HIGHLIGHTS

TECHNOLOGY

A bigger needle in a smaller haystack

For many decades, fruitfly chemical mutagenesis has followed a standard protocol: collect some flies, feed them a mutagen (such as EMS), select among their progeny those with a phenotype of interest, then breed and study the chosen few.

There are obvious shortcomings to this approach. If the aim is to mutate a specific gene sequence — an increasingly common occurrence now that the *Drosophila melanogaster* genome sequencing project is complete — then the traditional approach requires sorting through thousands of mutagenized flies, to recover perhaps only a handful of mutations in the selected gene. A more reliable method of selecting specific mutations has become urgent in flies, and Bentley *et al.* report an economical and time-efficient way to recover mutations in any gene of interest.

The technique combines a classical chemical approach with a molecular detection technique. Flies are mutagenized in the traditional way, but the genomic region of interest is amplified from the mutagenized lines by PCR. The PCR product (or products) is then analysed by DHPLC (denaturing high performance liquid chromatography), which can resolve homoduplexes and heteroduplexes of DNA molecules. The presence of heteroduplexes suggests that the amplified region carries a point mutation.

But how well does it work? Bentley and colleagues tested the technique on the *abnormal wing discs* (*awd*) gene. Sixteen independent *awd* mutations were picked up from screening fewer than 5,000 flies. If this seems like a lot of flies, then consider that an average screen would recover mutations at a rate of one in several thousand flies. Because the DHPLC method identifies mutations irrespective of whether the mutation causes a phenotype (typically the result of a loss-of-function mutation), an allelic series of mutations can be obtained. In the case of *awd*, both loss-of-function and dominant alleles were found and will be valuable for studying protein function.

By tweaking the mutagenesis protocol, the new detection method should be just as useful for recovering mutations on other chromosomes and, indeed, it can also be extended beyond *Drosophila* to other genetically tractable organisms. A modified version of the technique has already been applied to *Arabidopsis* and to mouse embryonic stem cells.

For *Drosophila*, the snag lies in having to extract and analyse DNA from individual flies. Such problems should be ironed out, because clever shortcuts — such as pooling flies before PCR — are expected to make the technique easier, quicker and cheaper.

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References and links

ORIGINAL RESEARCH PAPER Bentley, A. et al. Targeted recovery of mutations in *Drosophila*. *Genetics* **156**, 1169–1173 (2000)

FURTHER READING McCallum, C. M. *et al.* Targeted screening for induced mutations. *Nature Biotechnol.* **18**, 455–457 (2000) | Chen, Y. D. *et al.* Genotype-based screen for ENU-induced mutations in mouse embryonic stem cells. *Nature Genet.* **24**, 314–317 (2000)

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