

Tamoxifen Administration for Lineage Tracing Using CreERT2 mice

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Method Article

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Abstract

A requirement for lineage analysis is tight temporal control of label induction. In the context of the tamoxifen-inducible cre/lox system, this means that there can be no residual tamoxifen to activate cre driven by promoters that might become upregulated over time or following injury. However, the protracted half-life of tamoxifen can allow recombination longer than generally appreciated. This protocol describes the appropriate "chase" times necessary to ensure accurate lineage analysis.

Introduction

A requirement for lineage analysis is tight temporal control of label induction. In the context of the tamoxifen-inducible cre/lox system, this means that there can be no residual tamoxifen to activate cre driven by promoters that might become upregulated over time or following injury. However, the protracted half-life of tamoxifen can allow recombination longer than generally appreciated. This protocol incorporates the longer "chase" time necessary after tamoxifen administration in order to get meaningful fate mapping data.

Reagents

Tamoxifen, Toronto Reasearch Chemicals, cat# T006000 Corn Oil, Sigma, cat# C8267-500ML 200 Proof Ethanol 3/10cc Insulin Syringe, BD, cat #309301

Equipment

Vacuum Centrifuge

Procedure

1. Tamoxifen Storage: Make a stock solution of 20mg/ml dissolved into 100% ethanol in a cell culture hood. Make 1ml aliquots and store at -80C.
2. Tamoxifen Preparation: Tamoxifen is administered at a standard dose of 0.25mg/g body weight. Weigh mice and aliquot the appropriate volume into 50ul of corn oil. Vortex the corn oil/ethanol mixture and place in vacuum centrifuge for 30 min to evaporate the ethanol⁽¹⁾. Aspirate the tamoxifen into an insulin syringe.
3. Tamoxifen Administration: All injections are delivered intraperitoneally. For most transgenic mice, between 3 and 5 doses of tamoxifen are appropriate. Recombination efficiency should be quantified in a known cell type expressing the gene driving the CreER.
4. A chase period of at least 21 days is used to insure the absence of residual tamoxifen before injury⁽²⁾, but this chase period is highly dependent on the gene driving CreER, tamoxifen dose, and injury model. Ultimately the chase period will have to be determined empirically by end user for their particular experimental design.

Timing

Preparation and administration of tamoxifen will take approximately 1.5 hours. Plan to wait a minimum of 3 weeks after the final dose of tamoxifen before inducing injury.

Troubleshooting

1. Tamoxifen is light sensitive. It may be useful to store in opaque tubes. 2. Before corn oil can be used it must first be autoclaved or run through a 0.22uM filter. 3. Tamoxifen should not be used after 1 freeze-thaw.

Anticipated Results

Determine recombination efficiency in the appropriate control cell type depending on the gene of interest. We typically aim for >80% recombination.

References

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