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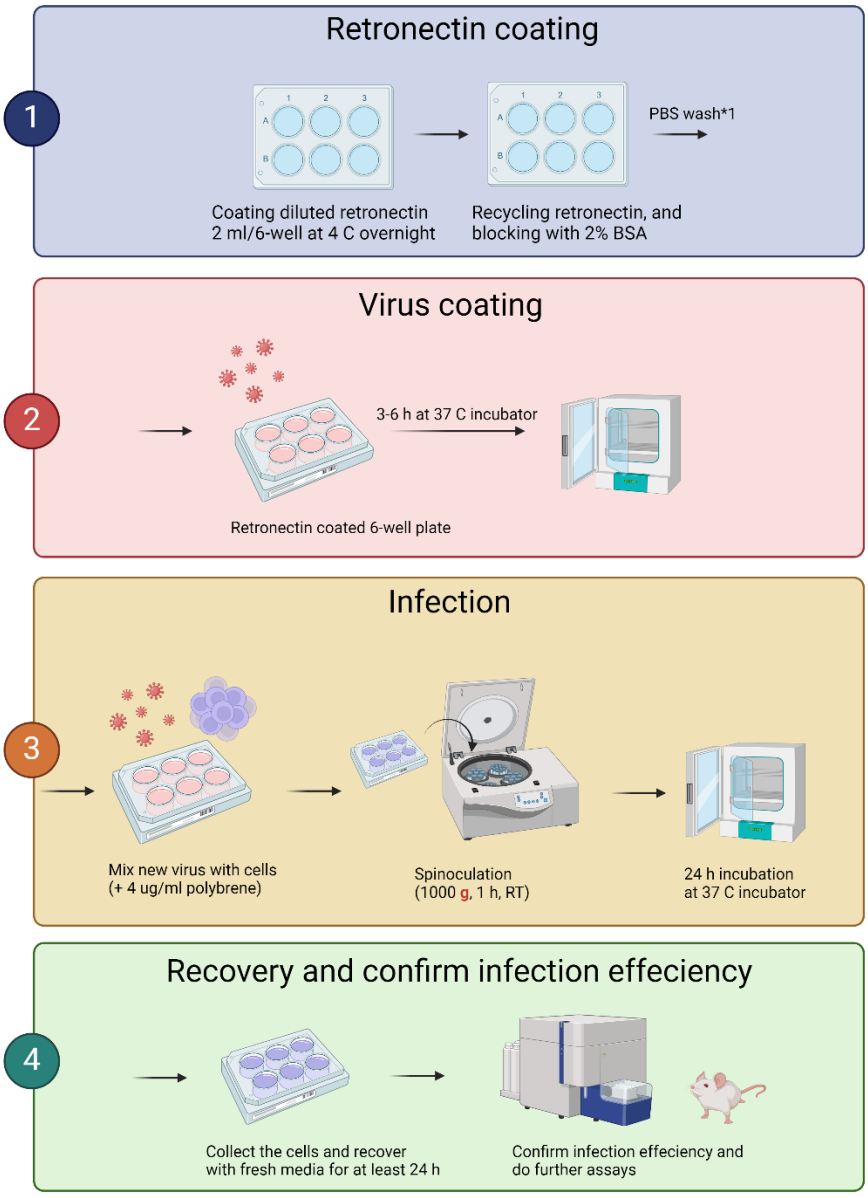
# Viral Infection using patient-derived xenograft (PDX) cells

## ➢ Materials

1. PBS
2. Retronectin (100 ul of 1 mg/ml retronectin diluted with 8 ml PBS)
3. 6-well non-treated plates (or any other small scale)
4. 2% BSA (sterile) in PBS
5. Polybrene (10mg/ml)
6. Virus (Cas9-mCherry as an example)
7. PDX cells

## ➢ Flow chart



- **Procedures**

6-well plate as an example (please scale-down if you use smaller plates.)

1. Preparation of diluted retronectin:
   retronectin (1mg/ml, 100 ul/tube) is stored at -30°C common freezer.
   Add 8 ml PBS into a 15 ml tube and then put 100 ul of retronectin into PBS for dilution.
2. Coating retronectin:
   Add 2 ml/well diluted retronectin into a 6-well plate.
   Seal the plate with parafilm to prevent evaporation.
   Put the plate into 4°C fridge for overnight.
3. Coating virus:
   Recycle diluted retronectin into a 15 ml tube (can be reused for 2 times).
   Add 2 ml of 2% BSA in PBS for blocking at room temperature for 30 min.
   Aspirate gently.
   Add 2 ml of PBS for washing.
   Aspirate gently.
   Add 2-4 ml of virus into wells.
   Incubate the plate at a 37°C incubator for 3-6 h.
4. Infection:
   Aspirate virus gently.
   Mix 1 ml of cells (1-2 million) and 1 ml of new virus and 0.8 ul of polybrene stock to make final 4 ug/ml of polybrene.
   * Add extra cytokines if needed.
5. Spinoculation:
   Put the plate into a centrifuge (attention to balance).
   1000 **g**, room temperature, 1 h
   Incubate the plate at a 37°C incubator for 24 h.
6. Collect the cells from the 6-well plate with PBS or trypsin:
   Collect suspension cells into a 15 ml tube.
   Add 2 ml PBS into wells and pipet several times to detach the cells from bottom.
   Collect all cells into the same 15 ml tube as the previous step.
   Spin down (1500 rpm, 5 min, 4°C)
   Resuspend the cells with fresh media and perform cell counting.
   Put the cells into an appropriate dish/plate and recover for at least 24 h before further assays.