

Retroviral infection of mouse and human myoblasts

Materials:

1. collagen coated 6 or 12 well plate
2. mouse or human myoblasts at lowish passage
3. Frozen RV supe (kept at -70°C)
4. $0.8\ \mu\text{g/ml}$ polybrene stock= 100x (kept at -20°C)
5. fetal bovine serum
6. $25\ \mu\text{g/ml}$ basic fibroblast growth factor (kept in frost free -30°C)
7. appropriate growth medium for cells
8. G418 stock (in -20°C freezer)
9. Table top centrifuge in common equipment room equilibrated to 32°C

1. The day before infection: Trypsinize cells and count.
For mouse myoblasts: Plate $1.5 - 2 \times 10^5$ cells/well of 6 well plate
For human myoblasts: Plate at 1×10^5 cells/well of 6 well plate

For 12 well plate: use half as many cells

2. On the day of RV infection:

- a. Make Infection Cocktail as follows:

For mouse mbs:

Make 10x Infection Cocktail: (scale recipe up or down depending on needs)

FBS	4.5 ml
Polybrene stock	0.5 ml
<u>bFGF stock</u>	<u>10 μl</u>
FINAL VOL	5 ml

For human mbs:

Make 5x Infection Cocktail: (scale recipe up or down depending on needs)

FBS	800 μl
Polybrene stock	200 μl
<u>bFGF stock</u>	<u>4 μl</u>

FINAL VOL 1 ml

- b. Thaw RV supe in 37°C waterbath. Remove as soon as thawed.
- c. Aspirate media from well to be infected.
- d. Add 2 ml of RV supe/well of 6 well size plate. (use 1 ml for 12 well size)
- e. Add 200 μ l of 10x Infection Cocktail/well of 6 well size plate of MOUSE myoblasts
(or 50 μ l of 5x Infection Cocktail/well of 6 well size plate of HUMAN myoblasts)
- f. Place plate in 32°C incubator (lower) for 15 mins to equilibrate pH.
- g. Remove and seal edges with parafilm strip.
- h. Spin plate (along with balance plate) at 2500 rpm for 30 mins at 32°C. Stop without brake.
- I. Aspirate RV supe. Refeed with fresh GM + bFGF
- J. Repeat infection protocol 8-12 hrs later.
 - For pTJM9 vectors: 2 rounds of RV infection is sufficient
 - For MFG-lacZ vector: Need 6 rounds to get >90% infection
- K. Two days after the last RV infection-refeed the cells with GM + 50 μ l G418 (mouse) or 400 μ l /ml G418 (human). Should start to see cell death in 2-4 days.
- L. Expand cells after selection and freeze away.