High-throughput mapping of transposable *P* elements in *Drosophila melanogaster*

As part of the international DrosDel project funded by the European Union, we have set up a high-throughput facility for mapping *P* elements in *Drosophila*. The DrosDel project involves use of RS3 and RS5 *P* elements[1] to produce targeted 1 megabase tiled deletions with a 0.5 megabase overlap, the ultimate use of which is to facilitate gene function research in the *Drosophila* community.

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Transformed fly stocks are constructed by injecting embryos from an isogenic background stock with the transposable *P* element, which inserts itself into a random part of the genome. The isogenic lines used for the transformations lack a functional *white* gene and develop white eyes as adults (w^-). The RS elements however carry a functional copy of the *white* gene, and therefore flies that possess a successful *P* element insertion develop red eyes (w^+). After successful transformation, the *P* elements are mobilised with transposase to produce many stocks with random insertions.

Flies are sorted on the basis of the presence of a *P* element insert and 15 individual adults transferred into 96-well 1.2ml Storage Plates (ABgene®) with one well representing one stock. The flies are homogenised using a Retsch MM300 and DNA prepared using a modified cell lysis and LiCl / KAc precipitation method[2]. DNA is cut using *Msp*1, with one restriction site at a known point near each end of the *P* element, and another at a random location in the genomic sequence near to the insertion (Figure 1). The restricted DNA is ligated with T4 DNA Ligase (ABgene®) for two hours at room temperature in a large volume to promote intramolecular ligation events, and the completed reactions precipitated and re-suspended in 50µl H_2O.

Inverse PCR is performed in a final volume of 25µl in a Thermo-Fast® 96 PCR Plate (ABgene®) using 5µl of ligated DNA and 0.5U of ABgene® Thermo-Start® DNA Polymerase*. Each end of the *P* element is amplified in a separate reaction using different primer pairs, although only the 3′ end is amplified routinely. Amplified products are purified via gel filtration and sequenced on an ABI-3100 automated sequencer using BigDye™ v2 terminator chemistry (ABI). Analysed sequences are mapped on to the *Drosophila* genome using Blast*n* and processed using custom software developed at Cambridge University. Additional software pairs up RS3 and RS5 elements in the correct orientation for use in deletion experiments. Results are transferred into a MySQL relational database and made available on the internet at http://www.flyseq.org.uk.

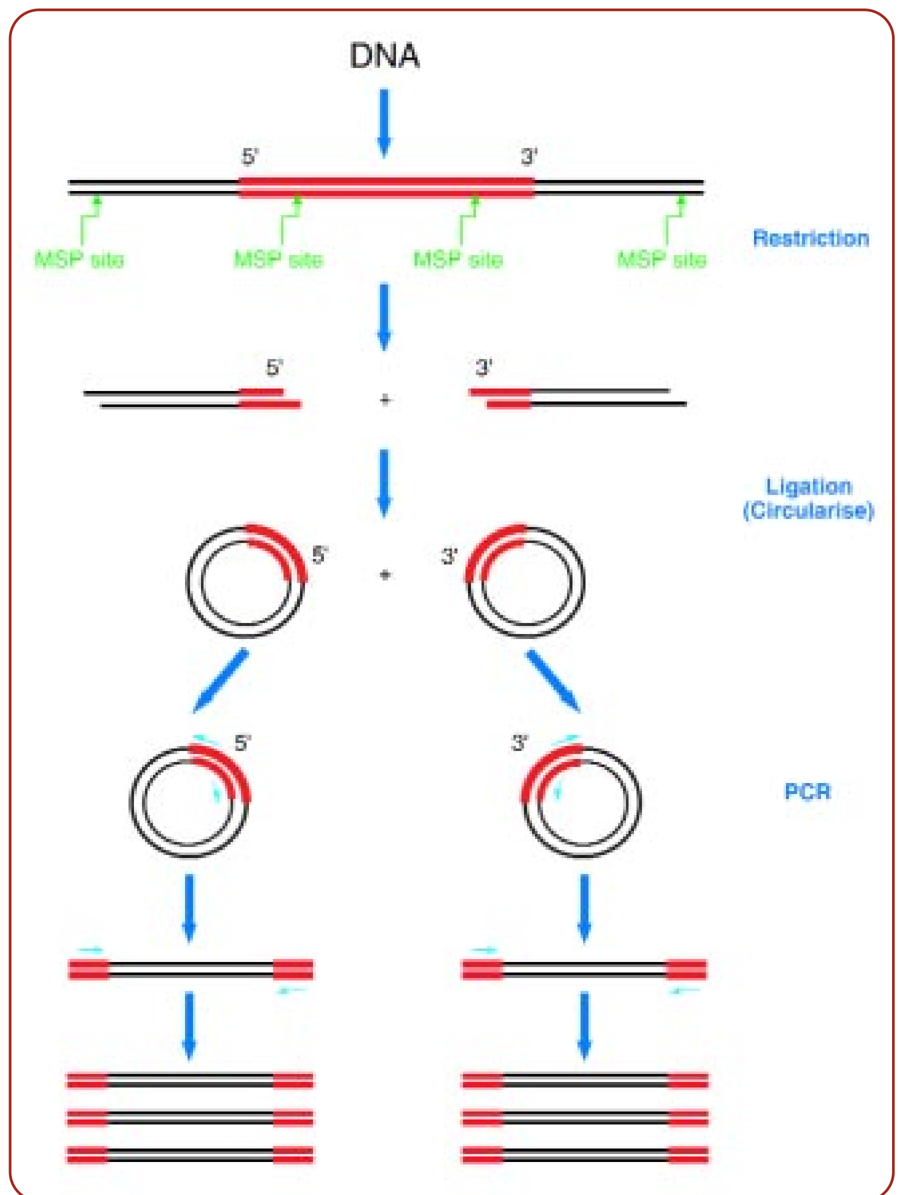

Figure 1: Overview of the inverse PCR method. The P *element DNA is represented in red and genomic DNA in black. DNA is cut with* Msp1 *and the resulting fragments, which contain either the 5′ or 3′ end of the element and the flanking genomic sequence, are circularised by ligation. Inverse PCR, which uses primers that face away from each other, is then performed to amplify the fragment ready for purification and sequencing.*

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Results and discussion

An agarose gel, showing inverse PCR products from a plate prior to purification, is shown in Figure 2. A success rate of 65–75% is frequently observed, and the high activity of the Thermo-Start® enzyme means that PCR reactions can be performed in a smaller volume using less enzyme and increasing cost-efficiency. A sample of sequence obtained is shown in Figure 3.

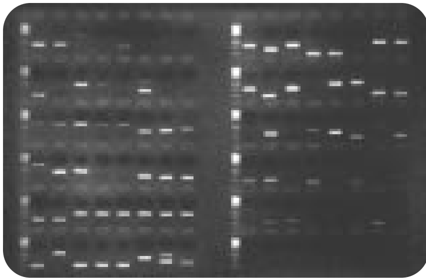

Figure 2: Agarose gel produced using ABgene®'s Electro-Fast® Wide *high-throughput gel system. The electrophoresis results show inverse PCR products from the 3' end of the* P *element insertions. Each well represents one fly line, and successful amplifications are visible as single bright bands. Double bands represent two* P *element insertion events in the same fly line and result in a mixed sequence. The last row contains negative controls and empty wells, and the first well in each row contains a 1kb DNA ladder for sizing amplified products.*

Successful inverse PCR is difficult due to a high failure rate of reactions and non-specific amplification of products. The PCR relies not only on a successful DNA extraction but also a successful restriction and ligation. We have found that using ABgene® Thermo-Start® DNA Polymerase* gives a good success rate of PCR amplification and a high product specificity.

Acknowledgements

I would like to thank Dr Steven Russell (PI for the FlyChip group) and Fiona Blows (FlySeq senior technician). FlySeq is a part of the Ashburner group at the University of Cambridge Department of Genetics.

References

1. **Golic, K.G., Golic, M.M. (1996)** Engineering the *Drosophila* genome: chromosome rearrangements by design. *Genetics* Dec;144(4):1693-711.

2. **Audrey, M., Huang, E., Rehm, J., Rubin, G.M.** Recovery of DNA sequences flanking P-element insertions: inverse PCR and plasmid rescue. In *Drosophila protocols*, W. Sullivan, M. Ashburner and R. S. Hawley eds. (Cold Spring Harbor Laboratory Press), pp 429-437.

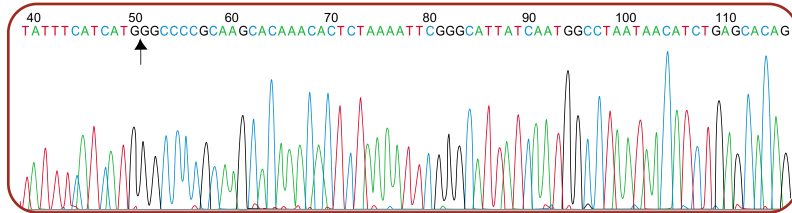

Figure 3: Sample of sequence obtained from a fly line with an RS3 P *element insertion. The 3' end of the* P *element is displayed as CATCATG (marked by an arrow).*

Cat. No.	Description	Quantity
AB-0908	Thermo-Start® DNA Polymerase	250 units
AB-0324	T4 DNA Ligase	500 units
AB-0800	Thermo-Fast® 96 Skirted PCR Plate	25 Plates
AB-0564	1.2ml Storage Plate	50 plates
AB-0826	Electro-Fast® *Wide* 108 Complete	1 unit

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