

Kotton Laboratory Method for Intratracheal Lentivirus Instillation

We find the best alveolar macrophage transduction efficiency occurs when we instill 5x 10⁷ virions into a mouse in 100 mcl of fluid in a single bolus.

This is accomplished as follows:

1. Titer lentivirus as usual on FG293 cells (we do this by FACS). Calculate titer (TU/mL). Most of our concentrated (ultracentrifuged) lentiviral stocks are 1-5x10⁹ TU/mL.
2. When ready to infect mice, calculate the dilution factor needed to dilute the titer down to precisely 5x10⁸ TU/mL for injection.
3. To prepare the virus for injection thaw the viral stocks and then add cold PBS or complete media to achieve the goal dilution factor (actually we use complete 293 media: 10% FBS in DMEM with 1% pen-strep for our dilutions but it shouldn't matter). Leave on ice until you inject that day. (If you need to re-freeze virus, you can expect the titers to drop approximately 2 fold with the next thaw.)
4. Add 5% Lipofectamine 2000 (Invitrogen) to the viral fluid (i.e. 5 mcl of lipofectamine for every 100 mcl of final fluid volume that you'll put in the mice). Let incubate on ice for around 20-30 minutes before injecting into the mice. (If you forget the lipofectamine, the transduction will still work but the efficiency is about 2 fold lower).
5. Anesthetize mice with isoflurane and intratracheally instill 100 mcl of virus into each mouse as a single bolus using a blunt-end canula.

Resident alveolar macrophages should begin gene expression within 36 hours, peaking at around 48 hours. 20-50% of alveolar macrophages should be transduced if your technique is good. Note: acute inflammation follows intratracheal infection and takes 4-6 weeks to completely resolve back to baseline.