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An Improved Method for Chemical Devitellinization of X-gal Stained Drosophila Embryos

Amit Singh
University of Dayton, asingh1@udayton.edu

Madhuri Kango-Singh
University of Dayton, mkangosingh1@udayton.edu

P. Sinha
School of Life Science, Indora, India

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An improved method for chemical devitellinization of X-gal stained *Drosophila* embryos

A Singh, M Kango & P Sinha
Drosophila Stock Center, School of Life Science, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Khandwa Road Campus, Indore 452 001, India

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In *Drosophila* developmental biological studies, X-gal staining is commonly employed to study the spatio-temporal expression of the *lacZ* reporter gene in the transformed flies or their embryos. Study of the *lacZ* pattern in embryos often suffers from the lack of an efficient and high yielding technique for devitellinization of X-gal stained embryos. Devitellinization techniques employed during antibody staining, *in situ* hybridization or embryonic cuticular preparations generally do not give satisfactory results when used for similar purpose in X-gal stained embryos. This results in the flaky appearance of the blue stain. We present here an improved chemical devitellinization technique which gives a high yield of devitellinized embryos and a better resolution of the X-gal staining pattern.

In *Drosophila* development bilogical investigations, X-gal (5-bromo-4-chloror-3-indolyl-β-D-galactopyranoside) staining is frequently required. These include enhancer trap studies that involves mobilization of P-*lacZ* transposon in the vicinity of desired genes[1] and in studies involving fusion of the *lacZ* gene to the promoters of the various developmentally expressed genes[2]. Developmental expression of the *lacZ* reporter gene generally mimics that of the gene of interest which can be detected by histochemical staining for the enzyme β-galactosidase using the chromogenic substrate X-gal[3,4]. X-gal staining is economical, reliable time saving, convenient and provides an excellent method for study of developmental expression of the *lacZ* reporter gene.

However, the step of devitellinization of the *Drosophila* embryo which is commonly followed during antibody staining or *in situ* hybridization or embryonic cuticular preparations is not practiced during X-gal staining. This is presumably due to the fact that the chemical devitellinization[6] protocol results in a flaky and diffused appearance of the blue stain. On the other hand, the hand peeling technique which may circumvent this problem is time consuming, cumbersome and needs additional skill to avoid damage to the embryos. Absence of a technique for fast and effective devitellinization of X-gal sained embryos limits the scope of the technique in the study of *lacZ* expression in embryonic cell types.

We present here a chemical devitellinization technique for X-gal stained embryos where the major hurdles of time consumption, low yield of devitellinized embryos, chances of loss of morphology of the embryos or quality of stain have been overcome. The protocol involves the following steps:

1. Dechorionation of the embryos in bleach (5% Na hypochlorite) and subsequent treatments with 0.7% KCl and Triton X-100 (1%) for duration of 5 min each.

2. Embryos are then fixed in 3.7% formaldehyde in citric phosphate buffer[4] [(*p*H 7.6:9 vol. of fresh solution of Na_2HPO_4.0.2*M* (0.89 g $Na_2HPO_4.2H_2O$ in 25 ml water), 1 vol of citric acid 0.1*M*, 10 vol of water. The *p*H of the buffer is not critical and may range from 7-8)] and equal volumes of heptane for 15 min. The solution is then drained out and embryos are gently dried to allow traces of heptane to evaporate.

3. The fixed embryos are then rinsed in citric phosphate buffer[4] and incubated overnight at 30°C in incubation buffer[4] (Citric phosphate buffer containing 5 m*M* each of potassium ferri- and ferrocyanides) with an increased concentration of Triton X-100 (0.5% which is critical as higher concentrations of Triton X-100 may turn the embryos brittle) as compared to usual 0.2% and a saturating amount[4] of X-gal.

4. Stained embryos are then fixed again with 3.7% formaldehyde, 50 m*M* EGTA and equal volume of heptane for 15 min.

5. After adding 1 ml of 5% TCA[6] the embryos are shaken for 2 min and then allowed to stand for 5 min. The solution is then removed and embryos are flushed with methanol and shaken vigorously; the devitellinized embryos will sink down. The duration of this exercise must not last longer than 3-4 min.

The step of exposure of the stained embryos to methanol should be optimum as it is crucial to the efficacy of this technique. Prolonged exposure of the embryos to methanol results in flaky appearance of the stain (Table 1, Fig. 1b) possibly due to precipitation of the proteins while shorter periods of exposure give very poor yield of devitellinized embryos (Table 1).

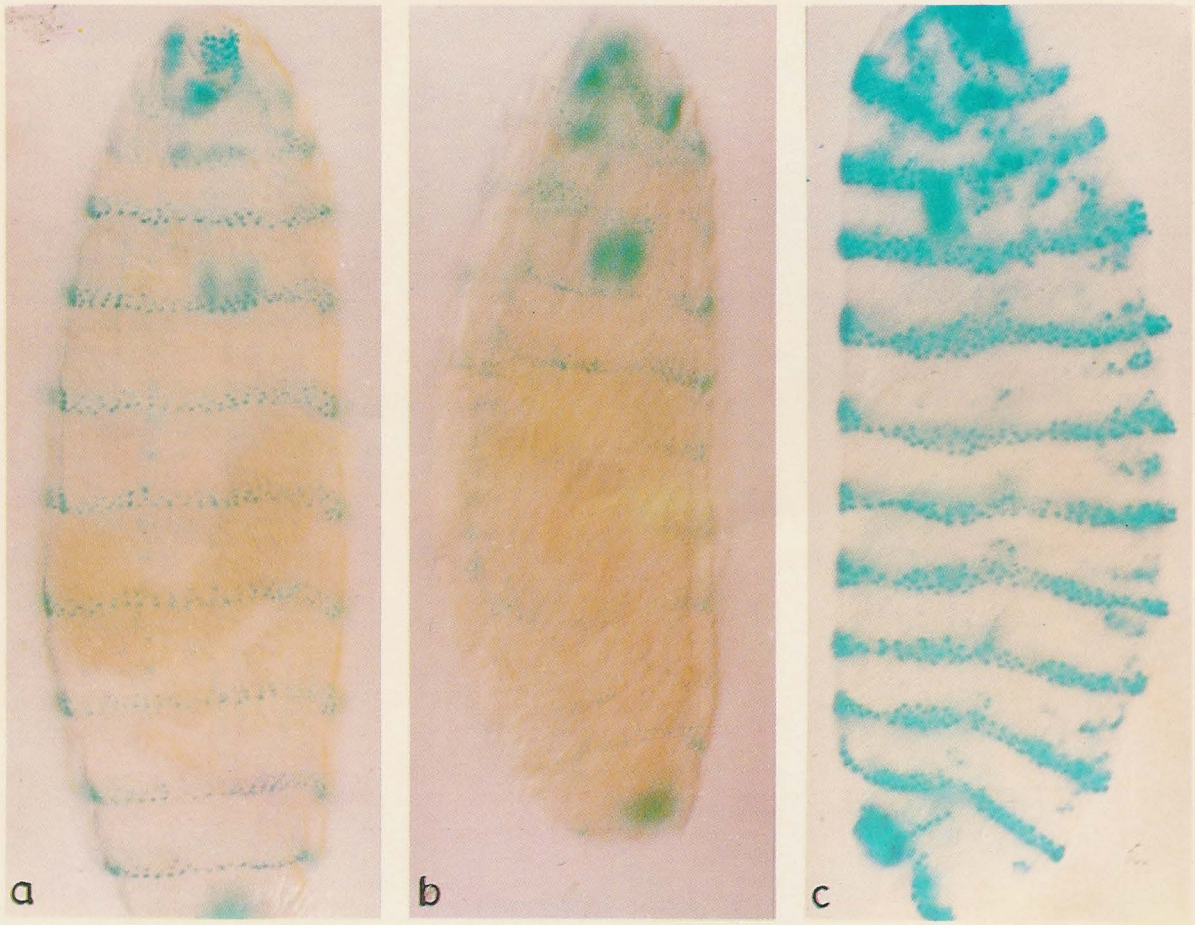

Fig. 1—Efficient detection of *lacZ* expression pattern in embryos bearing a P-*lacW* transposon inserted at *engrailed, en* locus following chemical devitellinization. (a) Non-devitellinized X-gal stained embryos. (b) X-gal stained embryos devitellinized by prolonged exposure (nearly 5 min) to methanol. Note the flaky appearance of the stain. (c) X-gal stained embryos devitellinized by the protocol described here with an optimum duration of exposure (3 min) to methanol. Stripe pattern of the *lacZ* expression is characteristic of the *en* is confined to the posterior compartment of all embyronic segments

Table 1—Efficiency of devitellinization of X-gal stained embryos following various lengths of methanol treatments of embryos of *en-lacZ* stock

Duration of treatment	Stained embryos	No of embryos devitellinized	Percentage yield	Qualitative measure of X-gal staining
Smaller duration (1-2 min) exposure to methanol	694	73	10.52	No loss of staining but very low yield of devitellinized embryos
Optimum duration (3 min) exposure to methanol	433	303	69.98	No loss of X-gal staining with increase yield of devitellinized embryos
Prolonged duration (4-5 min) exposure to methanol	374	262	70.05	Loss of X-gal staining due to flaky appearance of the stain with high yield of devitellinized embryos

The stock of *en-lacZ* was kindly provided by Judith Kassis and is available in the Drosophila Stock Center, Indore.

6. The embryos are then rinsed again in the incubation buffer. The incubation buffer is then replaced with a mixture of fresh incubation buffer and glycerol (1:1). The embryos will first shrink and then recover their normal shape in 1-2 hr.

7. The devitellinized X-gal stained embryos are then mounted in a mountant[4] (7 g of gelatin swelled for a few min in 42 ml of water, dissolved in boiling water bath. Added 63 g of glycerol and a crystal of phenol as bactericide. Stored at 4°C and warmed to 45°C).

Use of the higher concentration of Triton X-100 in

the incubation buffer, post staining fixation and optimization in the duration of the methanol treatment appeared to be key to the efficacy of this technique in retaining the X-gal staining in devitellinized embryos (Fig. 1, Table 1). This chemical devitellinization procedure is a modification of the earlier used procedure[6] employed for devitellinization of embryos for antibody staining. This technique overcomes the limitation of handling embryos by the hand peeling technique as well as provides an improved resolution of the X-gal stained embryonic cell type.

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