Lentivirus production in HEK-293T/17 by calcium phosphate transient transfection

- Plate cells **1/8** (the day before) or **1/6** (the same day) from a 90% confluent plate.*
- Change media the following morning (**20ml per p150**) [see Calcium phosphate protocol for the corresponding volume].
- Calcium phosphate transfection early in the evening:

  36.25ug Transfer vector
  27.50ug Packaging plasmid (8.9)
  11.00ug VSVg coding vector (we employ pMD2-G from Addgene).
  in 1.25ml 1xHBS (see recipe in protocol).

  Add **75ul 2.5M $CaCl_2$**
  Let sit for 20min @ tissue culture hood.
  Add dropwise to cells.
  Incubate cells overnight @ incubator.

Note: We routinely prepare the volume for 3 p150 per transfer vector in a 50ml falcon tube. This is very convenient as 3p150 will fill up one ultracentrifuge tube. Therefore, we place 3.75ml 1xHBS, add the corresponding amount of transfer and helper plasmids (3 times the amount typed above), add 225ul of CaCl2 and follow the protocol.

- Remove media and add **12.5ml** 10% serum-containing media per p150.
- Incubate cells for 48h @ incubator**.
- Collect virus-containing media.
- Filter it through a disposable sterile 0.45um PVDF membrane (i.e., Steriflip from Millipore)***.

*If you want to split cells and perform the transfection for the same day, split cells 1/6 early in the morning. Allow cells to attach to the plate (3h) before the transfection.
**A second supernatant can be collected at 72h. This second virus harvest contains less viruses (lower titer). We keep both supernatants separately.
***As an alternative to filtering, the supernatants can be centrifuged for 10min at 740xg (4ºC) to discard cell debris.

Note: This supernatant is ready to use. It can be also ultra-centrifuged to increase the virus concentration (see protocol for details).