Histological Techniques

What's here

whole mount preparations

image of a whole mount from a 4 wk old virgin

Protocol

Spread tissue on glass slide
Fix in Carnoy's fixative for 2 to 4 hours at r.t.
Wash in 70 % EtOH for 15 min
Change gradually to distilled water
Rinse in distilled water for 5 min
Stain in carmine alum O/N
Wash in 70 % EtOH 15 min
Wash in 95 % EtOH 15 min
Wash in 100 % EtOH 15 min
Clear in xylene and mount with Permount

After photographic documentation the tissue can be embedded in paraffin for sectioning and conventional histological staining.

Immerse slide into xylene to remove mounting medium
Change xylene 2 times
Transfer to 1:1 xylene:paraffin (60°C)
Change xylene 2 times and embed in paraffin

Carnoy's Fix:
6 parts 100 % EtOH
3 parts $CHCl_3$
1 part glacial acetic acid

Carmine Alum Stain:
Place 1 g carmine (Sigma C1022) and 2.5 g aluminum potassium sulfate (Sigma A7167) in 500 ml distilled water

and boil for 20 min. Adjust final volume to 500 ml with water. Filter and add a crystal of thymol as preservative. Refrigerate. Can be used for several months. Discard when color becomes weak.

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lacZ staining in whole mammary tissue

Summary
The bacterial beta-galactosidase gene lacZ is frequently used as a reporter gene. The expression of transgenic constructs can be monitored by histochemistry with the chromogenic substrate X-gal. This allows precise cellular localization of gene activity. Under certain conditions (see below), no background staining is detectable in mammary tissue. Beta-galactosidase activity can be assayed in small pieces of tissue or on cryosections.

Method
1. Spread gland tissue on a piece of paper and fix for 1-2 hrs in 2% paraformaldehyde, 0.25% glutaraldehyde, 0.01% NP-40 in PBS (use 10 to 20 ml in a scintillation vial)
2. Rinse in PBS, remove from paper
3. Add 10 ml of PBS with 2 mM MgCl2, 0,01% Na-deoxycholate, 0.02% NP-40 and rock for 2 hrs
4. Add 10ml of X-gal staining buffer with 1 mg/ml X-gal (make 40mg/ml stock in DMF, store at -20OC; don't use if discolored). Incubate at 30OC for 24 -48 hrs, or less if expression is strong
5. Clear in acetone, rehydrate and stain in carmine alum O/N.
6. Dehydrate, clear in xylene and mount with Permount

Alternative:
6. Fix again in 4% PFA, dehydrate, embed in paraffin and section

Staining buffer:
30 mM K4Fe(CN)6 [4.983 g/500 ml]
30 mM K3Fe(CN)6.3H2O [6.336 g/500 ml]
2 mM MgCl2 [1 ml 1M/500 ml]
0.01 % Na-desoxycholate [50 mg/500 ml]
0.02 % NP-40 [100 microl/500 ml]
1xPBS [50 ml 10x/500 ml]

Comments
Fixation time is critical. Overfixation inhibits enzyme activity. Close control of reaction temperature (30OC) and pH (PBS 7.2) are critical for elimination of endogenous beta-galactosidase activity. In cases of high enzyme activity the X-gal product forms a precipitate on the surface of the tissue which prevents penetration of substrate into deeper regions. When such tissues are sectioned only the outer layers of cells will show staining. To evalute transgene expression in the center of the tissue it will be necessary to perform the staining procedure on frozen sections.

image of a section from mammary tissue of a WAP-lacZ mouse at day 10 of lactation (see Robinson et al., 1995; 1996).

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lacZ staining of tissue sections

1. Embed tissue in OTC and freeze. Optionally, tissues can be fixed before freezing. This makes it easier to section but may reduce sensitivity.
2. Prepare 10-20 micron sections on gelatine-coated slides, let air dry.
3. Fix sections 5 min in 2% paraformaldehyde, 0.125% glutaraldehyde in PBS. (Omit this step if tissues are fixed before freezing)
4. Aspirate fixative and incubate slides 3 x 1 min in PBS with 2 mM MgCl2.
5. Incubate 3 x 2 min in PBS, MgCl2, NP40, desoxycholate.
6. Incubate 2 min with staining buffer without x-gal.
7. Add x-gal to staining buffer and incubate at 30 or 37OC. Several hours to over night.

All incubations are performed in a moist chamber.

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To home page

last update: May 1999