# Nanoblades production protocol (RMI2 lab)

**Day1:**

- Plate HEK293T cells in a 10 cm dish (preferably in the afternoon) in Glutamax DMEM containing 10% serum and antibiotics (Use 4/10 of a 80-90% confluent 10 cm dish for plating one dish).

**Day 2:**

- Cells should be at 70~80% confluence in the morning. Change the medium before transfection.

- Transfect plasmids using jetPRIME (Polyplus Transfection). Use 500 µL jetPRIME buffer and 20 µL jetPRIME reagent (following the manufacturer's protocol) for each 10 cm plate as well as the following amounts of plasmids:

0.3 µg VSV-G
0.7 µg BRL
2.7 µg MLV Gag/Pol
1.7 µg BIC-Gag-Cas9
4.4 µg sgRNA coding plasmid (or 2.2µg each if using two gRNAs)

**Day 4:**

40h after transfection, most of the cells will have fused together because of expression of the fusogenic viral envelopes.

- Collect the culture medium.
- Centrifuge at 500g for 5 min to remove cellular debris and recover the supernatant.
- If Nanoblades are meant to be used on primary cells, filtration is advised. Filter the supernatant using a 0.45 or 0.8 µm filter. Be aware that this step drastically reduces Nanoblades titer as a significant fraction will be blocked in the filter membrane. Thus, filter only if needed.
- Pellet Nanoblades either at 35,000 rpm on a SW41 Beckmann rotor for 1:15 h at 4°C or overnight in a swinging bucket rotor at max speed (~4,500 rpm) at 4°C.
- Slowly remove the medium by aspiration.
- Slowly resuspend the white pellet with 100 µL PBS by pipetting up and down.

Alternatively, if the target cells can grow in DMEM, you can directly use the supernatant from producer cells after removing the cellular debris.

**Storage:**
Nanoblades can be stored at 4C for at least 1 month. They can be stored at -80C as well but the freeze/thawing cycle will significantly affect their efficiency.

**Transduction protocol:**

For transduction, it all depends on the target cell type. It is important to have the smallest possible volume of medium so that Nanoblades are highly concentrated. Typically, we add between 5 to 10µl of concentrated Nanoblades to 500µl of medium. Adherent cells have to be transduced directly while attached to the plate (do not transduce in suspension as this will significantly decrease transduction efficiency). Some cells are OK with prolonged exposure to Nanoblades (24-48h), others are very sensitive so Nanoblades have to be incubated with cells only for 4-6 hours before replacing the medium. Spinoculation can also improve transduction for cells grown in suspension. Adjuvants such as Polybrene or retronectin can also improve transduction efficiency in some cell types. Transduction has therefore to be optimised for each cell type.

**Materials:**
jetPRIME Transfection Reagent (Polyplus, Cat number: 114-07)
Lenti-X 293T Cell Line (Clontech Takara, Cat number: 632180)
Retronectin (Clontech Takara, Cat number: T100A)
Polybrene (Sigma, Cat number: TR-1003-G)