**Table S3: SNP analysis data for phasing of alleles around each LTGC**

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| **Chromosome III extracted from CHEF gel a** | **SNP analysis near centromereb and other SNPs centromere-proximal to the LTGC on chromosome III** | **SNP analysis centromere-distal to LTGC on chromosome III** | **Interpretation of SNP phasing around the LTGC on chromosome III** |
| LTGC 1, larger chr III band | SNP at 113543b is uncut by *Mnl*I (462 bp); indicates “S” homolog | SNPs fail to be amplified by PCR | LTGC 1 is atypical |
| LTGC 1, smaller chr III band | SNP at 113543b is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | SNP at 298875c is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 2, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Next-generation sequencing of this cut-out chr III band indicates all SNPs up to 168371 are “S” homolog. | Next-generation sequencing of this cut-out chr III band indicates SNP 190660 and all SNPs after it are “S” homolog; Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 2 is coupling |
| LTGC 2, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 3, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog | SNPs fail to be amplified by PCR | LTGC 3 is atypical |
| LTGC 3, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 4, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 217266 is “Y” allele and SNP 221332 is “Y” allele. Restriction digest of SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog | LTGC 4 is repulsion |
| LTGC 4, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 217266 is “Y” allele and SNP 221332 is “S” allele. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog |
| LTGC 5, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Next-generation sequencing of this cut-out chr III band indicates all SNPs up to 167724 are “S” homolog. | Next-generation sequencing of this cut-out chr III band indicates that after chr III base 167724, there is a translocation to chr II | LTGC 5 is atypical |
| LTGC 5, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 6, larger chr III band | Restriction digest of SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 158956 is “S” allele and SNP 159894 is “Y” allele | Sanger sequencing of PCR product across Junction #2 indicates SNP 175328 is “S” allele. SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 6 is coupling |
| LTGC 6, smaller chr III band | Restriction digest of SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 158956 is “Y” allele and SNP 159894 is “Y” allele. | Sanger sequencing of PCR product across Junction #2 indicates SNP 175328 is “Y” allele. SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 7, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 175328 is “S” allele. SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 7 is coupling |
| LTGC 7, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 175328 is “Y” allele. SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 8, larger chr III band | Restriction digest of SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Next-generation sequencing of this cut-out chr III band indicates all SNPs up to 217266 are “Y” homolog. | Next-generation sequencing of this cut-out chr III band indicates SNP 221332 and all later SNPs are “S” homolog. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 8 is repulsion |
| LTGC 8, smaller chr III band | Restriction digest of SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Next-generation sequencing of this cut-out chr III band indicates all SNPs up to 148615 are “S” homolog. | Next-generation sequencing of this cut-out chr III band indicates SNP 221332 and all later SNPs are “Y” homolog. Restriction digest of SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 9, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 241516 is “Y” allele and SNP 241753 is “Y” allele. Restriction digest of SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog | LTGC 9 is repulsion |
| LTGC 9, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog | Sanger sequencing of PCR product across Junction #2 indicates SNP 241516 is “Y” allele and SNP 241753 is “S” allele. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog |
| LTGC 10, larger chr III band | Restriction digest of SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 144359 is “S” allele and SNP 144921 is “Y” allele. | Sanger sequencing of PCR product across Junction #4 indicates SNP 231858 is “Y” allele and SNP 232905 is “S” allele. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 10 is coupling |
| LTGC 10, smaller chr III band | Restriction digest of SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 144359 is “Y” allele and SNP 144921 is “Y” allele. | Sanger sequencing of PCR product across Junction #4 indicates SNP 231858 is “Y” allele and SNP 232905 is “Y” allele. Restriction digest of SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 13, larger chr III band | Restriction digest of SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 144359 is “S” allele and SNP 144921 is “Y” allele. | Sanger sequencing of PCR product across Junction #2 indicates both SNP 187932 and SNP 188418 are “Y” alleles. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 13 is repulsion |
| LTGC 13, smaller chr III band | Restriction digest of SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 144359 is “Y” allele and SNP 144921 is “Y” allele. | Sanger sequencing of PCR product across Junction #2 indicates SNP 187932 is “Y” allele and SNP 188418 is “S” allele. Restriction digest of SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog |
| LTGC 15, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 128824 is “S” allele and SNP 130242 is “Y” allele. | SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 15 is coupling |
| LTGC 15, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 128824 is “Y” allele and SNP 130242 is “Y” allele. | SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |
| LTGC 16, larger chr III band | SNP at 113543 is uncut by *Mnl*I (462 bp); indicates “S” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 128824 is “S” allele and SNP 130242 is “Y” allele. | Sanger sequencing of PCR product across Junction #2 indicates SNP 182576 is “Y” allele and SNP 183385 is “S” allele. SNP at 298875 is uncut by *Rsa*I (353 bp); indicates “S” homolog | LTGC 16 is coupling |
| LTGC 16, smaller chr III band | SNP at 113543 is cut by *Mnl*I (335 + 127 bp); indicates “Y” homolog. Sanger sequencing of PCR product across Junction #1 indicates SNP 128824 is “Y” allele and SNP 130242 is “Y” allele. | Sanger sequencing of PCR product across Junction #2 indicates SNP 182576 is “Y” allele and SNP 183385 is “Y” allele. SNP at 298875 is cut by *Rsa*I (226 + 127 bp); indicates “Y” homolog |

a Chromosome III bands to be extracted were identified by Southern blot with a *CHA1* probe.

b The SNP on the left arm of chromosome III near the centromere at base 113543 was used to confirm whether the particular band extracted is the “S” or “Y” homolog of chromosome III. This SNP results in a *Mnl*I site on the YJM789-related chromosome but not on the S288c-related chromosome. This region was amplified by PCR, generating a 462 bp product. After digest with *Mnl*I, we observe either the uncut 462 bp product or the cut 335 bp and 127 bp products.

c A SNP on the right arm of chromosome III that is centromere-distal to the end of all LTGC events analyzed in this manuscript at base 298875 results in a *Rsa*I site on the YJM789-related chromosome but not on the S288c-related chromosome. This region was amplified by PCR, generating a 353 bp product. After digest with *Mnl*I, we observe either the uncut 462 bp product or the cut 226 bp and 127 bp products.