Transfection of HEK293 cells with Lentiviral DNA to produce lentivirus in a 10cm plate

1. Eighteen to twenty-four hours prior to transfection, seed 1-2x10^6 cells/ml HEK293 cells (HEK293T cells can also be used and may provide as higher virus titre) per well in a Poly-L-Lysine 10 cm plate in 15ml of High Glucose Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS).

• Swirling of the well should be avoided to prevent cells clumping in the centre.

• After incubation at 37˚C for 18-24 hours, wells are checked visually to have ~70-80% confluency (cells at 100% confluency should not be used as this will reduce transfection efficiency).

2. For each 10 cm dish:

• Tube A: Into a 1.5ml polypropylene tube add 150µl of pALD Lenti Mix (Kan) or (Amp) and 6.25µg of your lentiviral vector genome plasmid. Make the total tube volume up to 1.1ml with Opti-MEM® (Thermo Fisher Scientific®) and thoroughly mix by pipetting up and down.

• Tube B: Into a 1.5ml polypropylene tube add 55µl of 25 kDa branched polyethylenimine (PEI) (Sigma-Aldrich®) (stock concentration 1mg/ml) and 1.1ml of Opti-MEM®.

• Tube C: Mix 1.05 ml from both tube A and B into a 15ml polypropylene Falcon tube. Invert the tube multiple times to ensure the DNA:PEI is mixed. Avoid vortexing or vigorous pipetting. Allow the DNA:PEI to complex at room temperature for 20 minutes.

3. Before transfection, replace the tissue culture media of each well to be transfected with 8ml of DMEM (10% FBS) and place back in the incubator for ten minutes to allow the temperature to recover.

4. After tube C has complexed, add 2ml in a drop wise fashion to the well.

5. Incubate the plate in a humidified CO2 incubator at 37˚C for 72 hours.

6. Collect the supernatant into a poly-propylene tube and spin at 5000g for 2 minutes pellet any cells that have dislodged from the plate during virus production. Harvest the supernatant and avoid disturbing any cells that may be at the bottom of the tube.

Transfection of HEK293 cells with Lentiviral DNA to produce lentivirus in a 6-well plate

1. Eighteen to twenty-four hours prior to transfection, seed 1x105 cells/ml HEK293 cells (HEK293T cells can also be used and may provide as higher virus titre) per well in a Poly-L-Lysine 6-Well Plate in 3ml of High Glucose Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS).

• Swirling of the well should be avoided to prevent cells clumping in the centre.

• After incubation at 37˚C for 18-24 hours, wells are checked visually to have ~70-80% confluency (cells at 100% confluency should not be used as this will reduce transfection efficiency).

2. For each single well of a 6-well plate:

• Tube A: Into a 1.5ml polypropylene tube add 30µl of pALD Lenti Mix (Kan) or (Amp) and 1.25µg of your lentiviral vector genome plasmid. Make the total tube volume up to 220µl with Opti-MEM® (Thermo Fisher Scientific®) and thoroughly mix by pipetting up and down.

• Tube B: Into a 1.5ml polypropylene tube add 11µl of 25 kDa branched polyethylenimine (PEI) (Sigma-Aldrich®) (stock concentration 1mg/ml) and 209µl of Opti-MEM®.

• Tube C: Mix 210µl from both tube A and B. Invert the tube multiple times to ensure the DNA:PEI is mixed. Avoid vortexing or vigorous pipetting. Allow the DNA:PEI to complex at room temperature for 20 minutes.

3. Before transfection, replace the tissue culutre media of each well to be transfected with 2ml of DMEM (10% FBS) and place back in the incubator for ten minutes to allow the temperature to recover.
4. After tube C has complexed, add 400µl in a drop wise fashion to the well.
5. Incubate the plate in a humidified CO2 incubator at 37˚C for 72 hours.
6. Collect the supernatant into a poly-propylene tube and spin at 5000g for 2 minutes pellet any cells that have dislodged from the plate during virus production. Harvest the supernatant and avoid disturbing any cells that may be at the bottom of the tube.

Notes:
The supernatant can be stored for up to a week at 4˚C or indefinitely at -80˚C.
Using other transfection reagents can significantly improve yields but will increase the cost of vector production significantly.
Use poly-propylene where possible and avoid using polystyrene tubes where possible because the surfaces can carry a charge which can cause viruses to stick.

Measurement of Titre by Infection of HEK293 cells with Lentivirus Produced by Transfection in a 48-Well Plate.
1. Twenty-four hours prior to virus infection, seed 3x104 HEK293 cells per well in approximately 300-400µl of DMEM (10% FBS) in a Poly-L-Lysine 48-well plate.
• After overnight incubation at 37˚C, wells are checked to have approximately 70-80% confluency.
2. Change the media of each well with 200µl of 10% FBS DMEM and place back in the incubator for ten minutes to come back up to temperature.
3. To measure infectivity, we recommend performing serial dilutions of your virus supernatant across multiple wells. In the first 3 wells add 50µl of virus supernatant, in the second row add either a 5- or 10-fold dilution and repeat this across multiple rows in the plate. The aim is to achieve approximately 10-20% infected cells, significantly more or less levels of infectivity can result in inaccurate titre calculations.
4. Ensure to leave some wells uninfected to act as negative controls. Incubate the plate for 72 hours.
5. If the lentivirus vector contains a reporter gene then infectivity can be measured by flow-cytometry.
6. Titre can be worked out by determining:
7. Volume of supernatant tested in mL

% of cells / 100 x number of cells initially in the well