**1. Lentivirus Production**

**D1~D3 Lentivirus harvest (caution: Biosafety!!!)**

- At 12 hours post-transfection, discard media and add **gently** 7 ml/10cp of Full DMEM Media back (for 10 cm plate).

- About every 12 hours, collect media and add **gently** 7 ml of DMEM Media back (3 times total > thus, final ~21 ml).

- Keep it at 4°C over the collecting period and pool the collected media (=lentivirus).

-Spin down media 5 min 400G 4C

- Filtrate through **0.45 μm** filter and keep the viruses at 4C until use. (0.22 μm filter may damage viruses)

- Add 10 ml of all filtered lentiviruses in addition to 5 ml fresh Full DMEM MEdia on each 10cp of target cells, include polybrene using 1000x stock) for 24h

-Change media, if doing puro selection wait at least 24h to start