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

- Plate cells **1/8** from a 90% confluent plate.*
- Change media the following morning (8ml per p100) [see Calcium phosphate protocol for the corresponding volume].
- Calcium phosphate transfection early in the evening (30ug per p100):

  14.5ug Transfer vector or carrier plasmid (pGL3Basic or pBlueScript)
  11ug Packaging plasmid (8.9)
  4.4ug VSVg coding vector
  in 0.5ml 1xHBS (see recipe in protocol).

  Add 30ul 2.5M $CaCl_2$
  Let sit for 20min @ tissue culture hood.
  Add dropwise to cells.
  Incubate cells overnight @ incubator.
- Remove media and add **5ml** 10% serum-containing media per p100.
- 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.
**As an alternative to filtering, the supernatants can be centrifuged for 10min at 740xg (4ºC) to discard cell debris.
***A second supernatant can be collected at 72h. This second virus harvest contains less viruses (lower titer). We keep both supernatants separately.
Note: This supernatant is ready to use. It can be also ultra-centrifuged to increase the virus concentration (see protocol for details).