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Generation of stable cell lines using viral infection

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for generation and precipitation of retrovirus, and infection of HeLa cells to generate stable cell lines.

Day 1

1 Seed HEK293T cells into a 10cm tissue culture plate (6.1 million cells/plate), seeding one plate per construct you are generating virus for.

Day 2

2 Transfect cells with viral and helper vectors using lipofectamine LTX. In a 15mL falcon tube, combine the following:

	A	B	C
		Reagent	**Amount**
	1	Viral vector construct containing cDNA of interest	9 ug
	2	Gag-pol vector	6 ug
	3	VSV-G vector	3 ug
	4	Opti-MEM	3 mL

3 Add 18 µL of PLUS reagent and vortex for ~ 00:00:20 . Incubate at Room temperature for 00:05:00 . 5m 20s

4 Add 54 µL of Lipofectamine LTX , vortex for ~ 00:00:20 . Incubate at Room temperature for 00:20:00 . 20m 20s

5 Once the 20 minute incubation starts, replace the media on the HEK cell plates with 7 mL media/plate .

6 At the end of the incubation, add the lipofectamine/OptiMEM mix to the plate using a sterile transfer pipette, in a drop wise fashion across the plate. Gently rock the plate to mix, and return the plate to the incubator.

Day 3

7 In the morning, remove the media from the HEK cell plate and dispose of the media in a beaker containing bleach. Add 6 mL of fresh growth media to the HEK cell plate.

Day 4

8 In the late afternoon, collect the media from the HEK cell plate into a falcon tube. Add back 8 mL fresh growth media to the HEK cell plate.

9 Centrifuge the media collected at maximum speed for 00:05:00 at Room temperature to pellet any debris. Pass the media through a 0.45 mm syringe filter into a clean falcon tube. Parafilm the falcon tube, and place store the media at 4 °C in the fridge. 5m

Day 6

10 In the morning, collect the media from the HEK cell plate into a falcon tube. Centrifuge the collected media at maximum speed for 00:05:00 at Room temperature. Pass the media through a 0.45 mm syringe filter into a clean falcon tube. 5m

11 Combine the media collected in step 9 and in step 10 into one tube. Add Lentivirus Precipitation Solution (ALSTEM) to the media at a ratio of 1:4 (Lentivirus precipitation solution : viral media).

12 Gently invert the tube 8 – 10 times to mix the precipitation solution and media together. Parafilm the tube and leave on a rocker gently rocking at 4 °C for 48:00:00. 2d

Day 7

13 If intending to infect cells with fresh virus, seed the cells to be infected into a 6 well plate (~220K cells/well).

Day 8

14 After the 48 hour rocking incubation, centrifuge the viral precipitation mix at 1600 rcf, 4°C for 01:00:00 1h

15 Remove the supernatant, disposing of it into a beaker containing bleach. Resuspend the precipitated pellet in 3 – 6 mL of fresh growth media (3 mL if you require high construct

expression).

16 Replace the media on the cells to be infected with 1.5 mL fresh growth media/well. Add 500 µL of the precipitated virus to each well to be infected.

17 Add polybrene at a final concentration of 8 mg/mL to each well, and rock the plate to mix. Place the cells back into the incubator.

18 Any leftover virus can be frozen at -80 °C for use at a future date

Day 9 - 10

19 After 24 – 48h (depending on the construct and expression required), remove the media from the infected cells and place in bleach to dispose. Add fresh growth media back to the cells.

Day 15 - 17

1h

20 After 5 - 7 days after the virus has been removed (and after three passages), using FACS, sort the cells populations to the desired fluorescent expression level.