Mega Lentivirus Transfection (onto 15cm plate)

Materials:

15 cm plate of 293T cells (Falcon)
48 ul Mirus LT1 tranfection reagent
1300 ul Serum Free MEM or DMEM
8 ug dR8.91 (gag & pol expression plasmid)
1ug MD2G (env expression vector, VSVG)
8 ug of lentiviral plasmid (sgRNAs)

Methods:

*The day before, plate 7.5 x 10^6 293T cells in 30 mL medium on to a 15 cm plate (next day, 25~30% confluent on the day of transfection)

1. In 1.5 ml tubes, mix 1300ul SF-MEM with 48 ul Mirus and incubate for 5 minutes at room temp.
2. Mix lentiviral plasmid with packaging vectors.
3. Pipet gently to mix 1&2 completely and incubate 20-30 minutes at room temperature.
4. Add the mix drop-wise onto 293T plate.
5. Allow viral production to continue for 72 hours before harvest (about 100% confluent in 3 days) (Note: Virus producing cells have a distinct rounded phenotype)
6. Filter supernatant through 0.4 uM filter
7. Decontaminate filters and syringes with 10% bleach solution

Mirus transfection reagent:
https://www.mirusbio.com/assets/protocols/ml009_transit_293_transfection_reagent.pdf