Preparation of Lentivirus by Transfection of 293T Packaging Cells: Trans-IT 293 (cationic liposomal) transfection

10/2003

Reagents:
293-T cells 70-80% confluent at time of transfection – pass the day before (use 1 P-100 plate at 90-100% confluence to pass to 15 cm plate in 25 cc media)
Trans-IT 293 from Mirus cat#Mir2700
DMEM high glucose
Complete media (e.g. for 293T cells use 10% FBS in high glucose DMEM with 1% pen/strep and 1x L-glutamine (5cc from a 200mM stock)
DNA plasmids (backbone/insert, tat, rev, gag/pol, vsv-g)

DNA proportions

20	:	1	:	1	:	1	:	2	
backbone	:	tat	:	rev	:	gag/pol	:	vsv-g	
24ug		1.2ug		1.2ug		1.2ug		2.4ug	=30ug total DNA

prepare trans-IT/DNA/media mix: 2ml DMEM per 15cm plate and 3 (ul) volumes of trans-IT per 1ug of DNA (e.g for 1x 15cm2 plate that will receive 30ug of DNA you need 3x30=90 ul of trans-IT in 2ml of DMEM and 30ug of DNA.

PROTOCOL:

1. prepare 293T cells the day before in 15 cm plates
2. Prepare DNA in an eppendorf by mixing together the 5 plasmids in the proportions above
3. Put amount of trans-IT needed into DMEM (2ml DMEM per 15cm plate). Put the trans-IT directly into the media! Don't touch the walls of the container. Plastic de-activates the reagent. Vortex and let stand at RT for 10 min.
4. Add 2ml of trans-IT/DMEM to the 30ug DNA plasmid mix ,vortex and let stand 15 min at RT
5. Meanwhile take plate of 293T cells, aspirate off old media and pour 11cc of complete media (e.g 10% DMEMetc) into each 15cm plate.
6. Add the 2ml of trans-IT/DNA/DMEM mix to each plate drop-wise
7. Mix gently by back and forth motion in two directions
8. Incubate
9. Start collecting supernatants 36 or 48 hours after transfection, and collect every 12 hours (4-5 collections =12.5cc supernatant collected each time; options are to do 4 collections from 4 x 15 cm plates to get 200cc of supernatant= 6 centrifuge tubes {33cc/tube} to get around 1.5cc of concentrated virus. OR can do 1 plate and 3 collections to fill one centrifuge tube getting around 180mcl of concentrated virus.). Use 0.45 bottletop filter. Re-feed cells with 12cc of complete media.
10. Concentrate by spinning for 3 hours at 15k at 4C (modified 12/03: 16.5K for 90 minute spin; =48960g on Beckman SW28 rotor).
11. Aliquot and store at –80C: Pour off all of supernatant after spinning and let stand on ice for 30 minutes prior to resuspending with P200 pipette and aliquoting virus.