08. (August) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

TUESDAY, 13/8/2019

Johanna

cell culture: transfection for Purification and qPCR

- 550 µL medium were exchanged before transfection
- 2 24-well plates seeded the 12th of August

Transfection scheme - qPCR and Purification 13/08/19 (ng per well)

	Condition	V8	V10	V11	V14	V15	V27	V28	V30
1	2	200 ng	-	-	-	-	-	-	300 ng
2	4	200 ng	-	100 ng	100 ng	-	100 ng	-	-
3	6	200 ng	100 ng	-	100 ng	-	100 ng	-	-
4	7	200 ng	100 ng	-	-	100 ng	100 ng	-	-
5	9	200 ng	-	100 ng	-	100 ng	100 ng	-	-
6	4H	200 ng	-	100 ng	100 ng	-	-	100 ng	-
7	V14	-	-	-	500 ng	-	-	-	-

- Transfection:

Transfectionmix 24-well plate 13/0…

	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.

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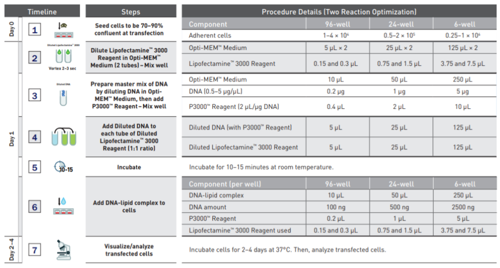

Timeline	Step	Steps	Procedure Details (Two Reaction Optimization)			
			Component	96-well	24-well	6-well
Day 0	1	Seed cells to be 70–90% confluent at transfection	Adherent cells	1–4 × 10⁴	0.5–2 × 10⁵	0.25–1 × 10⁶
Day 1	2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) – Mix well (Vortex 2–3 sec)	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
	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)	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	7	Visualize/analyze transfected cells	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			

- transfection finisthed at 10:00

Alejandro

cell culture: transfection for HiBit Assay in 96-well plates

- cells were at 50-60 % confluence (rather 50 % after medium eschange)
- 100 µL medium over the cells were exchanged before transfectin
- transfection was finished at 11:30
- 8 replicates

Transfection scheme - HiBit 96-well plate 13/08/19 (ng per well)

	Condition	V8	V10	V11	V14	V15	V17	V30
1	1	-	-	-	-	-	-	100 ng
2	2	40 ng	-	-	-	-	20 ng	40 ng
3	4	40 ng	-	20 ng	20 ng	-	20 ng	-
4	6	40 ng	20 ng	-	20 ng	-	20 ng	-
5	7	40 ng	20 ng	-	-	20 ng	20 ng	-
6	9	40 ng	-	20 ng	-	20 ng	20 ng	-

- Transfection:

Transfectionmix 96-well plate 13/0…

	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.

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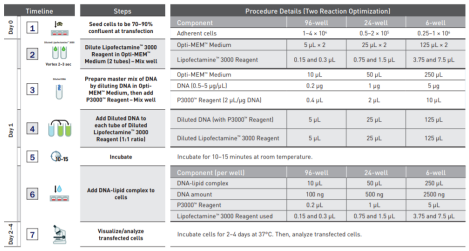

Timeline	Steps	Procedure Details (Two Reaction Optimization)			
Day 0	1	Seed cells to be 70–90% confluent at transfection	Component	96-well	24-well	6-well
			Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
	2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes)—Mix well	Opti-MEM™ Medium	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	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)	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
Days 2–4	7	Visualize/analyze transfected cells	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			