

For early postnatal induction (P1-P5) we use intragastric injection. We use an insulin syringe 29-31 gauge needle, 1 cc. (<https://www.nature.com/article-assets/npg/nprot/journal/v5/n9/extref/nprot.2010.113-S1.mov>)

Aiming for the milk spot.

P1: [inject 20ul of 2mg/mL stock]

P2-P3: [inject 30ul of 2mg/mL stock]

P4-P5: 1 [inject 40ul of 2mg/mL stock]

For postnatal Tamoxifen induction (P6-adult), we use subcutaneous injection of Tamoxifen [20 mg/ml] at 8 mg/40gm of body weight ([Cell. 2006 Dec 15; 127\(6\): 1253–1264.](#))

Adult mice [IMG injection] = 50uL of 20mg/ml stock

The duration and frequency of TAM injections is to be determined empirically for each strain. To begin we will assay for maximal recombination after 2 ip injections (given 1 per day) of 1 mg/ mouse.

Preparation of TAM for injection

We are using a non-pharmaceutical grade of Tamoxifen that we dissolve in filter-sterilized peanut oil. We obtain the highest quality Tamoxifen available from Sigma (Product #T5648), which is $\geq 99\%$ pure (determine by TLC) and we do not use this past its expiration date. Dissolve tamoxifen (Sigma-Aldrich) in corn oil at a concentration of 20 mg/ml by shaking overnight at 37°C. Tamoxifen is light sensitive, and should be made and stored in a light- blocking vessel (amber, or foil wrapped). After tamoxifen is in solution, store at 4°C for the duration of injections or store aliquots for longer at -20C. We will use a NF grade peanut oil from Spectrum (https://www.spectrumchemical.com/OA_HTML/chemical-products_Peanut-Oil-NF_PE090.jsp?section=22373). Prior to injection it is wise to warm up aliquots to 37°C to ensure proper mixing, especially after removing an aliquot from the freezer.

Tamoxifen is a hazardous substance. Review the MSDS, wear appropriate PPE, and, house mice in disposable pens in a separate animal room for the duration of injections. All materials must be disposed of properly.

- Following the final injection, mice should be quarantined for 72 hours before returning to their normal animal room. Disposable pens and bedding should be discarded at this time.
- Throughout the course of tamoxifen injections and any post-injection wait period, mice should be closely monitored for any adverse reactions to the treatment.