

# 09. (September) 2019

Project: iGEM\_Munich2019 Shared Project

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TUESDAY, 3/9/2019

Johanna

cell culture: transfection: Purification

- finished at 9 a.m.
- medium exchange: 1.5 mL out, 2 mL new medium in
- 6-well plate

Transfectionmix 6-well plate 03/09...		
	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		Procedure Details (Two Reaction Optimization)			
Day	Step	Component	16-well	24-well	48-well
Day 1	1	Seed cells to be 70-90% confluent at transfection	1-4 × 10 <sup>6</sup>	0.5-2 × 10 <sup>6</sup>	0.25-1 × 10 <sup>6</sup>
	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
Day 1	3	Prepare master mix of DNA to diluting DNA in Opti-MEM® Medium, then add P3000® Reagent - Mix well	Lipofectamine™ 3000 Reagent 0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
	4	Add diluted DNA to each tube of diluted Lipofectamine™ 3000 Reagent (1:1 ratio)	Opti-MEM® Medium 10 µL	50 µL	250 µL
Day 1	5	Incubate	DNA (0.5-5 µg/µL) 0.2 µg	1 µg	5 µg
	6	Add DNA-lipid complex to cells	P3000® Reagent (2 µL/µg DNA) 0.4 µL	2 µL	10 µL
Day 2-4	7	Visualize/analyze transfected cells	Diluted DNA (with P3000® Reagent) 5 µL	25 µL	125 µL
			Diluted Lipofectamine™ 3000 Reagent 5 µL	25 µL	125 µL

- Transfection scheme

Transfection scheme - Purification 03/09/19 in ng per well (1: control, 2: Biotin, 3: His)							
	wells	V8	V11	V14	V27	V28	V
1	2	1000 ng	500 ng	500 ng	-	-	500 ng
2	2	1000 ng	500 ng	500 ng	500 ng	-	-
3	2	1000 ng	500 ng	500 ng	-	500 ng	-

Alejandro

cell culture: transfection: qPCR

- finished at 12.20 p.m.
- medium exchange: 1.5 mL out, 2 mL new medium in
- 6-well plate

Transfectionmix 6-well plate 03/09...		
	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.

image.png

Timeline		Steps		Procedure Details (Two Reaction Optimization)			
				Component	16-well	24-well	6-well
Day 0	1	Seed cells to be 70–90% confluent at transfection	Adherent cells		1.4 × 10 <sup>6</sup>	0.5–2 × 10 <sup>6</sup>	0.25–1 × 10 <sup>6</sup>
	2			Opti-MEM® Medium	5 µL × 2	25 µL × 2	125 µL × 2
Day 1	3	Prepare master mix of DNA by diluting DNA in Opti-MEM® Medium, then add P3000® Reagent - Mix well	Lipofectamine™ 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL	
	4		Opti-MEM® Medium	10 µL	50 µL	250 µL	
Day 2	5	Add diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)	DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg	
	6		P3000® Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL	
Day 3	7	Visualize/analyze transfected cells	Diluted DNA (with P3000® Reagent)	5 µL	25 µL	125 µL	
	8		Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL	

Incubate for 10–15 minutes at room temperature.

Component (per well)	16-well	24-well	6-well
DNA-lipid complex	10 µL	50 µL	250 µL
DNA amount	100 ng	500 ng	2500 ng
P3000® Reagent	0.2 µL	1 µL	5 µL
Lipofectamine™ 3000 Reagent used	0.15 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 (pos. control for Western Blot was transfected on Joshi's plate)

Transfection scheme - qPCR 03/09/19 in ng per well										
	condition	V4	V8	V9	V10	V11	V14	V15	V27	V
1	2	-	1000 ng	-	-	-	-	-	500 ng	1000 n
2	3	-	1000 ng	-	-	500 ng	500 ng	-	500 ng	-
3	4	-	1000 ng	-	-	500 ng	-	500 ng	500 ng	-
4	5	-	1000 ng	-	500 ng	-	-	500 ng	500 ng	-
5	6	-	1000 ng	-	500 ng	-	500 ng	-	500 ng	-
6	3*	-	-	1000 ng	-	500 ng	500 ng	-	500 ng	-
7	pos. control for western blot	1500 ng	-	-	-	-	500 ng	-	500 ng	-