

09. (September) 2019

Project: iGEM_Munich2019 Shared Project

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TUESDAY, 10/9/2019

Johanna

cloning: mouse Gag

- gBlock:
 - spin down
 - resuspend in 50 μ L MiliQ -> 1000 ng in 50 μ L
 - vortex
 - 20 min @ 50 °C
- Backbone: PacI-V4-MluI (23 ng/ μ L)
- insert digestion

digestion gBlock mouse Gag			^
	A	B	
1	5 μ L	10X CutSmart Buffer	
2	1 μ L	MluI-HF	
3	1 μ L	PacI	
4	25 μ L	Insert (gBlock)	
5	18 μ L	MiliQ	

- 20 min @ 37 °C
- clean up with NEB Monarch PCR & DNA Cleanup Kit
 - Binding Buffer: 250 μ L, sample: 50 μ L; wash 2x with 200 μ L was buffer, elute with 12 μ L MiliQ
 - 31 ng/ μ L (Insert)
- Ligation
 -

ligation mouse Gag			^
	A	B	
1	T4 Buffer	2 μ L	
2	T4 Ligase	1 μ L	
3	Vector V4	2 μ L	
4	Insert	1.2 μ L	
5	MiliQ	13.8 μ L	

- 10 min @ RT, 10 min @ 60 °C
- Transformation in NEB stable: plating of 50 μ L bacterial suspension

Alejandro

cell culture: Transfection for qPCR

- finished at 10:15 a.m.
- 6-well plates

Transfectionmix 6-well plate 10/09...		
	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.

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Timeline		Steps	Procedure Details (Two Reaction Optimization)			
Day	Step	Steps	Component	10-well	15-well	6-well
Day 0	1	Seed cells to be 70-90% confluent at transfection	Adherent cells	1-4 x 10 ⁶	0.5-2 x 10 ⁶	0.25-1 x 10 ⁶
	2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) - Mix well	Opti-MEM™ Medium	5 µL x 2	25 µL x 2	125 µL x 2
Day 1	3	Prepare master mix of DNA to diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent - Mix 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 2-4	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 2-4	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	15-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 (pos. control for Western Blot was transfected on Joshi's plate)

Transfection scheme - 10/09/19 in ng per well - qPCR											
	condition	V4	V5	V8	V9	V10	V11	V14	V15	V27	V
1	2	-	-	1000 ng	-	-	-	-	-	500 ng	1000 ng
2	3	-	-	1000 ng	-	-	500 ng	500 ng	-	500 ng	-
3	4	-	-	1000 ng	-	-	500 ng	-	500 ng	500 ng	-
4	5	-	-	1000 ng	-	500 ng	-	-	500 ng	500 ng	-
5	6	-	-	1000 ng	-	500 ng	-	500 ng	-	500 ng	-
6	3*	-	-	-	1000 ng	-	500 ng	500 ng	-	500 ng	-
7	F1	1500 ng	-	-	-	-	-	500 ng	-	500 ng	-
8	F2	500 ng	1000 ng	-	-	-	-	500 ng	-	500 ng	-








Johannacell culture: Transfection for Purification

- finished at 13:00
- 6-well plates
- medium exchange: 1.5 mL Medium out, 2 mL new Medium in

Transfectionmix 6-well plate 10/09...		
	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.

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Timeline		Details		Procedure Details (Two Reaction Optimization)			
Day 0	1	 Seed cells to be 70–90% confluent at transfection	Adherent cells	Component		Amount	
	2			1 × 10 ⁶		2 × 10 ⁶	
Day 1	3	 Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (1:1000) – Mix well	Opti-MEM™ Medium Lipofectamine™ 3000 Reagent	5 µL + 2		25 µL + 2	
	4			0.10 and 0.3 µL <td data-kind="day"></td> <th data-cs="2" data-kind="parent">0.75 and 1.5 µL<td data-kind="day"></td></th>		0.75 and 1.5 µL <td data-kind="day"></td>	
Day 2	5	 Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent – Mix well	Opti-MEM™ Medium DNA (0.5–5 µg/µL) P3000™ Reagent (2 µL/µg DNA)	10 µL		50 µL	
	6			0.2 µg		1 µg	
Day 3–4	7	 Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)	Diluted DNA (with P3000™ Reagent) Diluted Lipofectamine™ 3000 Reagent	0.4 µL		2 µL	
	8			5 µL		25 µL	
Day 3–4	9	 Incubate	Incubate for 10–15 minutes at room temperature	25 µL		125 µL	
	10			5 µL		25 µL	
Day 3–4	11	 Add DNA-lipid complex to cells	Opti-MEM™ Medium DNA-lipid complex DNA amount P3000™ Reagent Lipofectamine™ 3000 Reagent used	10 µL		50 µL	
	12			100 ng		500 ng	
Day 3–4	13	 Visualize/analyze transfected cells	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.	0.2 µL		1 µL	
	14			0.10 and 0.3 µL		0.75 and 1.5 µL	

- Transfection scheme

Transfection scheme - 10/09/19 in ng per well - Purification							
	condition	B7	B9	E10	E16	V15	V
1	Bioinfo 1	2000 ng		-	-	-	500 ng
2	Bioinfo 2	1000 ng	1000 ng	-	-	-	500 ng
3	Bioinfo 3	-	-	-	1250 ng	-	1250 n
4	Purificaiton 1	-	-	-	750 ng	500 ng	1250 n
5	Purification 2	-	-	-	750 ng	500 ng	1250 n
6	Purification 3	-	-	750 ng	-	500 ng	1250 n

Alejandro

cell culture: MIN6-K

- medium over MIN6-K cells was exchanges (+ β-Mercaptoethanol)
- 20:00