Document Number:	**SASoM/METHOD/073.v4**
Title:	**Lentivirus production from HEK293T cells**
Version:	**v4**
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SOP History		
Number	Date	Reason for Change
v1	10/10/2014	Original
V2	10/10/2016	Update
V3	10/10/2018	Update
V4	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for Lentivirus production from HEK293T cells for use in Laboratory 248K at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in Lentivirus production from HEK293T cells.

3.0 Responsibilities –
All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

[A]: Lentivirus production from 293T cells

Early passage HEK293T cells (designated 293T(SD)) should be used for lentiviral production. Later passage cells will be inefficient at producing virus.

Day 1 – Plating Cells:

Plate 4e6 293T cells per 10cm T/C plate (in a volume of 10mL).

Day 2 – Cell Transfection:

Put 50mL aliquot tube containing OPTIMEM into the water bath to pre-warm. Then add 1.5mL of this OPTIMEM into a 15mL tube (1 tube for each petri dish of cells). Add the appropriate amount of Mirus LT1 to the OPTIMEM as required (according to Paul Reynolds Excel file protocol).

Gently pipette Mirus LT1 directly into the media without touching the plastic of the tube. (NEVER vortex).

Wait 20 minutes. While waiting, make up DNA mixture in sterile eppendorf tube as required (again according to Paul Reynolds Excel file protocol). Use pVSV.g and psPAX2 for packaging plasmids (2nd generation packing) plus the expression plasmid of interest.

After the 20 minutes has elapsed, add DNA mix to the Mirus LT1-OPTIMEM solution. Gently pipette. (NEVER vortex). Wait 30 minutes.

Add DNA-Mirus LT1-OPTIMEM solution dropwise evenly across the surface of a 10cm T/C plate of 293T cells containing 10 mL DMEM/10%FBS/1% PS. Place in incubator labeled "viral' overnight.

Day 3 – Change medium to reduce volume and increase viral particle concentration:

Change media at <24 hrs post-transfection, adding 4 mL DMEM / 10%FBS / 1% PS.

From this point onwards, live virus is being produced – take appropriate safety measures!

Use the "viral" safety cabinet and "viral" labeled equipment for all manipulations.

Day 4 – Collect viral particles

Collect media containing viral particles from cells, 48hr post-transfection into a 15mL tube. CAREFULLY add new 4 mL DMEM/10%FBS/1% PS to the plate (these cells detach easily). The viral particle medium should be a clear, slightly orange colour

(cloudy = contamination). Store 15mL tube in a box labeled “virus” in the fridge in 248K.

Day 5 - Collect viral particles and infect recipient cells

Collect media containing viral particles from cells, 72hr post-transfection. The viral particle medium should be a clear, slightly orange colour (cloudy = contamination). Mix with viral particles from cells, 48hr post-transfection. Pass ppoled 48hr and 72hr viral particle media through a 0.45um filter using a 10ml syringe into a 50mL tube containing 8ug/ml polybrene (1000x stock). This step removes cellular debris. Add 4mL of lentiviral particles to each pre-prepared 10cm T/C plate of adherent recipient cells (see below). Place into “viral” incubator for > 4 hr, gently rocking occasionally to aid viral attachment. Dispose of 293T cell plates by autoclaving. After >4hr, aspirate viral particle media to liquid viral waste and replace with 10mL culture media.

[B] **Lentiviral infection/ transduction of adherent recipient cells.**

Day 4 – Plate ‘recipient’ cells

Seed 5e5 cells (e.g. MCF10A) per 10cm T/C plate – 2 plates for each plasmid (=1e6 cells). This is based on appropriate MOI.

Day 5 - Collect viral particles and infect recipient cells

Collect media containing viral particles from the cells, 72hr post-transfection. The viral particle medium should be a clear, slightly orange colour (cloudy = contamination). Combine with viral particles from cells, 48hr post-transfection. Pass the pooled 48hr and 72hr viral particle media through a 0.45um filter using a 10ml syringe into a 50mL tube containing 8ug/ml polybrene (1000x stock). This step removes cellular debris. Add 4mL of lentiviral particles to each pre-prepared 10cm T/C plate of adherent recipient cells (see below). Place the T/C plates into “viral” incubator for > 4 hr on a plastic tray, gently rocking occasionally to aid viral attachment. Dispose of 293T cell plates by autoclaving. After >4hr, aspirate viral particle media to liquid viral waste and replace with 10mL culture media.

Day 7 – Select recipient cells (e.g. with puromycin)

After 48h post-infection, select cells (e.g. with puromycin).
Puromycin works most efficiently on floating, trypsinized cells.
Therefore, aspirate media, add 3mL of PBS, aspirate PBS and incubate at 37°C with 2mL TrypLE Express (Invitrogen) until cells are detached (about 10min). Add 8mL of culture media and transfer to 50mL tube. Centrifuge to pellet cells (1,200 rpm for 3 min). All 20mL culture media to a T-75 flask and add required amount of selection drug (e.g. puromycin). Do this by dropping from a micropipette from the top of the flask. Do not contaminate the flask by placing a micropipette into the flask.

Resuspend cells in 5mL culture media and transfer to the T-75 flask. Incubate at 37°C in the "viral" incubator.

Day 8-11 – Select recipient cells (e.g. with puromycin)

Change media if required due to dead, floating cells. Split cells if they become confluent. 4 days after addition of puromycin selection only puromycin resistant cells will remain. Continue with downstream applications. Cell lines are "safe" after 2 changes of plastic.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with water.

7.0 Training -

All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments – CHARM RA1386: Generating and working with viral particles at Biosafety Level 2

8.2 SOP - SASoM-METHOD-009-Generating and working with viral particles at Biosafety Level 2

9.0 Approval and sign off –

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