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**Titration of Lentivirus**

##All procedure must be done at BL2 and all used materials must be bleached until cells are prepared for facs##

**Materials**
- 6 well plate
- Concentrated virus
- 293 FT cells (4x10^5 cells/well): Prepare healthy cells
- Polybrene
- cDMEM media for 293T cells:
  - 10% heat-inactivated FCS
  - 1% Sodium Pyruvate
  - 1% L-glutamine

**day 1: Dilution of Virus and infection to 293FT cells**
1. Place 4x10^5 293FT cells/2 ml media/well in 5 wells. You will need 4 wells/virus plus one well for a negative control.
2. 6 hours later, check under a microscope to be sure that cells have adhered to the plate.
3. Add 1 ul polybrene/well (10 mg/ml stock solution. Final concentration is 5ug/ml)
4. Make serial dilutions of virus in 1.5 ml eppendorf tubes as follows:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Description</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x: VIRUS STOCK (usually 10-15 ul aliquot)</td>
<td>5 ul</td>
<td></td>
</tr>
<tr>
<td>1/10x:</td>
<td>5 ul virus + 45 ul cDMEM</td>
<td>5 ul</td>
</tr>
<tr>
<td>1/100x:</td>
<td>5 ul 1/10x virus + 45 ul cDMEM</td>
<td>5 ul</td>
</tr>
<tr>
<td>1/1000x:</td>
<td>5 ul 1/100x virus + 45 ul cDMEM</td>
<td>5 ul</td>
</tr>
</tbody>
</table>

4. Add serial dilutions of virus to pre-plated 293FT cells, 5 ul/well.

Mix gently. Wrap plate with Saran Wrap and spin 2000 rpm at 32 C for 90 minutes. Incubate 37C for 48 hours exactly in order to make reproducible data.

**Day3: Flow Cytometry and calculation of virus titer**
1. Put plate on ice after exact 48 hours of 37C incubation.
2. Carefully wash cells 1x with PBS.
3. Trypsinize attached cells by adding 100 ul 0.05% Trypsin-EDTA solution.
4. Quench Trypsin by adding 1 ml/well FACS Buffer. Resuspend cells, wash 2x. Wash buffer 2x and filter it. Run flowcytometry to detect GFP positive cells.
Calculation of virus titer
Take numbers from data between 1-10% infection rate.
For example,

if infection rate is 90% at 1st row (2.5ul of virus/ml), 55% at 2nd row (0.25ul of virus/ml), 5.00% at 3rd row (0.025ul of virus/ml) and 0.50% at 4th row (0.0025ul of virus/ml), use 5.00% at 3rd row and calculate

4x10^5 cells x5.0%/100 = 2x10^4 positive cells
2x10^4 cells/0.025ul = 8x10^5 viral particles/ul

It means, also,

Titer in IU/mL =
{(# cells at starting time) * (dilution factor) * (percent infection) / (vol virus solution added expressed in mls)}

{(4x10^5 cells/ml) * (100 times dilution) * (5.0%/100)} / (0.0025ml) = 8.0 x 10^8 IU/ml

In general, you should have at least 5.0x10^8 IU/ml for embryo infections.