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Whole-Mount X-Gal Staining of Mouse Tissues

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Abstract

Although the development of improved mouse models, including conditional deletions, marks an exciting time in mouse genetics, it is important to characterize and validate these models. Cre reporter strains allow researchers to assess the recombinase expression profile and function in individual Cre mouse lines. These strains are engineered to express a reporter gene (usually *LacZ*) following the removal of a floxed STOP cassette, thus marking cell lineages that can be targeted with a given Cre line. This protocol provides a detailed method for the histochemical detection of β -galactosidase activity in Cre mouse strains.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

CO₂ for euthanization

Ethanol (70%)

Formalin (10%)

Mice (Cre reporter strains that express *LacZ*)

Paraformaldehyde (PFA) (3.7% in PBS)

Dissolve the PFA by heating the solution to 65°C in a heat block (use a stir bar to mix). Adjust the pH to 7.0–7.5 with 1 N NaOH. Store on ice until the solution is to be used. Prepare fresh before each experimental procedure. PFA is a suspected carcinogen and should be handled with care.

Phosphate-buffered saline (PBS)

X-Gal rinse buffer for Cre expression <R>

X-Gal staining solution for Cre expression <R>

Equipment

Aluminum foil

Bibulous paper

Forceps, blunt

Forceps, sharp

Incubator at 37°C

Needles (26 gauge)

Parafilm

Petri dish (15 cm) coated with paraffin

Platform rocker

Scalpel

Scissors, dissecting

METHOD

1. Euthanize a mouse with CO₂. Remove the desired tissue from the mouse. Clean the tissue with PBS and place on bibulous paper. Cut around the tissue eliminating as much bibulous paper as possible, and, if more than one mouse is being dissected, label the paper for animal identification.
 1. See Troubleshooting
2. Move tissue to a 15-cm dish coated with paraffin and pin the tissue with 26-gauge needles.
3. Fix the tissue in fresh 3.7% cold paraformaldehyde for 1 h at 4°C.
 1. One 15-cm dish requires at least 150 mL of solution to cover the tissue.
4. Properly discard the paraformaldehyde and incubate the tissue in rinse buffer for 30 min at 4°C on a platform rocker.
5. Rinse two additional times for 30 min each with rinse buffer at room temperature on a platform rocker.
6. Discard the rinse buffer and add staining solution to cover the tissue. Wrap the Petri dish and lid with Parafilm, cover with foil, and incubate overnight at 37°C. The plate does not to be agitated.
 1. See Troubleshooting

7. Fix the tissue in 10% formalin for 1 h at room temperature on a platform rocker.
8. Wash with 70% ethanol until the tissue is bleached. Replace with fresh 70% ethanol and wrap the covered Petri dish with Parafilm.
9. Monitor *LacZ* expression by looking for the appearance of blue spots on the tissue (see Fig. 3 in Strategies to Achieve Conditional Gene Mutation in Mice [Gierut et al. 2014]).

TROUBLESHOOTING

Problem (Step 1): The tissue tears during dissection from the mouse.

Solution: Use sharp forceps and scissors when removing the tissue. Tearing may also occur if the tissue is dry. Keep the tissue moist with PBS.

Problem (Step 6): The tissue dries out overnight.

Solution: Make sure the lid of the plate is covered tightly with Parafilm. It may also help to add more staining solution to ensure that the entire tissue is submerged in liquid.

RECIPES

X-Gal Rinse Buffer for Cre Expression

| Reagent | Final concentration |
|--|---------------------|
| Sodium phosphate dibasic (0.5 M; pH 7.0–7.5) | 0.1 M |
| Sodium phosphate monobasic (0.5 M; pH 7.0–7.5) | 5 mM |
| MgCl ₂ · 6H ₂ O (1 M) | 3 mM |
| Sodium deoxycholate | 1.5 mM |
| Octylphenoxypolyethoxyethanol (IGEPAL CA-630) | 3.0% |

Prepare in PBS.

X-Gal Staining Solution for Cre Expression

| Reagent | Final concentration |
|------------------------|---------------------|
| Potassium ferricyanide | 5 mM |
| Potassium ferrocyanide | 5 mM |
| X-gal solution <R> | 1 mg/mL |

Prepare the X-gal staining solution in X-gal rinse buffer <R>.

References

Gierut JJ, Jacks TE, Haigis KM. Strategies to achieve conditional gene mutation in mice. *Cold Spring Harb Protoc.* 2014;10.1101/pdb.top069807