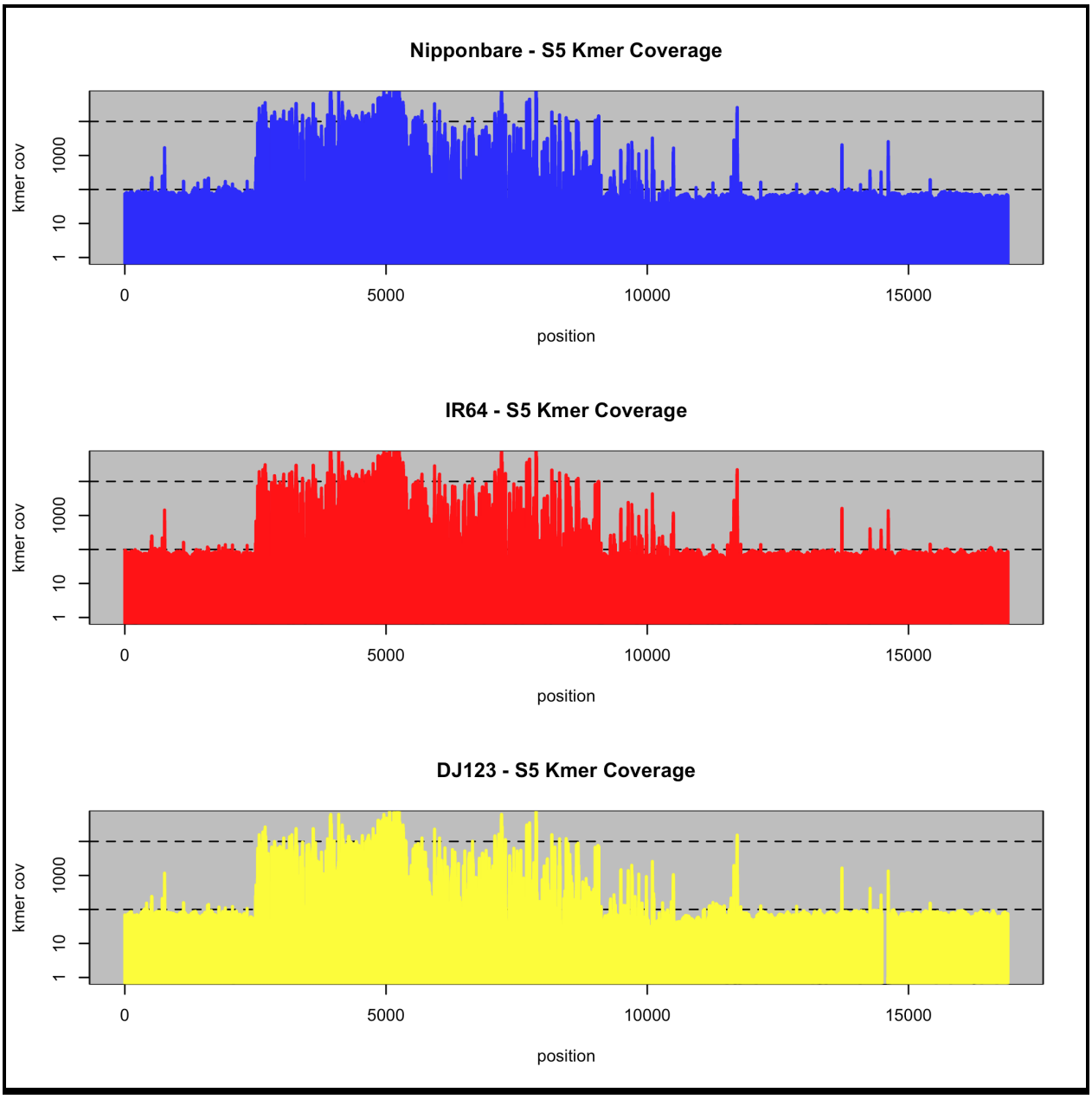
Primers for Sanger sequencing validation of *Pstol1* SNPs

Pstol-Fwd1	ATGGCCGTGAGATAGCCGTC
Pstol-Rev1	AAGCCCTTTTGGTGGCAACG



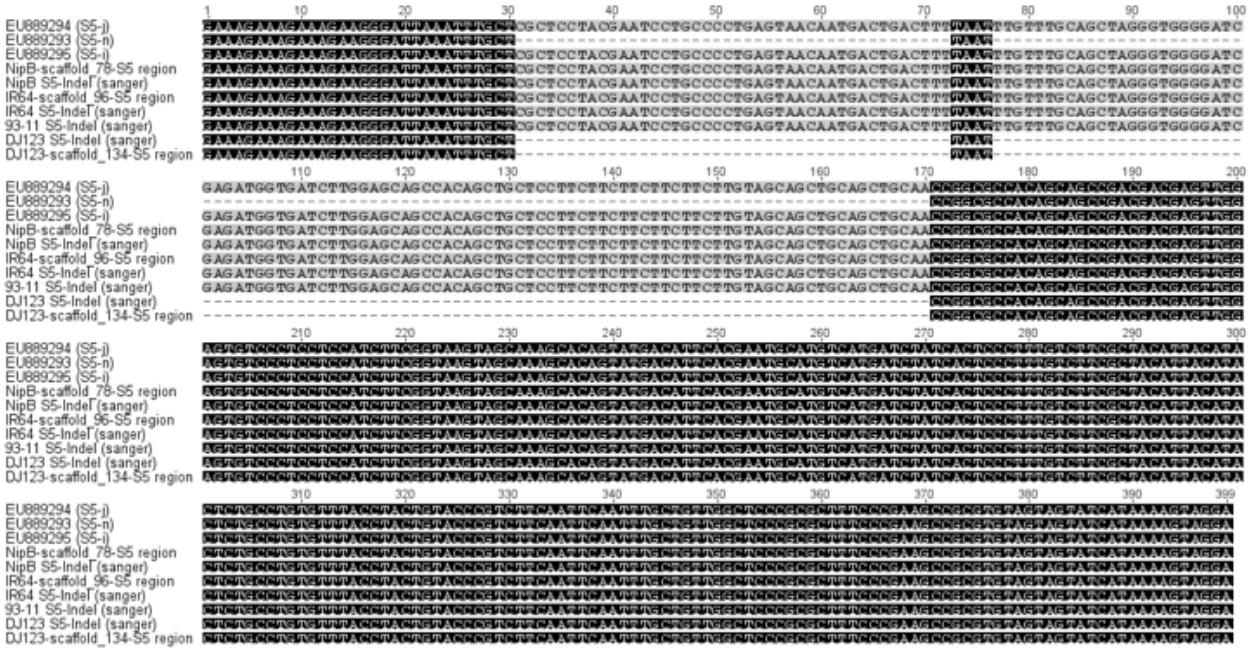
Supplementary Figure 1. K-mer frequency distributions for the three genomes.

The mode values of the distributions are highlighted as dashed vertical lines. DJ123 and IR64 have well resolved peaks from centered at their average coverage levels, Nipponbare has a broad peak because the library had the greatest proportion of duplicate reads.



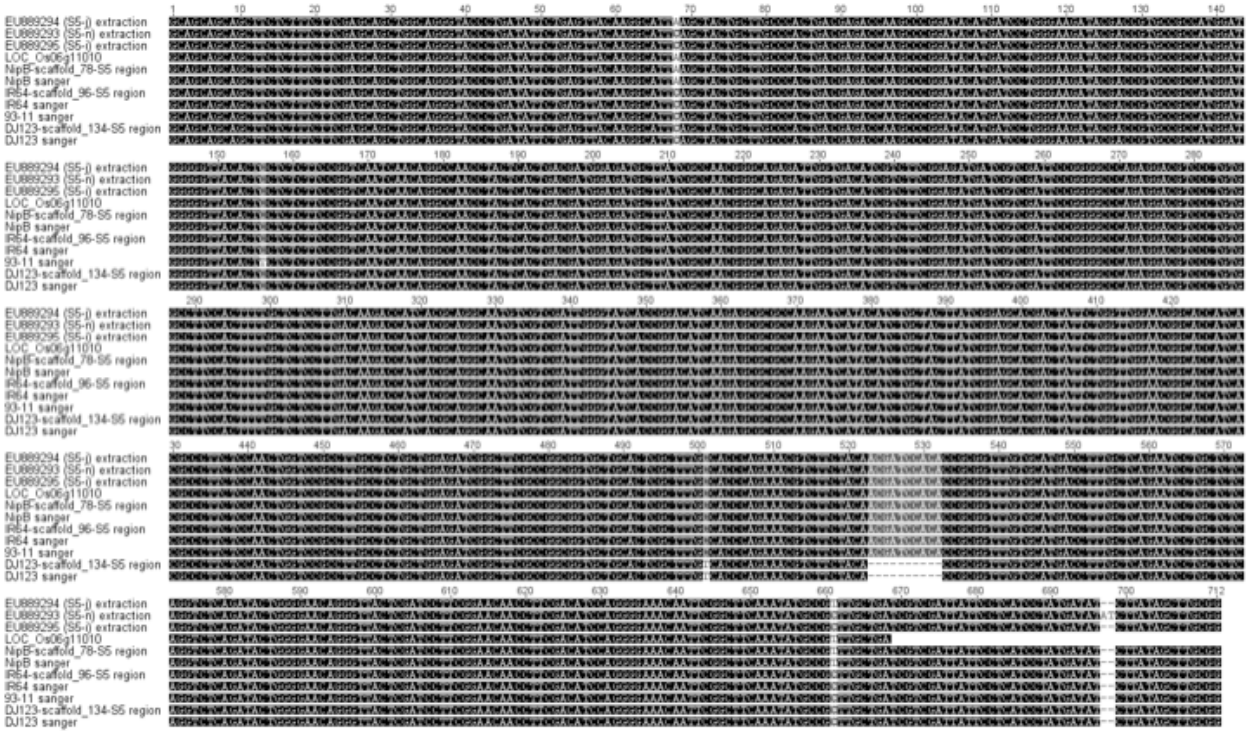
Supplementary Figure 2. K-mer coverage of the *S5* locus.

These plots display the k-mer coverage from the start of the CDS of ORF3 to the end of ORF5 of the Nipponbare reference sequence (NC_008399.2: 5744179-5761023). For clarity, 1x to 50,000x range (log scale) has been displayed in all the plots.



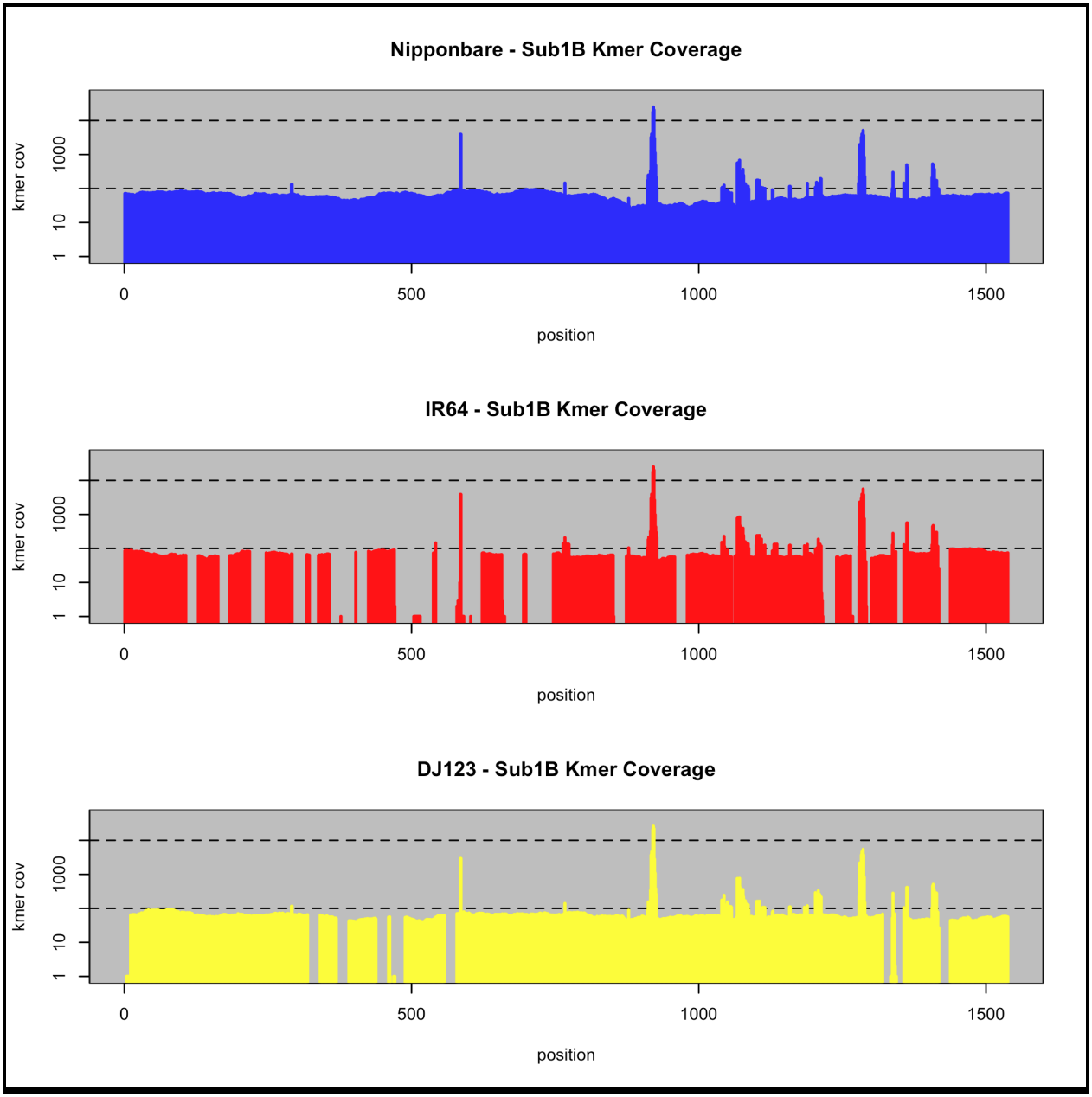
Supplementary Figure 3. Multiple alignment of *S5* Hybrid Sterility Locus: Indel Region

In particular, the indel predicted by the DJ123 scaffold was confirmed by Sanger sequencing.



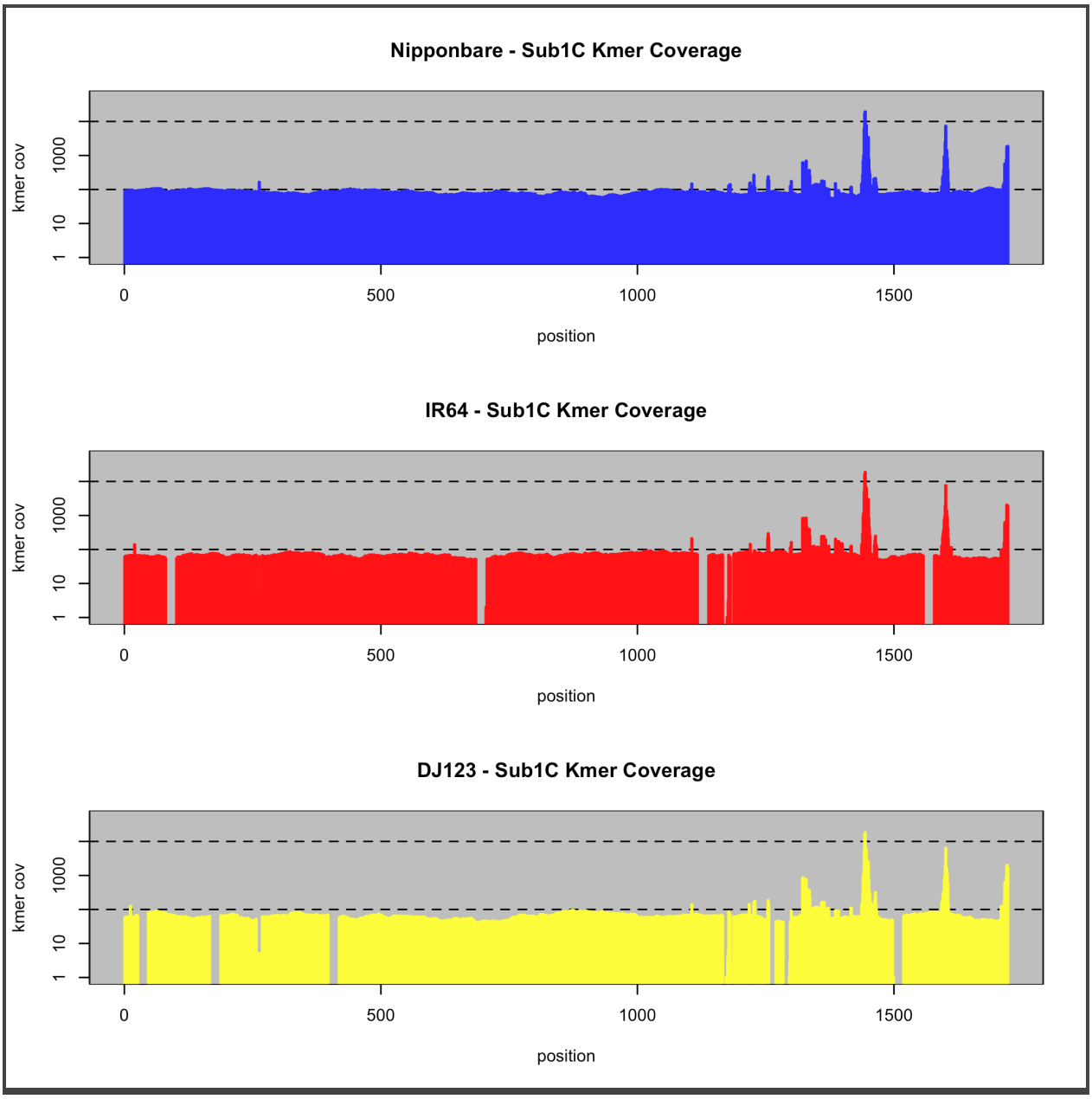
Supplementary Figure 4. Multiple alignment of *S5* Hybrid Sterility Locus: SNP region

In particular, the DJ123 scaffold shows a novel haplotype confirmed with Sanger sequencing.



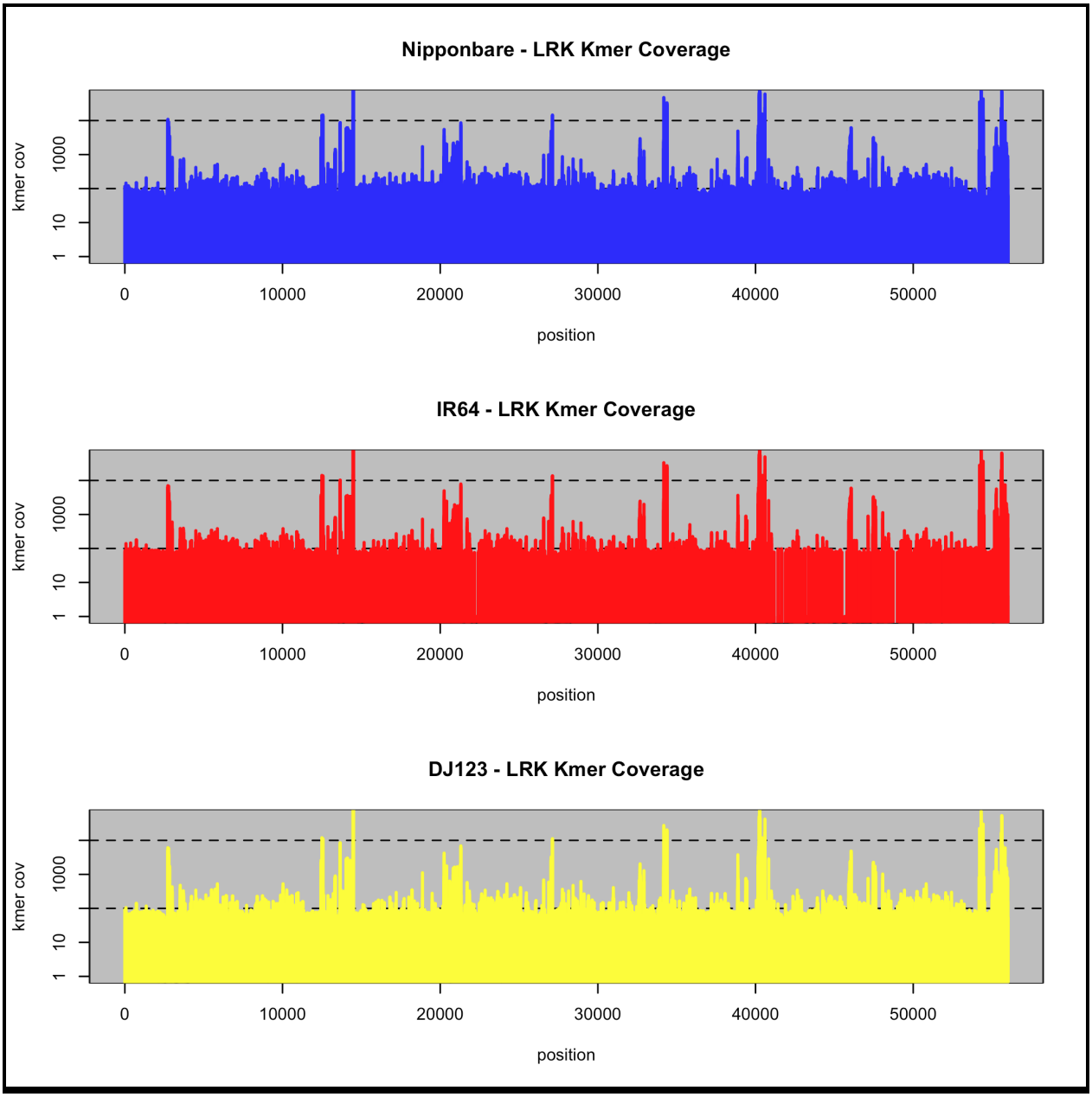
Supplementary Figure 5. K-mer coverage across the *Sub1B* gene.

For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots.



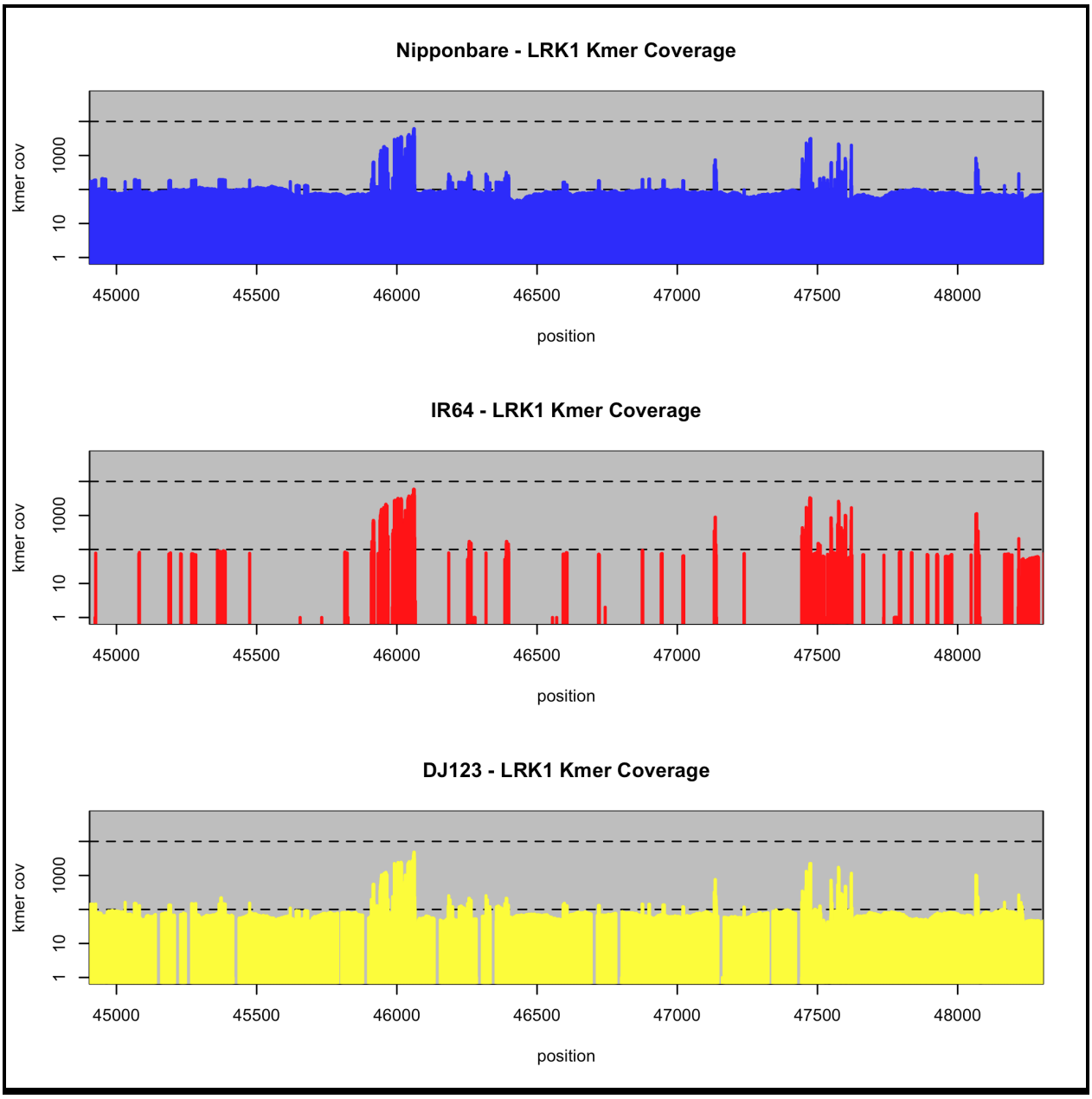
Supplementary Figure 6. K-mer coverage across the *Sub1C* gene.

For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots.



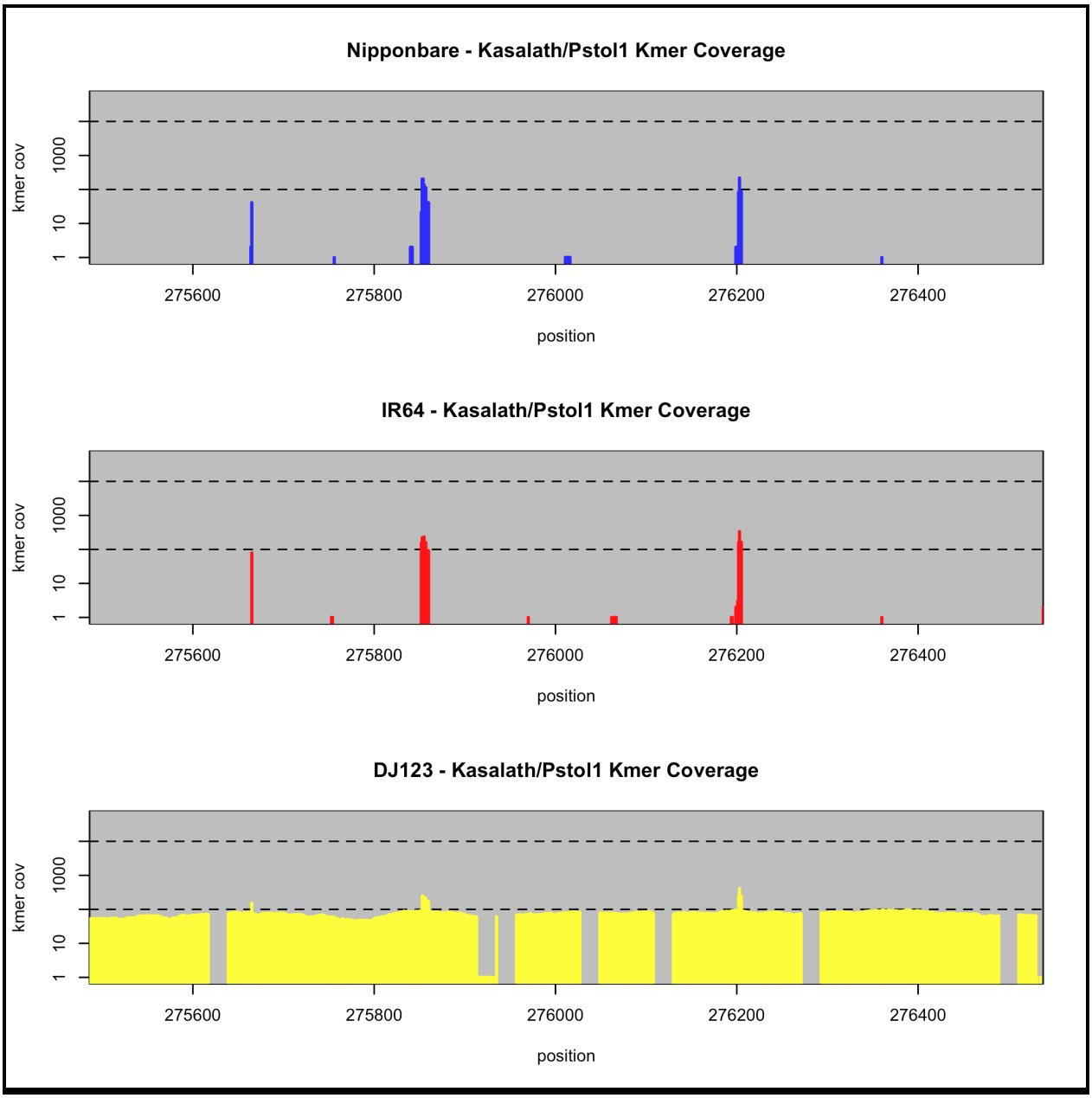
Supplementary Figure 7. K-mer coverage across the entire *LRK* region.

These plots display the k-mer coverage across the locus defined by the Nipponbare reference sequence (chr2:2930001-2986002). For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots.



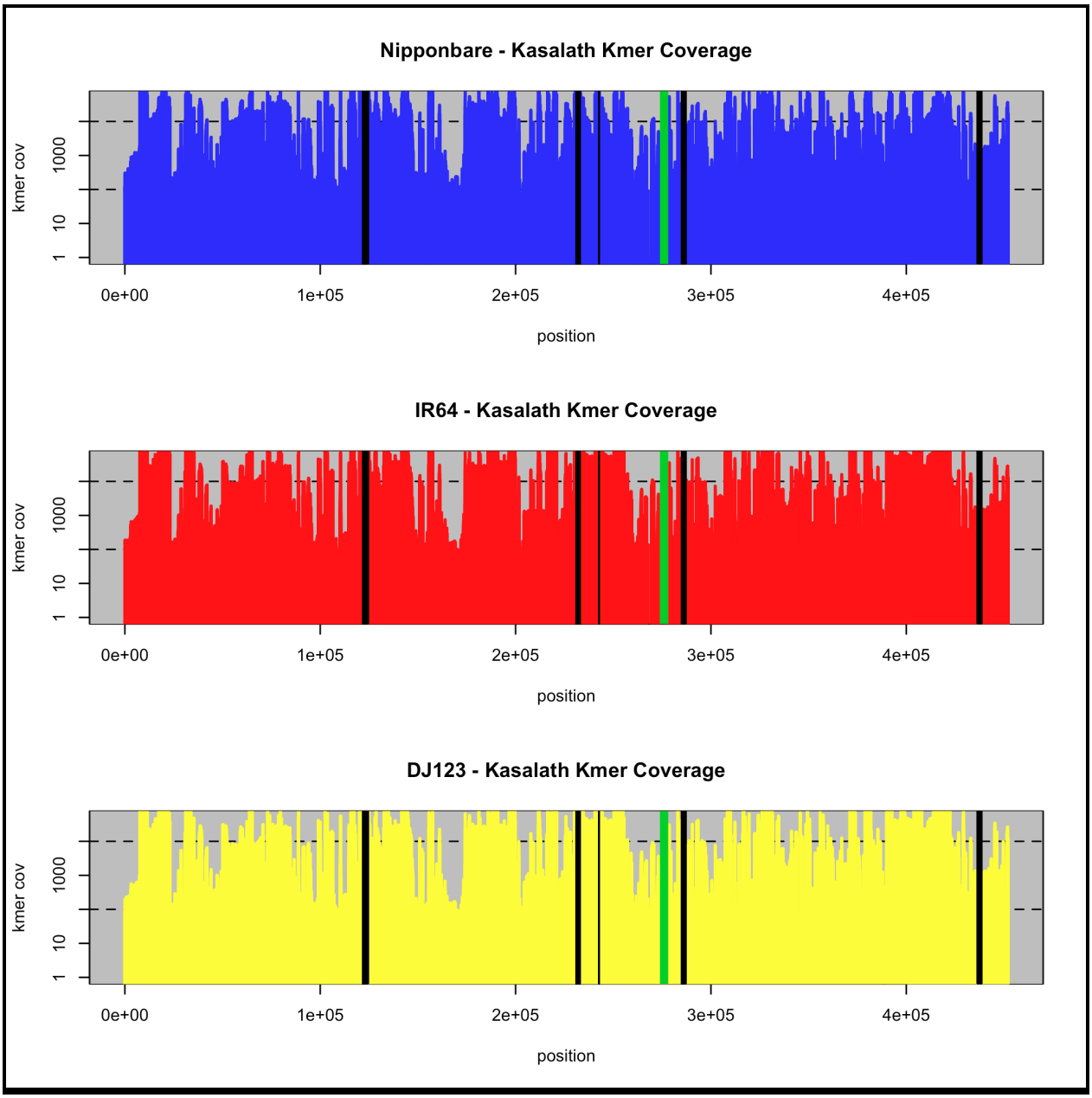
Supplementary Figure 8. K-mer coverage across the LRK1 gene

These plots display the k-mer coverage across the locus defined by the Nipponbare reference sequence (chr2:2930001-2986002) focusing on the interval for LRK1 (45030-48179 of this region). For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots. Nipponbare and DJ123 have nearly uniform coverage across the gene except for SNPs in DJ123 shown as abrupt drops in coverage. The sparse coverage in IR64 indicates the presence of isolated k-mers and repeats shared with the other genomes, but an overall absence of the gene.



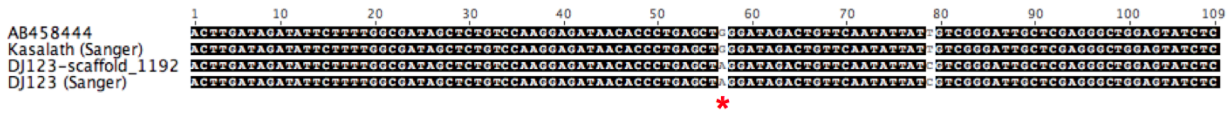
Supplementary Figure 9. K-mer coverage across the *Pstol1* gene

The k-mer coverage is plotted with respect to the reference Kasalath sequence (AB458444.1) across the *Pstol1* gene. For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots.



Supplementary Figure 10. K-mer coverage across the entire Pup1 region in Kasalath

The k-mer coverage is plotted with respect to the reference Kasalath sequence (AB458444.1) The total sequence span is 452 kbp. For clarity, the range 1x to 50,000x (log scale) is displayed in all the plots. Black vertical lines indicate gaps (Ns) in the reference sequence. The position of the *Pstol1* gene is highlighted in green. See Supplementary Figure 9 for just the coverage of *Pstol1*.



Supplementary Figure 11. Multiple alignment of *Pstol1* sequence fragment.

In particular, the SNP that introduces a premature stop codon in the DJ123 allele (red asterisk) was confirmed with Sanger sequencing.