

TRANSFECTION

Reagents

HEPES-buffered saline (HEBS) – 1000 mL, pH to 7.1, autoclave, store at 4°C.

Hepes	5.0 g
NaCl	8.0 g
KCl	0.37 g
Na ₂ HPO ₄ ·7H ₂ O	0.188 g
Glucose	1.0 g

2.5M CaCl₂

2% FBS / 1% P/S DMEM

10% FBS / 1% P/S DMEM

Cell Lines

Human Embryonic Kidney Cell (HEK 293) -

- Prepare 60 mm plates the day before the start of transfection.
- Plate HEK 293 cells at 1.5×10^6 cells/plate.
- Cells should be 50 - 70% confluent at time of use.

Digestion

Enzymes:

- Pac I; OR
- Nhe I

Shuttle: 15.0 μ g of plasmid DNA.

Backbone: 4.0 μ g of plasmid (calculate number of transfections plus one and digest backbone as a master mix).

Prepare 50.0 μ l digestion reactions.

	Shuttle	Backbone
DNA	15.0 μ g	4.0 μ g \times (n+1)
Buffer	5.0 μ l	5.0 μ l
BSA	0.5 μ l	0.5 μ l
Enzyme	1.0 μ l	2.0 μ l
Water	To 50.0 μ l	To 50.0 μ l

n = # of transfections

Procedure

Label 75 x 100 mm test tubes.

Backbone master mix:

- Add 500.0 μ l HEBS per transfection plus one, to a 15.0 ml conical tube.
- Add backbone 50.0 μ l digestion reaction to buffer.
- Vortex.
- Aliquot 500.0 μ l of master mix in each 75 x 100 mm test tube.

Transfection

- Add 50.0 μ l digestion reaction of each virus to its corresponding 75 x 100 μ m test tube.
- Vortex for 2 sec.
- Add 25.0 μ L of 2.5M CaCl₂.
- Vortex for 2 sec.
- Incubate at room temperature for 25 min.
- Change media on 60 mm plates to be transfected to 2.0 mL of 2% FBS / 1% P/S DMEM.
- After incubation, add all of each sample to its corresponding plate in a slow spiral motion.
- After 4 h, change the media to 4.0 mL of 10%FBS/1% P/S DMEM and incubate overnight at 37°C with CO₂.
- The next day, aspirate the media and add 4.0 mL of 10%FBS/1% P/S DMEM.
- Incubate at 37°C with CO₂.
- On day 7, feed the cells with 1.0 mL of 2% FBS / 1% P/S DMEM. Incubate at 37°C with CO₂.
- Check for the presence of plaques. If plate ready for harvest, (> 60% of cells lifted), do so.
- If not, incubate at 37°C with CO₂.
- On day 10, feed the cells with 1.0 ml of 2% FBS/1% P/S DMEM.
- Check for the presence of plaques. If plate is ready for harvest (> 60% of cells lifted), do so.
- If not ready, keep checking plate for the presence of plaques. Do not keep plate more than 15 days. At 15 days, if no growth, harvest and transfer lysate and media to 1, 150 mm plate.

Harvest

- Using a 5.0 ml sterile serological pipet, collect cells and media. Transfer to a 15.0 ml conical sterile tube.
- Freeze media lysate at -20°C.