08. (August) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

TUESDAY, 20/8/2019

Johanna

Cell culture: Reuptake assay transfection:

- 500 ng/well, triplicates, 24-well plate (coated with poly-L-Lysine)

Transfection scheme - reuptake assay 20/08/19

	condition	V8	V10 (L7Ae)	V11 (MCP)	V14 (MS2)	V15 (C/D Box)	V30
1	1	250 ng	-	-	-	-	250 ng
2	2	-	-	-	125 ng	-	375 ng
3	3	250 ng	-	125 ng	125 ng	-	-
4	4	250 ng	125 ng	-	-	125 ng	-

- 1: negative control, 2: positive control (fluc), 3: MCP/MS2-System, 4: L7Ae/C/D-Box-System
- exchange 550 µL medium
- Transfection:

Transfectionmix 24-well plate 20/0…

	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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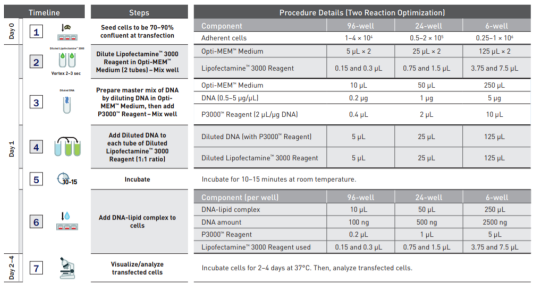

Timeline	Steps	Procedure Details (Two Reaction Optimization)			
		Component	96-well	24-well	6-well
Day 0	1 Seed cells to be 70–90% confluent at transfection	Adherent cells	1–4 × 10⁴	0.5–2 × 10⁵	0.25–1 × 10⁶
Day 1	2 Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) – Mix well	Opti-MEM™ 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 30–15 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 time: 11:30 a.m.
- attention: no Biotin-tag (V27)

Alejandro

cell culture: Transfection for Purification and qPCR

- time finished: 13:20 p.m.
- note: in condition 6: 6 µL V10 were given mistakenly, not the supposed 5.25 µL
- 6-well plate

Transfectionmix 6-well plate 20/08…

	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.

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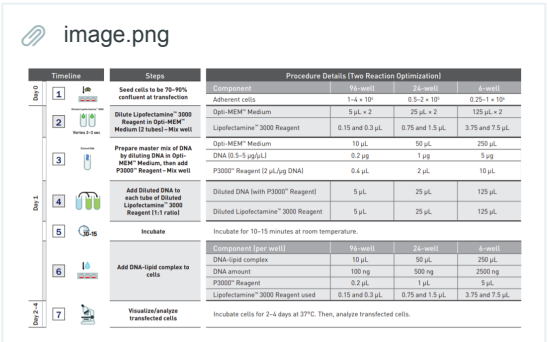

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		Lipofectamine™ 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
	3 Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent – Mix well	Opti-MEM™ Medium	10 µL	50 µL	250 µL
		DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 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
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		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
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Day 2–4	7 Visualize/analyze transfected cells	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			

- Transfection scheme

Transfection scheme - Purification and qPCR 20/08/19 in ng per well

	condition	wells	V8	V10	V11	V14	V15	V27	V28	V30
1	2	3	1000 ng	-	-	-	-	-	-	1500 ng
2	3	2	1000 ng	-	500 ng	500 ng	-	500 ng	-	-
3	4	1	1000 ng	-	500 ng	-	500 ng	500 ng	-	-
4	5	2	1000 ng	500 ng	-	-	500 ng	500 ng	-	-
5	6	1	1000 ng	500 ng	-	500 ng	-	500 ng	-	-
6	3H	2	1000 ng	-	500 ng	500 ng	-	-	500 ng	-
7	5H	2	1000 ng	500 ng	-	-	500 ng	-	500 ng	-

- qPCR-plate: 1 well with cond. 2, 3, 4, 5, 6 each
- purification-plates
    - scAvidin: 1 well with cond. 2, 3, 5 each
    - His: 1 well with cond. 2, 2 wells with cond. 2H and 5H each

Agar plate

- Chloramphenicol (3 plates; 25 µg/mL)
- Ampicillin (8 plates; 1:500 Ampicillin)