Heather LaMarca 6/07

Adenovirus Transduction

Transductions are performed on freshly isolated primary MECs that are in single cell suspension.

- **Transduce cells in a 50ml conical tube. This size tube will allow for larger surface area.**
- **Dilute the virus in growth media (or wash buffer).**

1. Thaw virus on ice.

2. Transduce with Ad.Cre at an MOI of 50**.**
   - Add the indicated amount of virus to 2ml (for 3 mice) of growth media/cells in a **50ml** conical.

Note: Depending on the size of the pellet, I typically transduce in 1-5ml volume for up to 10 mice. A minimal volume is optimal.

3. Incubate cells with virus for 1 h at 37°C on a 45° angle, rocking every 15′. After 30 min, briefly pipet up and down.

4. Transfer cells/virus to a 15ml conical tube. Bring the volume up to 15ml with wash buffer. Centrifuge at 300 × g for 5′ at RT to remove any unbound virus.

5. Wash cells with 15ml of wash buffer.
   Centrifuge at 300 × g for 5′ at RT.
   Resuspend each pellet in the appropriate media.

If transplanting at limiting dilution, re-count cells at this step. Cell number is usually much less at this stage.

<u>To calculate how much virus you need</u>:

(Number of Cells)×(MOI)/Titer (pfu/ml) = ____ ml of virus