

***Drosophila melanogaster* control vectors and genes**

Outline

A selection of commonly used *Drosophila* control vectors and genes were prepared for the FlyChip cDNA microarrays using the protocols defined below.

- Vectors: pBluescript, pOT2a, pUAST, pLacW and pGawB
- Genes: white, lacZ, gal4, P transposase, FLPase and GFP

Linearisation of vectors

DNA for the control vectors, pOT2a, pBluescript, pUAST, pLacW and pGawB was transformed by standard methods into XL Gold cells and plasmid containing colonies selected on either LB-Ampicillin or LB-Kanamycin plates.

Plasmid preps were prepared using Qiagen plasmid purification spin columns and the purified DNA was then linearised with the restriction enzyme Xba I.

Protocol:

1. Make the following restriction digestion mixture:
 - ◆ 20 to 40 µg DNA
 - ◆ 20 µl NEB Buffer 2
 - ◆ 20 µl 10 x BSA (1 mg / ml)
 - ◆ 4 µl XbaI enzyme (2U per µl)
 - ◆ Make up to 200 µl with MilliQ water
2. Incubate each restriction digestion at 37 °C for 2 hours
3. Heat denature enzyme at 65 °C for 20 minutes
4. Phenol:Chloroform extraction and ethanol precipitation of DNA
5. Centrifuge at 13000 rpm for 10 minutes, perform an ethanol wash, and then precipitate using 70% ethanol
6. Resuspend in MilliQ water

20 to 30 µg of linearised vector were generated from several Xba I digests. The DNA was then pooled and concentrated to make a stock of approximately 0.2 to 0.3 µg / µl.

PCR of Control Genes

The control genes white, lacZ, gal4, P transposase, FLPase and GFP were amplified by PCR from the following vectors using the specified gene-specific forward and reverse primer pairs:

Gene	Template	Forward primer	Reverse primer	Annealing Temp.
white	pUAST	AAGTATCGCCATCCGGGATGCG	TAAGCGTCTCCAGGATGACCTTG	68 °C
lacZ	pLacW	TATCCCATTACGGTCAATCCGCC	ACCGTCGATATTCAGCCATGTGC	60 °C
gal4	pGawB	GTATCGATTGACTCGGCAGCTC	AGTTTGGTCCGTCCAACCAGG	68 °C
P transposase	p 25.7wc	TTGCTGCAAAGCTGTGACTGGAG	TTCGGACGGCTTAATAAGTCCG	60 °C
FLPase	phsFLP	CAGCAATCAAGAGAGCCACA	CTGCCACTCCTCAATTGGAT	60 °C
GFP	pUASmGFP6	GGAATTCATGAGTAAAGGAGAAGAAC	CTAGATCTCATCATTATTTGTATAGTTCATCC	60 °C
pOT2a	pOT2a	AATGCAGGTAAACCTGGCTTATCG	AACCGCGGCTACAATTAATACATAACC	68 °C
pBlueScript	pBlueScript	GGCGTAATCATGGTCATAGCTGTTTC	GAGTCGTATTACAATTCACTGGCCG	68 °C

Protocol:

PCR Reaction mix:

- 10 μ l 10 x Stratagene Yield Ace reaction buffer (or ABgene ThermoStart standard buffer)
- 2 μ l 25 mM dNTP mix
- 2 μ l 25 pmol / μ l forward and reverse primer mix
- 84 μ l MilliQ water
- 1 μ l Stratagene Yield Ace polymerase (or ABgene ThermoStart DNA polymerase)
- 1 μ l DNA template

PCR cycle:

All PCR reactions were performed in 0.2 ml microfuge tubes with a Dyad thermal cycler with the following PCR cycle.

1. 94 °C for 3 minutes
2. 94 °C for 30 seconds
3. Annealing temperature for 30 seconds
4. 72 °C for 4 minutes
5. Cycle between steps 2 to 4, 34 times
6. 72 °C for 10 minutes
7. 4 °C cold storage before unloading

PCR products were purified using Qiagen QIA quick columns or Millipore Multiscreen-PCR plates and checked by both agarose gel electrophoresis and the Nanodrop. The DNA concentration was then adjusted to make a final stock concentration for printing.

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