07. (July) 2019

Project: iGEM_Munich2019 Shared Project
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TUESDAY, 30/7/2019

Transfection :

- 96 well plate for HiBit assay
- 6 well plate for Biotin purification
- 100µL medium out of the 150µL were exchanged in the 96 well plate. 1.5mL out of the 2mL medium were exchanged in the 6 well plate.
- time of transfection : 11:00 - 11:15

96 well plate for HiBit transfection scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		1	1	1	1	1	1	7	8	9	10	
C		2	2	2	2	2	2	7	8	9	10	
D		3	3	3	3	3	3	7	8	9	10	
E		4	4	4	4	4	4	7	8	9	10	
F		5	5	5	5	5	5	7	8	9	10	
G		6	6	6	6	6	6	7	8	9	10	
H												

6 well plate transfection s…

	1	2	3
A	2	2	5
B	4	4	5

conditions

	V8	V9	V10	V11	V14	V15	V27	V30
1	/	/	/	/	/	/	/	100
2	40	/	/	/	/	/	20	40
3	/	40	/	/	/	/	20	40
4	40	/	/	20	20	/	20	/
5	/	40	/	20	20	/	20	/
6	40	/	20	/	20	/	20	/
7	40	/	20	/	/	20	20	/
8	/	40	20	/	/	20	20	/
9	40	/	/	20	/	20	20	/
10	see below							

-> condition 10 is 40ng V25 + 60nG V30

-> for the 6.5 wells these ng respond to :

- 20ng = 1.3µL
- 40nG = 2.6µL
- 60ng = 3.9µL
- 100nG = 6.5µL

Alejandro

cell culture: Transfection

- 550 µL medium was exchanged before transfection

Table1

	condition	V8	V11	V14	V27	V28	V30
1	Mock	-	-	-	-	-	500 ng
2	His	200 ng	100 ng	100 ng	-	100 ng	-
3	scAvidin	200 ng	100 ng	100 ng	100 ng	-	-

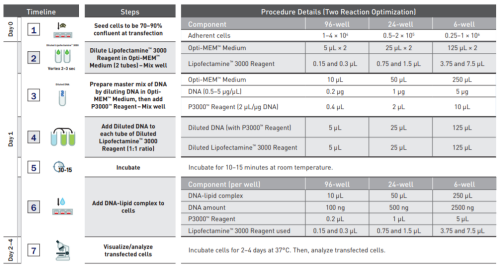
- Transfection:

Table2

	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.

image.png



Moritz

cell culture: transfection

- note: cell density seemed low (40 %)
- every condition was transfected in 10 wells

Transfection scheme - qPCR 96-well plate 30/07/19 (ng per well)

	Condition	V8	V10	V11	V14	V15	V17	V
1	1	-	-	-	-	-	-	100 ng
2	4	40 ng	-	20 ng	20 ng	-	20 ng	-
3	7	40 ng	20 ng	-	-	20 ng	20 ng	-

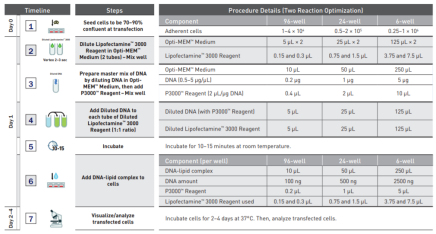
- Transfection:

Transfectionmix 96-well plate 30/0…

	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	Steps
Day 0	1 Seed cells to be 70–90% confluent at transfection
	2 Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes)—Mix well.
	3 Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent—Mix well
Day 1	4 Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)
	5 10–15 Incubate
	6 Add DNA–lipid complex to cells
Day 2–4	7 Visualize/analyze transfected cells

Procedure Details (Two Reaction Optimization)			
Component	96-well	24-well	6-well
Adherent cells	1–4 × 10⁴	0.5–2 × 10⁵	0.25–1 × 10⁶
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
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.			