Lentivirus Transfection (10cm)

Materials:

10 cm plate of 293T cells (Falcon)
25 ul X-tremeGENE HP transfection reagent
1500 ul Serum Free MEM or DMEM
8 ug of packaging vector pCMV-dR8.91
1 ug of packaging vector pMD2-G
9 ug of lentiviral plasmid

(Note this plasmid ratio is roughly a 1:1/4:1 molar ratio as the plasmid sizes of the packaging vectors are pCMV-dR8.91 12150 bp, pMD2G 5824 bp, and pHR-dCas9 is 14000 bp)

Methods:

*The day before, plate 5-6 x 10^6 293T cells in 10-12 mL medium

1. In 1.5 ml tubes mix 1500ul serum free DMEM with 25 ul Mirus and incubate for 5 minutes at room temp
2. In cap mix lentiviral plasmid with packaging vectors
3. Close lid and mix
4. Incubate 30 minutes at room temperature
5. Pipette into 293T plate
6. Allow viral production to continue for 72 hours before harvest (Note: Virus producing cells have a distinct rounded phenotype)
7. Centrifuge briefly (500xg for 10 min)
8. Transfer clarified supernatant to a sterile 15ml focol tube and mix with Lenti-X concentrator (Cat#631232, ratio 3:1).
9. Incubate mixture at 4 °C for overnight.
10. Centrifuge sample at 1,500xg for 45 minutes at 4 °C. After centrifugation, an off-white pellet will be visible.
11. Carefully remove supernatant (not to disturb the pellet). Resudal supernatant removed by brief centrifugation at 1,500xg.
12. Gently resuspend the pallet in 200ul of complete DMEM and immeidately use it or store at -80°C in single use aliquotes .

Scaled to 6 well for each well add

600k-900k cells the day before in 3mL then transfect

1.35 ug of packaging vector pCMV-dR8.91
165 ng of packaging vector pMD2-G
1.5 ug of lentiviral plasmid

with 7.5uL of mirus in 300uL serum free media

The viral titer depends on the size of the lentiviral construct. Large constructs (dCas9 fusions have 5-10 fold lower titer than smaller constructs (like sgRNAs). Your titer also depends on your HEK293 cell health and transfection efficiency. Do not let your HEK293 get over confluent and be sure to trypsinize well to single cells!!! Clumpy 293s don't produce high titer virus.

For sgRNA constructs in K562 cells I generally add twice 150uL of fresh virus to 100,000 cells a total volume of 1mL in a well of a 6 well plate at day1 and day2, which results in 80%~90% infection. As well as, I add 8ug/mL polybrene final concentration and don't spin.

Use 1ug/ml Puromycin to select for 6 days.
Add 1ug/ml tetracycline to treat cells and collect cells at day 2 and day6 for RNA-seq library preparation, saparatly.