10. (October) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

SATURDAY, 5/10/2019

Alejandro

Cell Culture: Transfection

- 96-well plate
  - transfect triplicates

Transfectionmix 96-well plate 05/1…

	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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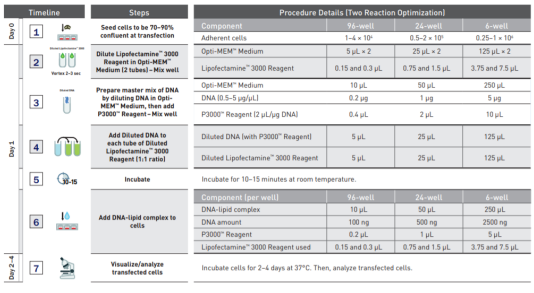

Timeline	Steps	Procedure Details (Two Reaction Optimization)			
Day 0	1 Seed cells to be 70–90% confluent at transfection	Component	96-well	24-well	6-well
		Adherent cells	1–4 × 10⁴	0.5–2 × 10⁵	0.25–1 × 10⁶
	2 Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) – Mix well	Opti-MEM™ Medium	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
Days 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.			

  - Transfection scheme

Transfection scheme - 05/10/19 in ng per well - 96-well plate- HiBit

	condition	wells	V8	V41	V42.1	V42.2	V15	V34
1	1	6	-	-	-	-	-	-
2	2	6	60 ng	-	-	-	40 ng	-
3	3	6	-	60 ng	-	-	40 ng	-
4	4	6	-	-	-	-	40 ng	60 ng
5	5	6	-	-	60 ng	-	40 ng	-
6	6	6	-	-	-	60 ng	40 ng	-

Joshi

Cell Culture: Transfection

- min6 VLP transfection for qPCR
- 550 µL medium were exchanged before transfection

transfection scheme qPCR min6 05/10/2019 - in ng per well

	condition	V30	V15	V42.1	V42.2	V14
1	Mock	500 ng	-	-	-	-
2	Only V15	300 ng	200 ng	-	-	-
3	Fusion 1 o.k.	-	200 ng	300 ng	-	-
4	Fusion 1 x	-	-	300 ng	-	200 ng
5	Fusion 2 o.k.	-	200 ng	-	300 ng	-
6	Fusion 2 x	-	-	-	300 ng	200 ng

- Transfection:

Transfectionmix 24-well plate 05/1…

	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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