**PRODUCTION OF RETROVIRAL SUPERNATANT USING PACKAGING CELL LINES**

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A few recommendations to get a very nice titer.

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**MATERIALS**

DMEM 10% FCS
Puromycin
Blasticydin

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**METHOD**

1. Culture Platinum-E-based packaging cell lines in DMEM 10%FCS supplemented with 1µg/ml Puromycin and 10µg/ml Blasticydin (to ensure selection of virion-producing cells). Also ensure the cell line is 100% positive for the reporter gene.

2. Seed $18x10^6$ cells per 15cm Petri dish in DMEM 10%FCS (ABSOLUTELY NO PURO/BLASTI FROM THIS STAGE ON, otherwise your supernatant will kill target cells!!).

3. Let cells grow to confluence (1-2 days).

4. When confluent, put 11ml of DMEM 10%FCS, let it to be conditioned for 24 hours, clarify supernatant by centrifugation, to spin down contaminant dead cells, and then use it on target cells and/or freeze it down.

5. Repeat step 4 for as many days you want. Usually cells resist 5 days before dying.