THE GOODELL LABORATORY

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Title	**Production of MSCV Retrovirus**	

	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