

# 10. (October) 2019

**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Johanna Wallner

MONDAY, 7/10/2019


**Johanna**







Cell Culture: Transfection

- finished at 09:30
- medium exchange: 1.5 mL out, 2 mL new medium in
- mi6-medium: add beta-mercaptoethanol (1:700)
- 6-well plate

Transfectionmix 6-well plate 07/10...		
	A	B
1	DNA per well	2500 ng
2	P3000 Reagent per well	5 µL
3	Lipofectamine 3000 reagent per well	3.75 µL
4	OptiMEM per well	2 x 125 µL

- Transfect cells according to the following table. Use the indicated volume of DNA and P3000™ Reagent with each of the two volumes of Lipofectamine™ 3000 (when performing optimization). Each reaction mix volume is for one well and accounts for pipetting variations. Scale volumes proportionally for additional wells.



Timeline		Steps		Procedure Details (Two Reaction Optimization)			
Day 0	1		Seed cells to be 70–90% confluent at transfection	Component	96-well	24-well	6-well
				Adherent cells	1–4 × 10 <sup>6</sup>	0.5–2 × 10 <sup>6</sup>	0.25–1 × 10 <sup>6</sup>
Day 1	2		Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes)–Mix well	Opti-MEM™ Medium	5 µL × 2	25 µL × 2	125 µL × 2
				Lipofectamine™ 3000 Reagent	0.10 µL × 2	0.75 µL × 2	3.75 µL × 2
Day 1	3		Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent–Mix well	Opti-MEM™ Medium	10 µL	50 µL	250 µL
				DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg
Day 1	4		Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)	P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
				Diluted DNA (with P3000™ Reagent)	5 µL	25 µL	125 µL
Day 2–4	5		Incubate	Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL
				Incubate for 10–15 minutes at room temperature.			
Day 2–4	7		Visualize/analyze transfected cells	Component (per well)	96-well	24-well	6-well
				DNA–lipid complex	10 µL	50 µL	250 µL
				Opti-MEM™	100 µg	500 µg	2500 µg
				P3000™ Reagent	0.2 µL	1 µL	5 µL
				Lipofectamine™ 3000 Reagent used	0.10 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
				Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			

- Transfection scheme

Transfection scheme - Purification (HEK) 07/10/19 in ng per well											
	wells	V8	V14	V15	V41	E10	E11	E16	E17	E19	E
1	3	1500 ng	-	1000 ng	-	-	-	-	-	-	-
2	3	-	-	1000 ng	1500 ng	-	-	-	-	-	-
3	1	-	-	1000 ng	-	1500 ng	-	-	-	-	-
4	1	-	1000 ng	-	-	-	1500 ng	-	-	-	-
5	1	-	-	1000 ng	-	-	-	1500 ng	-	-	-
6	1	-	1000 ng	-	-	-	-	-	1500 ng	-	-
7	1	-	-	1000 ng	-	-	-	-	-	1500 ng	-
8	1	-	1000 ng	-	-	-	-	-	-	-	1500 ng

Transfection scheme - Purification (min6) 07/10/19 in ng per well											
	wells	V34	V14	V15	V11	E10	E11	E16	E17	E19	E20
1	3	1500 ng	-	1000 ng	-	-	-	-	-	-	-
2	3	1250 ng	625 ng	-	625 ng	-	-	-	-	-	-
3	1	-	-	1000 ng	-	1500 ng	-	-	-	-	-
4	1	-	1000 ng	-	-	-	1500 ng	-	-	-	-
5	1	-	-	1000 ng	-	-	-	1500 ng	-	-	-
6	1	-	1000 ng	-	-	-	-	-	1500 ng	-	-
7	1	-	-	1000 ng	-	-	-	-	-	1500 ng	-
8	1	-	1000 ng	-	-	-	-	-	-	-	1500 ng

**Alejandro**Cell Culture: Transfection

- VLPs: 8 wells: L7Ae fusion + Fluc-C/DBox
- Exosomes: L7Ae + Fluc-C/DBox
- finished at 12:30