

# Tech-Trends

## Volume 3, Series 5

### BTX electroporation of *Bombyx mori* embryos and larval tissues

#### Electroporation, an alternative to biolistics for transfection of *Bombyx mori* embryos and larval tissues

##### Abstract:

There are few powerful techniques available to transfect insect tissues. We previously used biolistics to transfect *Bombyx mori* embryos, and larval and pupal tissues (Thomas J-L et al. 2001. Journal of Insect Science 1/9, Kravariti L et al. 2001. Insect Biochemistry and Molecular Biology 31: 473-479). As the main limitation was the irregularity in results we explored electroporation as an alternative technique by adapting techniques used for chicken embryos to *B. mori* embryos. By injecting the DNA solution into the hemocoel of late embryos that were finishing organogenesis, we expressed marker genes in numerous tissues following electroporation. With some adaptation of the method this was also achieved for early embryos lacking a hemocoel. Some larval tissues were also transfected. During these technical studies we found that optimizing parameters such as electrical voltage, number of pulses and their frequency, and conductivity of the buffer was important. These results confirmed that electroporation is a reliable technique for transfecting *B. mori* tissues.

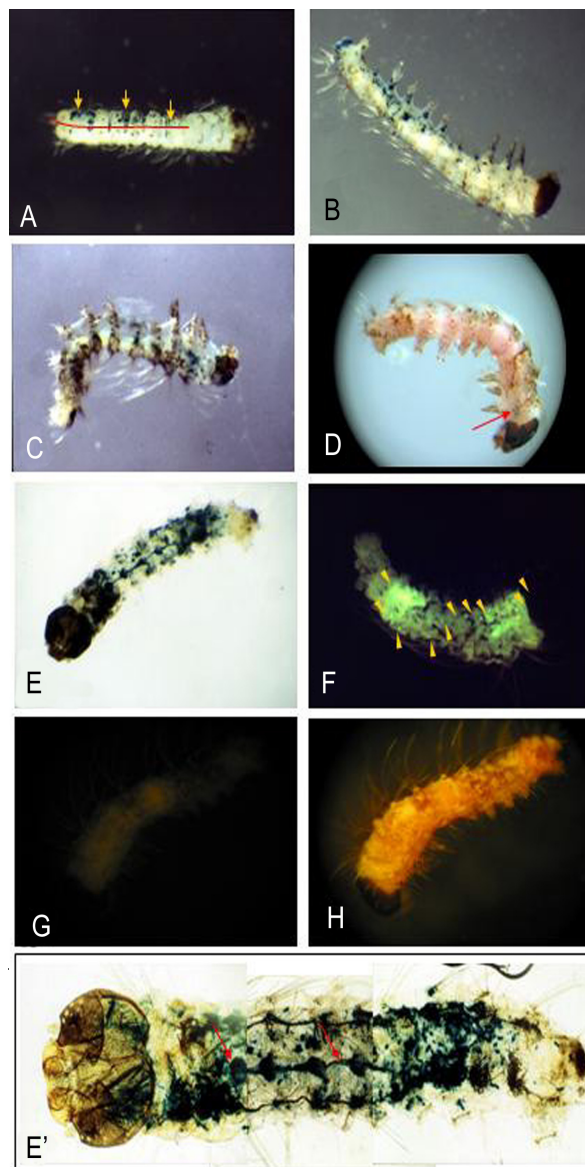
##### Results:

**Table 4.** Assays of different promoters and expression markers

Experiments	Assays	ADN	Number of electroporated embryos	Number of positive embryos	Percentage of positive embryos	Number of spots	Mean number of spots per electroporated embryo
1	1	<i>pBRJZ</i>	4	1	25	30	7.5
	2		5	4	80	129	25.8
	1	<i>pIE1BmZ</i>	5	4	80	47	9.4
	2		5	5	100	33	6.6
	3		5	5	100	146	29.2
	1	<i>pA3ΔSZ</i>	5	4	80	24	4.8
2	2		5	4	80	31	6.2
	3		6	6	100	94	15.6
	1	<i>pJGFP</i>	5	5	100	12	2.4
	2		5	5	100	8	1.6
	3		15	5	30	17	1.13

Electroporation program : P'2

The DNA solution were at 0.5µg/µl



6 day old electroporated embryos. P2 electroporation program in A and P'2 electroporation programs from B to F. A : *pBRJZ*: Arrows show the main distribution of the staining. The red line displays the longitudinal axis of the embryonic body. B : *pA3ΔSLacZ*: Xgal Control: *pBRJZ* injected embryo and Xgal stained without electroporation. Arrow shows the low Xgal staining background in the sub-oesophagian corpus. E et E': *pBRJZ* F : *pJGFP* G and H: GFP control: *pJGFP* injected embryo without electroporation, G : GFP filter, H : GFP filter in episcopic ordinary light. A-H (x25), E' (x50) embryos. E': magnification (photomontage) of the view E. Arrows show the ventral ganglionic chain

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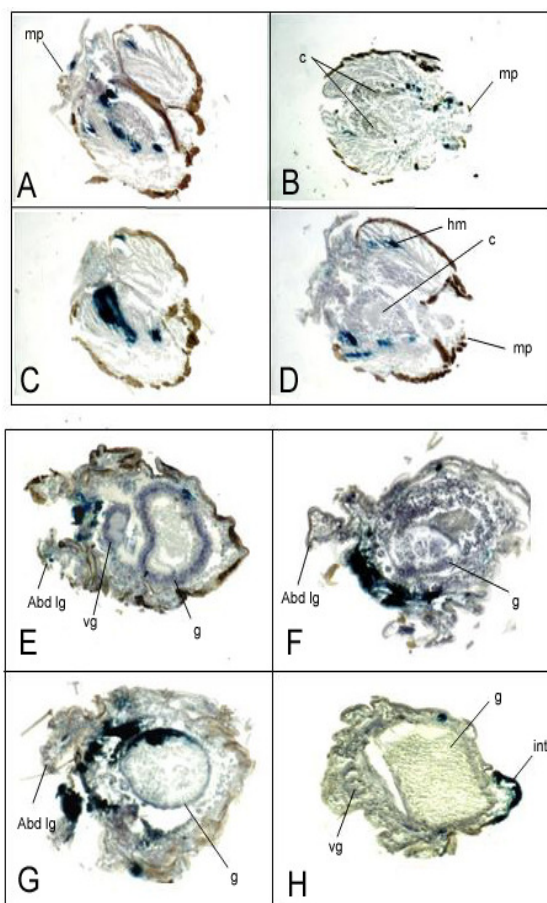
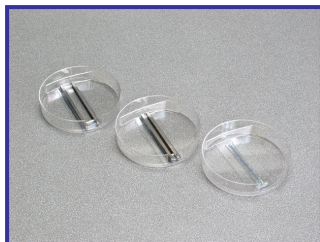
Volume , Series

## BTX electroporation of *Bombyx mori* embryos and larval tissues



ECM 830 SQUARE WAVE GENERATOR  
CATALOG: 450052

**MICROSLIDES**  
1 MM CATALOG: 450104  
3.2 MM CATALOG: 450105  
10 MM CATALOG: 450106



Histological sections of embryos electroporated with *pBRJZ*.  
Magnification x240. A to D: head. E to H: abdomen. **c**: cerebrum, **hm**: head muscles, **abd lg**: abdominal leg, **vg**: ventral ganglion, **g**: gut, **int**: integument, **mp**: mouth part.

**Cell Preparation:** Eggs newly laid on a sheet of paper were placed in an incubator at 25° C and 80% R.H. for 6 days and the occurrence of stemmata pigmentation (the first pigmented structures) was controlled. The eggs were used after the appearance of stemmata pigmentation until cephalic black pigmentation appeared. A constant supply of eggs was maintained by keeping them at 5° C for no longer than one week. This temperature stops development without disturbing the normal resumption. Eggs were collected by incubation for 5 minutes in tap water bath (approximately 20° C) and dried on paper towels before being glued onto Petri dishes with cyano-acrylate glue ensuring that they were laid flat. Eggs were disinfected with a 4% formaldehyde solution in PBS 10 mM, pH 7.4, for 10 min, rinsed with distilled water and finally dried with absolute ethanol. Eggs were dissected in Grace's medium containing antibiotics (Sigma). Just before being electroporated 6 day old embryos were put on a wet GF/C Whatman glass filter (diameter 25 mm) and injected with the DNA solution (0.5 µg/µl, 0.5% eosin) using a sharpened glass capillary (tip diameter: approximately 30 µm). Injection of DNA was carried out two ways; the first location was just behind the head through the translucent soft integument, the second, directly through the front of the head.

**Electroporation Settings** (using Program P'2, see detailed publication):

Set Voltage:	80 V
Set Pulse Length:	50 ms
Set # of Pulses:	5-10
Electrode gap:	3.2 mm
Desired Field Strength:	250 V/cm

**Electroporation Procedure:**

Temperature:	Room Temperature
Transfectant conc:	0.5 µg/µl, 0.5% eosin

**Pulse:** Press Start to Activate the Automatic Charge and Pulse Sequence

**Post Treatment:** The embryos were then placed in Grace's medium containing antibiotics for 2 days at 25° C for subsequent development. It was possible to cultivate embryos in wells of 16 mm or 35 mm in diameter, or in standard 1.5 ml micro-tubes filled with one ml of culture medium.

**Source:** <sup>1</sup>Thomas J-L. 2003. Electroporation, an alternative to biolistics for transfection of *Bombyx mori* embryos and larval tissues. 12pp. *Journal of Insect Science*, 3:17

<sup>1</sup>UNS/INRA, 25 quai J.J. Rousseau, 69350 La Mulatiere, France  
thomas@pop.univ-lyon1.fr

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