THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Package of Lenti-shRNA virus using HEK293T cells

1. Before seeding cells for virus package, make sure HEK293T cells are in rapid replication state by growth cells to approximately 75%~80% confluence in a 10 cm cell culture dish and passaging at 1:2 ratio for at least 2 consecutive days;
2. On day 0, Seed HEK293T cells at 8.5~9´10^6 cells in growth medium without antibiotics for a 10- cm culture dish to reach around 90% confluence on next day;
3. On day 1, Co-transfect pGIPZ-shRNA or pLOC-ORF with pCMV-DR8.2 and pCMV-VSV-G (ratio: 1:1:0.1 or 0.2) into HEK293T cells (adjust medium with 5 mL);
   A) Mix the plasmid DNAs (as indicated below) in 0.5 mL Opti-MEM;
      pCMV-D8.2: 10 mg
      Lenti-shRNA: 10 mg
      pCMV-VSVG: 1 mg
   B) Dilute Lipofectamine 2000 with Opti-MEM: 50 ml of Lipofeactamine in 0.5 mL Opti-MEM;
   C) Incubate at room temperature for 5min → Combine the DNA mixture with diluted Lipofectamine 2000;
   D) After 20 min incubation → Add the DNA-Lipofectamine mix to the cells (1 mL→ 5 mL, the total medium will be ~6 mL);
4. Change medium within 18hours (remove the old medium, add 10 mL fresh growth medium).
5. Check GFP expression around 24 hours after transfections. If GPF+ cells <50%, transfections are failed and please try again. Best practice is GFP+ cells>80-90%.
6. Harvest medium 48 hours after the medium change
7. Optional: harvest medium again 24 hours after harvesting the first batch of medium.
8. Perform virus concentration using Lenti-X Concentrator by following the protocol from Clonetech or home-made virus concentrator;
9. Aliquot and Store the concentrated lentivirus at -80°C. Avoid multi-cycle of freeze and thaw. One cycle will lead to 50-90% loss of lentivirus.

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Protocol for different transfection reagents for lentivirus package

Lipofectamine (for 10cm dish)

Plasmid/Reagent	DNA	
pGIPZ control	10μg	Total DNA 21 μg
dR8.2dvpr	10μg	
VSVG	1μg	
Lipofectamine2000	50μl	

- Change medium with fresh OPTI-MEM (5mL/10cm dish);
- Dilute DNA plasmids into 500μl OPTI-MEM and Lipofectamine2000 in 500μl, respectively;
- Incubate at room temperature for 5 min;
- Add diluted lipofectamine to DNA mixture;
- Incubate at room temperature for 20 min;
- Add the lipofectamine-DNA mixture onto cells drop-wise;
- Replace transfection medium with fresh completely culture medium after 5 hours;
- Change medium next day (about 18 hours culture);
- Collect the supernatant on 24 hours and on 48 hours after changing medium;

Xfect Polymer (for 10cm dish)

Plasmid/Reagent	DNA	
pGIPZ control	10μg	Total DNA 21 μg
dR8.2dvpr	10μg	
VSVG	1μg	
Xfect Polymer	6.3μl	

- Change medium with fresh culture medium containing FBS but no antibiotics (5mL/10cm dish);
- Dilute DNA plasmids into 600μl Xfect Reaction Buffer and vortex for 5 sec at high speed followed by brief spinning down;
- Add 6.3 μl Xfect Polymer to the diluted DNA mixture directly;
- Incubate at room temperature for 10 min;
- Add the Xfect-DNA mixture onto cells drop-wise;
- Replace transfection medium with fresh completely culture medium after 5 hours;
- Change medium next day (about 18 hours culture);
- Collect the supernatant on 24 hours and on 48 hours after changing medium;

Note: Thaw the reagents before use and then store at 4 °C.

LipoD293 (for 10cm dish)

Plasmid/Reagent	DNA	
pGIPZ control	7.2µg	Total DNA ~15 µg
dR8.2dvpr	7.2µg	
VSVG	0.72µg	
LipoD293	46µl	

- Change medium with fresh complete medium containing FBS & antibiotics (5mL/10cm dish) 0.5~1.0 hour transfection;
- Dilute DNA plasmids into 500µl Serum-free DMEM with high glucose (i.e. Hyclone DMEM), vortex gently and briefly spin down to bring drops to the bottom of the tube;
- Dilute 46 µl LipoD293 transfection reagent with 500 µl serum-free DMEM (Hyclone DMEM), vortex gently and spin down briefly;
- Add the diluted LipoD293 Reagent immediately to the diluted DNA mixture, mix well by pipette up and down 3~4 times;
- Incubate at room temperature for 15 min;
- Add the 1000 µl LipoD293-DNA complex drop-wise onto the cells;
- Replace transfection medium with fresh completely culture medium after 5 hours;
- Change medium next day (about 18 hours culture);
- Collect the supernatant on 24 hours and on 48 hours after changing medium;

Note: 1) Never use Opti-MEM to dilute DNA and LipoD293 reagent because it will disrupt transfection complex;

2) Add dilute LipoD293 to diluted DNA mixture not in a reverse order;

Plasmid/Reagent	DNA	
pGIPZ control	5µg	Total DNA ~11 µg
dR8.2dvpr	5µg	
VSVG	0.5µg	
LipoD293	22µl	

- Change medium with fresh culture medium containing FBS but no antibiotics (5mL/10cm dish);
- Add DNA plasmids into 500µl jetPRIME Buffer and mix by brief vortexing;
- Add 22 µl jetPRIME to the diluted DNA mixture directly, and briefly spin down;
- Incubate at room temperature for 10 min;
- Add the 500 µl of jetPRIME-DNA mixture onto cells drop-wise;
- Replace transfection medium with fresh completely culture medium after 4 hours;
- Change medium next day (about 18 hours culture);
- Collect the supernatant on 24 hours and on 48 hours after changing medium;