

09. (September) 2019

Project: iGEM_Munich2019 Shared Project

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TUESDAY, 17/9/2019

Johanna

cell culture: Transfection for VLP-Purification

- medium exchange: 1.5 mL Medium out, 2 mL new Medium in

Transfectionmix 6-well plate 17/09...			^
	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		Steps		
Day 0	1	Seed cells to be 70-80% confluent at transfection		
	2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (12-well) - Mix well		
Day 1	3	Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium. Then add P3000™ Reagent - Mix well		
	4	Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)		
Day 2	5	Incubate		
	6	Add DNA-lipid complex to cells		
Day 4	7	Visualize/analyze transfected cells		
	8	Incubate cells for 2-4 days at 37°C. Then, analyze transfected cells.		
Procedure Details (Two Reaction Optimization)				
Component		12-well	24-well	48-well
Adherent cells		1.4 x 10 ⁶	0.5 x 10 ⁶	0.25 x 10 ⁶
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)		12-well	24-well	48-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 (3 wells per condition)

Transfection scheme - 17/09/19 in ng per well - Purification						
	condition	V8	V10	V11	V14	V15
1	1	1250 ng	625 ng	-	-	625 ng
2	2	1250 ng	-	625 ng	625 ng	-
3	3	-	-	-	-	-
4	4	1250 ng	-	-	-	-

Alejandro

cell culture: Transfection

- 6-well plates

Transfectionmix 6-well plate 17/09...		
	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		Steps	Procedure Details (Two Reaction Optimization)			
Day 1	1	Seed cells to be 70-80% confluent at transfection	Adherent cells	1-4 x 10 ⁵	0.5-3 x 10 ⁵	0.25-1 x 10 ⁶
	2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (12.5mL - 10x well)	Opti-MEM™ Medium	5 µL x 2	25 µL x 2	125 µL x 2
Day 2	3	Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium. Then add P3000™ Reagent - 10x 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 3	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 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
			Incubate for 10-15 minutes at room temperature.			
			Component (per well)			
			10-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 - 17/09/19 in ng per well												
	condition	V8	V11	V14	V15	V26	V27	V30	V33	V36	V40	V41
1	A	-	-	-	-	-	500 ng	500 ng	-	400 ng	200 ng	-
2	B	-	-	-	-	-	500 ng	1500 ng	-	-	200 ng	-
3	C	-	-	500 ng	-	-	500 ng	-	-	400 ng	-	-
4	D	-	-	-	500 ng	-	500 ng	-	-	400 ng	-	-
5	E	-	-	-	500 ng	-	500 ng	-	-	-	-	650 ng
6	F	-	-	500 ng	-	-	500 ng	-	-	-	-	650 ng
7	NC	1000 ng	500 ng	500 ng	-	-	-	500 ng	-	-	-	-
8	V26	1000 ng	500 ng	500 ng	-	500 ng	-	-	-	-	-	-
9	V27	1000 ng	500 ng	500 ng	-	-	500 ng	-	-	-	-	-
10	BB	1000 ng	500 ng	500 ng	-	-	-	-	-	-	-	5
11	V33	1000 ng	500 ng	500 ng	-	-	-	-	500 ng	-	-	-

- 24-well plate with min6-K8 cells
 - time finished: 16:00

Transfectionmix 24-well plate 17/0...		
	A	B
1	DNA per well	500 ng
2	P3000 Reagent per well	1 µL
3	Lipofectamine 3000 reagent per well	0.75 µL
4	OptiMEM per well	2 x 25 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 0	Steps	Component	1-well	2-well	3-well
1	Seed cells to be 70-80% confluent at transfection	Adherent cells	1 × 10 ⁵	0.5 × 10 ⁵	0.25 × 10 ⁵
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 (2.5-5 µg/µL)	0.2 µg	1 µg	5 µg
		P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
4	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 10-15 minutes at room temperature.			
6	Add DNA-lipid complex to cells	Component (per well)	1-well	2-well	3-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
7	Visualize/transfect cells	Incubate cells for 2-4 days at 37°C. Then, analyze transfected cells.			

- Transfection scheme

transfection scheme 17/09/2019 - MIN6-K8									
	condition	V8	V10	V11	V14	V15	V27	V30	
1	2	400 ng	-	-	-	-	200 ng	400 ng	-
2	3	400 ng	-	200 ng	200 ng	-	200 ng	-	-
3	5	400 ng	200 ng	-	-	200 ng	200 ng	-	-
4	m2	-	-	-	-	-	200 ng	400 ng	4
5	m3	-	-	200 ng	200 ng	-	200 ng	-	4
6	m5	-	200 ng	-	-	200 ng	200 ng	-	4

- Problems:
 - not enough V36 in A
 - not enough V41 in F
 - Calculation error for the amount of OptiMEM in the 24-well plate. 57.2 µL per condition should have been used and not 29 µL