The method of DNA-seq analysis published in Okaiko-sama No.54.

Genomic DNA was extracted from CT05 and contaminated-p50 male larvae using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). DNA library preparation and sequencing were performed by Azenta (Beijing, China) using DNBSEQ (MGI, Shenzhen, China) and Novaseq(Illumina, San Diego, USA). The DNA sequence data for the Sakado strain (B. mandarina) was downloaded from the DDBJ DRA database (DRA004652). Raw DNA sequencing reads were subjected to quality checking and trimming to remove adaptor sequences, contamination, and low-quality reads using fastp version 0.22.0 (Chen et al., 2018) (-q 30). The trimmed reads were aligned to the publicly available reference genome of B. mori (Genome assembly (November 2016), http://silkbase.ab.a.u-tokyo.ac.jp) (Kawamoto et al., 2019) using minimap2 version 2.1.0 with default parameter settings (Li, 2018). SAMtools version 1.9 was used to convert SAM files and to sort and index BAM files (Li et al., 2009). Genomic variants including single nucleotide polymorphisms (SNPs) were called using SAMtools version 1.9, using the command line "mpileup -uf" and BCFtools version 1.9 (call -c) (Narasimhan et al., 2016). SNPs fewer than 20 mapped reads were filtered out using SnpSift version 4.3t using the following command: filter "(DP>=20)" (Cingolani et al., 2012a). Histograms were drawn using R packages (R Development Core Team, 2013)

References

Chen, S., Zhou, Y., Chen, Y., Gu, J. (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 32, i884–i890.

Cingolani, P., Patel, V.M., Coon, M., Nguyen, T., Land, S.J., Ruden, D.M. and Lu, X. (2012a) Using Drosophila melanogaster as a model for genotoxic chemical mutational studies with a new program, SnpSift. Frontiers in Genetics, 3. https://doi.org/10.3389/fgene.2012.00035.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X. and Ruden, D.M. (2012b) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly, 6, 80-92.

Kawamoto, M., Jouraku, A., Toyoda, A., Yokoi, K., Minakuchi, Y., Katsuma, S., Fujiyama, A., Kiuchi, T., Yamamoto, K. and Shimada, T. (2019) High-quality genome assembly of the silkworm, Bombyx mori. Insect Biochem. Mol. Biol., 107, 53-62.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25, 2078-2079.

Li, H. (2018) Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics, 34, 3094-3100.

Narasimhan, V., Danecek, P., Scally, A., Xue, Y., Tyler-Smith, C. and Durbin, R. (2016) BCFtools/RoH: A hidden Markov model approach for detecting autozygosity from next-generation sequencing data. Bioinformatics, 32, 1749-1751.

R Development Core Team. (2013) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.