**Using PiggyBac (PB) vector**:

1. Before transfection, prepare the feeders with appropriate drug-resistant markers (e.g. puromycin-resistant)
2. Prepare appropriate amount of ESC medium (M15+LIF) which doesn’t contain antibiotics (P/S)
3. Prepare Lipofectamine 2000 (Thermo # 11668027) transfection factions (format: one well of **12-well plate**):

|  |  |
| --- | --- |
| **Fraction A** | **Fraction B** |
| 0.5 g PB vector + 0.5 g pBase | 3.2 l Lipofectamine 2000 |
| 100 l OPTI-MEM | 100 l OPTI-MEM |

1. Combine A + B, mix by pipetting. Incubate in room temperature for 20 min
2. Within this 20 min, trypsinize ESCs/iPSCs with 0.25% red TE, after neutralization and spinning, resuspend cells in ESC medium (M15+LIF) without P/S.
3. Mix cells and transfection mixture in tubes or eppendorfs. Sit in room temperature for 10min.
4. Plate [cells+transfection mixture] on the drug-resistant feeders. Put cells in CO2 incubator.
5. After 5-6 hours, change the medium with fresh ESC medium containing P/S.
6. Select cells 24-48 hours after transfection.

**Transduction:**

Similar to the transfection protocol, ESCs are trypsinized, combined with retroviruses or lentiviruses supernatant, supplied with 1X polybrene, and then plated on the feeder with the appropriate selection marker.

*Making lentiviruses*.

1. For 6 cm 293T cells (50% confluency), transfect plasmids using calcium phosphate protocol with the following amount:
   1. Transfer vector: 5 g
   2. psPAX2: 4 g
   3. pMD2.g: 1.5 g

* The calcium phosphate protocol is described in the iPS generation protocol. That protocol is for 10 cm plates so cut every reagent by half here.
* Cells can be also transfected by PEI with the following amount mixed with this order (10cm plate):

1. OPTI-MEM: 165 l
2. PEI: 24 l
3. Transfer vector: 2 g
4. psPAX2: 1.6 g
5. pMD2.g: 0.7 g

* Sometimes lentiviruses need to be concentrated (when the viral titer is low due to the large size of the transfer vector). In that case, the protocol can be scaled-up to 10cm. For the concentration protocol, follow the supplemental *Nature Protocol*.

1. Lentiviral supernatant can be collected and used 48 after transfection.

*Making retroviruses*: follow the viral-making steps in the iPS generation protocol.