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Generation of stable cell lines using retroviral system

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nguyen.tha[1]

[1]Laboratory of Michael Lazarou, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

nguyen.tha

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

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Abstract

This protocol details generation of stable cell lines using retroviral system.

Attachments

698-1486.docx
19KB

Guidelines

Attention

- The HEK293T cells detach very easily, be extra gentle when changing the media.

Materials

Buffers and reagents:

- Polybrene (4 mg/mL)

Growth media:

	A	B
	DMEM with 10% FBS	
	Glucose	4.5 g/l
	GlutaMAXTM	1x
	MEM NEAA	1x
	HEPES	25 mM

45% D-( )-Glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G8769

GlutaMAX™ Supplement **Thermo Fisher Catalog #**35050061

MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Scientific Catalog #**11140050

HEPES Buffer 1M Solution Cell Culture Grade MP Biomedicals **Fisher Scientific Catalog #**ICN1688449

Lipofectamine™ LTX Reagent with PLUS™ Reagent **Thermo Fisher Catalog #**A12621

Gibco™ Opti-MEM™ I Reduced Serum Medium no phenol red **Fisher Scientific Catalog #**11-058-021

Millex-HV Syringe Filter Unit 0.45 µm PVDF 33 mm gamma-sterilizable sterilized **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SLHVM33RS

Troubleshooting

Safety warnings

Attention

- All viral waste must be bleached and left under UV light for at least 30′ after viral work in TC hoods before disposal.

Day 1

1 Seed NIH HEK293T cells into a 6-well plate (900k cells/well if set up in the morning, 950k cells/well if set up in the afternoon).

Note

Set up 1 well for each construct you wish to generate a virus harvest for, can be scaled up according to your need.

Day 2: The following protocol is designed for one well of the 6-well plate

2 Transfect cells with viral and helper vectors using lipofectamine LTX. Combine the following in a 1.5 mL tube:

	A	B
	viral vector construct (pBMN, pBABE or pMX) containing cDNA of interest	1.5 µg
	gag-pol vector	1.0 µg (amount for 1 well)
	VSV-G vector	0.5 µg (amount for 1 well)
	Opti-MEM (RT)	500 µL

3 Add 3 µL of Plus reagent and mix well. Incubate at Room temperature for 00:05:00 . 5m

4 Add 9 µL of Lipofectamin LTX (1:3 ratio of Plus:LTX is standard in the lab but can be adjusted for your own protocol) and vortex for 00:00:15 . Incubate at Room temperature for 00:20:00 . 20m 15s

5 Once the 20 min incubation starts, replace the media in each well with 1 mL DMEM/10% FBS media.

6 When the 20 min incubation finishes, add the optimum/liposome mix to the well.

Note

Do it gently on the side of the well.

Day 3

7 In the morning, remove the old media from the HEK293T cells which may contain viruses at this stage) into a beaker of beach and add 1 mL of fresh growth media. The next day, viruses can be harvested for infection.

8 Seed the target cells (about 100k-120k cells) into a 6-well plate if intending to do infection with fresh viruses.

Day 4

9 In the late afternoon, collect viral supernatant from HEK293Ts, spin down at max speed for 00:05:00 to pellet debris and filter through 0.45µm syringe filters. Viral particles can freshly be used for infection on the cells plated out on day 3 (see below) or can be frozen at -80 °C for future use. 5m

10 For second harvest, add 1.5 mL fresh growth media back to HEK293T cells for 2 days and harvest again (on Day 6).

11 For infection, harvested viruses are topped up with fresh growth media to make up a total of 2 mL .

12 Aspirate the media from the target cells.

13 Add the 2 mL of virus-containing media (from step 3) to the target cells. Add polybrene to a final concentration of 8 µg/mL to the well and mix well.

Days 5 and 6

14 The viruses can be removed from the cells into a beaker of bleach after 24 h (Day 5) or 48 h (Day 6) and fresh media can be added to the wells.

15 All waste must be treated as viral waste for at least 3 media changes over 3 days post-infection.