

## Athymic Nude Mice Intramuscular Heterotopic Ossification Protocol

By Mike Sun 2-21-2003; commented by TCH

### Direct Viral Injection (for 4 injections)

1. PBS+P/S solution added to virus to make to total of 200ul volume.
2. Prepare 1% agarose tubes (prepared in PBS) by removing the storage buffer completely using long-stem tips.
3. Dialyze virus samples in 1% agarose tubes @RT x 5min.
4. Recover the dialyzed samples and transfer to a new set of microfuge tubes (or use a 48-well plate if you have too many samples).
5. Divide into four 40ul injections (i.e., 40ul per injection).
6. Inject with 50ul Hamilton Syringes.

### Injection with C2C12 cells (for 4 injections)

1. Combine two T-75 flasks confluent C2C12 cells infected with adenovirus overnight (check GFP expression before collecting).
2. Spin at 1000 RPM for 5 minutes with Eppendorf Centrifuge (**the big one on the bench**) in Cold Room
3. **Completely** aspirate supernatant, then add 150ul of PBS/PS solution (**using p1000**), and resuspend cells completely by pipeting up-and-down multiple times (**again using p1000 tips**). The final volume should be slightly over 200ul.
4. Divide into four 50ul and inject with 50ul Hamilton Syringes.