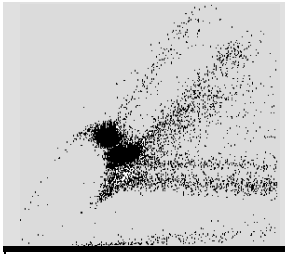

THE GOODELL LABORATORY

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Title	**Production of MSCV Retrovirus**	
Introduction	The objective of this protocol is to transfect 293T cells and produce high titer of MSCV viruses in a 6-well plate. To perform transfection in a plate with different size, it is necessary to scale up/down the amount of reagents. The size of MSCV vector is negatively correlate with the virus titer. A size of 8.5 kbs (about a 2kb gene insertion) is the maximal size of the retroviral vector we have succeeded.	
Materials	1. OPTI-MEM (Invitrogen 31985-062) 2. LipofectAmine 2000 (Invitrogen 11668-019) 3. Growth Medium: DMEM (Invitrogen 11965-092) with 10% FBS (with **NO antibiotics**) 4. 6-well plates 5. plasmid DNA (It is highly recommended that one does a Midi-prep to prepare for DNA. A good concentration with high quality of DNA usually results in good transfection, thus good virus titer)	
Protocol		*Notes*
1.	<u>d -1 Seeding 293T cells in a 6-well plate</u> The density is about $5x10^5$ cells /well in a 6-well plate. However, when cells are from a hyper-confluent flask, their growth rate will be slower in the beginning. In this case, more cells will be needed to give rise to 80-90% of cell density -usually $8x10^5$ cells /well. When seeding, cells can be cultured in growth medium containing antibiotics.	*In our experience, we found the health of 293T cells crucial for the titer of produced MSCV. Generally, 293T cells need to be grown and passaged for at least 2 generations after being thawed. In addition, 293T cells with slower growth rate or those show retardation in duplication is not suitable for virus production. To avoid aggregates of cells in culture, make sure 293T cells are well trypsinized before seeding.*
2.	<u>d 0 Transfection</u> 1. Change medium to an antibiotics-free medium. Plate 2ml/well 2. <u>Make DNA-OPTI mixture (Solution A)</u>	

	Dissolve DNA in OPTI medium, incubate for 5 minutes MSCV vector 2ug /RXN Eco-pCL vector 2ug /RXN OPTI medium 250ul/ RXN 3. <u>Make LipofectAmine-OPTI mixture (Solution B)</u> Dissolve LipofectAmine in OPTI medium 10ul LipofectAmine + 250 ul OPTI /RXN **Incubate for 5 minutes.** 4. Mix DNA (Solution A) and LipofectAmine mixture (Solution B), incubate in RT for 20 minutes. 5. Gently apply DNA-LipofectAmine mixture (500ul/RXN) onto the 293T cells in the 6 well plates. Avoid aspiration.	
3.	<u>d 1 Replace medium</u> Replace medium-containing medium, 2ml/ RXN	293T cells may be easily detached from the plate. Removed the old medium. Do not let cells sit without having new medium added so you will have to be quick. When adding new medium, gently tilt the plate, and add the medium to the wall of well so the medium drop would not disturb the cells.
4.	<u>d 2 Harvest virus</u> 48 hours after transfection, harvest surpernatant. To exclude cell debris, one can 1) spin supernatant in 4^{o}C, 2000rpm for 10 minutes, and transfer the supernatant to the freezing tubes; or 2) filter the virus through a 0.45uM syringe filter.	To prevent loosing viral titer in the future during viral titering and infection experiment, repeated thaw-freeze cycles need to be avoided. Therefore, it is highly recommended to aliquot viral supernatant before freezing them down at this step.