In situ hybridization with oligonucleotides: a simplified method to detect *Drosophila* transcripts

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We describe a simplified method for the *in situ* detection of *Drosophila* mRNA with oligonucleotides (45-mers) as probes using a protocol developed for use on vertebrate brain (1). This method requires no pretreatment or prehybridization of sections, involves a simple 5 minute labelling procedure for the probes, and is comparable in sensitivity and specificity to other protocols.

Stage 13 to 16 embryos were collected, dechorionated and embedded in cryoglue. 7 μ m sections were cut on the cryostat at -20°C and thaw-mounted onto slides coated with 0.1% (w/v) poly-L-lysine hydrobromide (Sigma, m.wt. 350 000). The sections were air-dried for one hour, fixed in ice-cold 4% (w/v) phosphate-buffered paraformaldehyde for 5 minutes, submerged in 1 $\times$ phosphate-buffered saline, rinsed with 70% ethanol and, finally, transferred into 95% ethanol. The sections can be stored at 4°C for several months.

An antisense 45-mer oligonucleotide, built to exon 5 of the *Antennapedia* gene (5'-ATG TCC GCC CCC ATG TAC GAG TTG GTG AAG TAC GAG GTC ATG CTC-3'; see ref. 4), was labelled by 3'-end tailing using a 30:1 molar ratio of 5'-[α -³⁵S]dATP to oligonucleotide. 1 μ l of terminal deoxynucleotidyl transferase (BRL, 14 U/ μ l) was added to 2 μ l of 5 $\times$ tailing buffer (BRL), 1 μ l of oligonucleotide (0.3 pmol/ μ l), 5 μ l water and 1 μ l of 5'-[α -³⁵S]dATP (1200 Ci/mmol, 10 μ Ci/ μ l, NEN, Dupont). This was incubated at 37°C for 5 minutes, after which time 40 μ l of TE was added. Unincorporated nucleotides were separated from the labelled probe using a 1 ml spin column filled with Sephadex G-25 (3). For hybridization, 1 μ l of the labelled probe was added per 100 μ l of oligonucleotide hybridization buffer to yield a probe concentration of 6×10^{-2} fmol/ μ l. The hybridization buffer consisted of 4 $\times$ SSC, 50% (v/v) deionized formamide, 10% (w/v) dextran sulphate, 25 mM sodium phosphate, pH 7.0, 1 mM sodium pyrophosphate, 5 $\times$ Denhardt's solution, 0.2 mg/ml acid-alkali hydrolyzed salmon sperm DNA, 100 μ g/ml polyadenylic acid (5' potassium salt, Sigma, P-9403), 120 μ g/ml heparin and 10 mM DDT (1). This was applied to a slide with the section of interest (when removing sections from the ethanol storage box sections were allowed to air-dry for half an hour) and Parafilm™ was lowered onto the hybridization mixture. No prehybridization step was found to be necessary. The slides were incubated at 42°C overnight. The Parafilm™ was removed under 1 $\times$ SSC at room temperature and sections were washed at 55°C for half an hour in 1 SSC (the addition of DTT was found to be unnecessary). After this, sections were rapidly taken through 1 $\times$ SSC (room temperature), 0.1 $\times$ SSC (room temperature), 70% ethanol, 95%

ethanol and then allowed to air-dry. Figure 1 shows a photomicrograph of a sectioned *Drosophila* embryo after hybridization to an antisense 45-mer *Antennapedia* oligonucleotide and emulsion autoradiography. Heavy labelling is observed over the ventral cord and the abundance of *Antennapedia* transcripts in this region is identical to that reported previously (4). Probe specificity was tested by using a 20-fold excess of unlabelled probe in addition to labelled probe. No labelling above background was found when performing these control experiments (not shown).

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Figure 1. *In situ* hybridization of *Antennapedia* transcripts in a parasagittal tissue section of a *Drosophila* stage 14 embryo. Emulsion autoradiography was for 6 weeks. This brightfield photomicrograph shows the segmental expression pattern of *Antennapedia* mRNA along the developing ventral cord. Intense labelling is observed for ganglionic cells in the meso- and metathoracic segments (arrowheads). Scale bar, 50 μ m. Abbreviation: vc, ventral cord.