

# 09. (September) 2019

**Project:** iGEM\_Munich2019 Shared Project  
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TUESDAY, 24/9/2019

**Alejandro**

Transfection

- transfection time: 14:00
- 6-well plates

Transfectionmix 6-well plate 24/09...		
	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.

**Transfection scheme**

**Timeline**

- Day 0: Seed cells to be 70-80% confluent at transfection
- Day 1: Prepare transfection mix
- Day 2: Add DNA-lipid complex to cells
- Day 3-4: Incubate
- Day 5-7: Visualize/analyze transfected cells

**Procedure Details (Two Reaction Optimization)**

Component	96-well	24-well	6-well
Adherent cells	1-4 x 10 <sup>5</sup>	0.5-2 x 10 <sup>5</sup>	0.25-1 x 10 <sup>5</sup>
Opti-MEM™ Medium	5 µL x 2	25 µL x 2	125 µL x 2
Lipofectamine™ 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
Opti-MEM™ Medium	10 µL	50 µL	250 µL
DNA (0.5-5 µg/µL)	0.2 µg	1 µg	5 µg
P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
Diluted DNA (with P3000™ Reagent)	5 µL	25 µL	125 µL
Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL

Incubate for 10-15 minutes at room temperature.

Component (per well)	96-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

Transfection scheme - 24/09/19 in ng per well - 6-well plate						
	condition	V5	V41	V14	V15	V30
1	Mock	-	-	-	-	2500 ng
2	V15	-	-	-	1000 ng	1500 ng
3	V41 ok	-	1500 ng	-	1000 ng	-
4	V41 x	-	1500 ng	1000 ng	-	-
5	1:1 ok	725 ng	725 ng	-	1000 ng	-
6	1:1 x	725 ng	725 ng	1000 ng	-	-

- 96-well plate
  - transfect triplicates

Transfectionmix 96-well plate 24/0...		
	A	B
1	DNA per well	100 ng
2	P3000 Reagent per well	0.2 µL
3	Lipofectamine 3000 reagent per well	0.15 µL
4	OptiMEM per well	2 x 5 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		Procedure Details (Two Reaction Optimization)			
Day 0	Steps	Components	96-well	24-well	6-well
1	Seed cells to be 70-90% confluent at transfection	Adherent cells	1-4 × 10 <sup>5</sup>	0.5-3 × 10 <sup>5</sup>	0.25-1 × 10 <sup>5</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
		Lipofectamine™ 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
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
		P3000® Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
Day 1	Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)	Diluted DNA (with P3000® Reagent)	5 µL	25 µL	125 µL
		Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL
5	Incubate	Incubate for 15-15 minutes at room temperature.			
6	Add DNA-lipid complex to cells	Components (per well)	96-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
Day 2-4	Visualize/analyze transfected cells	Incubate cells for 2-4 days at 37°C. Then, analyze transfected cells.			

- Transfection scheme

Transfection scheme - 24/09/19 in ng per well - 96-well plate								
	condition	V5	V8	V36	V41	V14	V15	
1	V41 ok	-	-	-	60 ng	-	40 ng	-
2	V41 x	-	-	-	60 ng	40 ng	-	-
3	1:1 ok	30 ng	-	-	30 ng	-	40 ng	-
4	1:1 x	30 ng	-	-	30 ng	40 ng	-	-
5	V8	-	60 ng	-	-	-	-	4
6	V36	-	-	60 ng	-	-	-	4