09. (September) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

TUESDAY, 10/9/2019

Johanna

cloning: mouse Gag

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

digestion gBlock mouse Gag

	A	B
1	5 µL	10X CutSmart Buffer
2	1 µL	MluI-HF
3	1 µL	PacI
4	25 µL	Insert (gBlock)
5	18 µL	MiliQ

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

ligation mouse Gag

	A	B
1	T4 Buffer	2 µL
2	T4 Ligase	1 µL
3	Vector V4	2 µL
4	Insert	1.2 µL
5	MiliQ	13.8 µL

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

Alejandro

cell culture: Transfection for qPCR

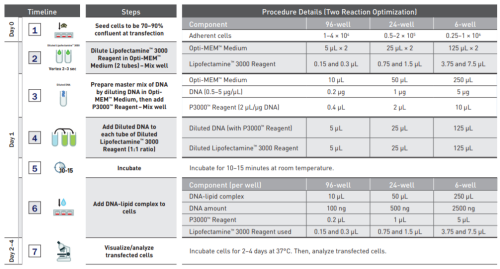
- finished at 10:15 a.m.
- 6-well plates

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.

image.png



- 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 n
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	-

Johanna

cell culture: Transfection for Purification

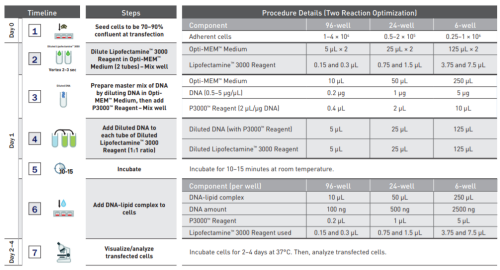
- finished at 13:00
- 6-well plates
- medium exchange: 1.5 mL Medium out, 2 mL new Medium in

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.

image.png



- 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 (+ ß-Mercaptoethanol)
- 20:00