# Transfection and Viral Titer

## Materials

* 293T cells
* D-10 media
* D-30 media
* Poly-D-lysine
* 15cm dish
* 10cm dishes
* Lipofectamine 2000
* Opti-MEM media
* Plasmid
* Packing plasmids (psPAX2 and VSVG, both gel-verified)
* 10ml syringes
* 0.45um syringe filter
* Cryo tubes and labels
* Mr Frosty
* 3T3 cells
* 6-well plates
* Polybrene
* Antibiotic

## Preparation

Day -3: Friday

* Thaw 293T cells and expand over the weekend in a 15cm dish
	+ D-10 media

## Protocol

Day 0: Monday

1. Coat plates with poly-d-lysine
	1. Dilute 1:100 in PBS (final conc 10ug/ml)
	2. 5ml per plate, incubate at 37°C for at least an hour
2. Aspirate poly-d-lysine and allow to dry under the hood
3. Trypsinize and resuspend 293Ts
4. Count cells
5. Plate 500,000 cells per plate in D-10
6. Pre-warm 6ml D-10 per plate

Day 2: Tuesday

1. Check that cells are ~70% confluent
2. Complete media change with 6ml D-10
3. Transfection per plate:
	1. 450ul Opti-MEM in a 1.5ml tube
	2. 5ug plasmid + 4ug psPAX2 + 1ug VSVG
	3. 30ul Lipofectamine 2000 (add to center of tube, do not let it touch the sides!!)
	4. Mix gently by flicking the tube, do NOT vortex
	5. Incubate at RT for 30’
	6. Add dropwise to plate and swirl
	7. Incubate overnight
4. Pre-warm 8ml D-30 per plate
5. Thaw 3T3s in 15cm dish
	1. D-10 media

Day 3: Wednesday

1. Full media change with D-30
2. Trypsinize and resuspend 3T3 cells
	1. Plate 50,000 cells per well in a 6-well plate (2ml per well)
3. Pre-warm D-10 with polybrene (8ug/ml)
	1. 2ml per well

Day 4: Thursday

1. Draw up supernatant with 10ml syringe
2. Attach 0.45um syringe filter
3. Create 1ml aliquots in cyro tubes
4. Snap freeze on dry ice
	1. Reserve one tube for titer
5. Transfer to -80
6. Set up titer
	1. Full media change with 2ml D-10 with polybrene
	2. Add virus to each well (500ul, 100ul, 50ul)

Day 5: Friday

1. Dilute antibiotic in D-10 at 2ug/ml
	1. 6ml per well
2. Full media change with antibiotic-treated media

Day 6+: Observation

Day 8-9: Staining and Counting

1. Once cells in control well (no virus, antibiotic added) are all dead, aspirate all media from all wells
2. Rinse wells with PBS
3. Stain with 500ul crystal violet
4. Rinse in the sink and dry
5. Count colonies
6. Calculate titer