09. (September) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

TUESDAY, 17/9/2019

Johanna

cell culture: Transfection for VLP-Purification

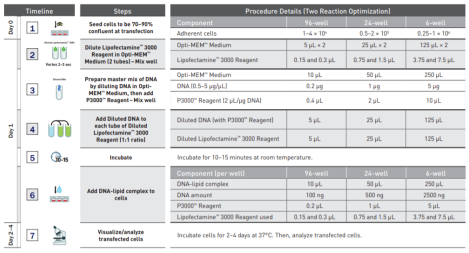
- medium exchange: 1.5 mL Medium out, 2 mL new Medium in

Transfectionmix 6-well plate 17/09…

	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 (3 wells per condition)

Transfection scheme - 17/09/19 in ng per well - Purification

	condition	V8	V10	V11	V14	V15	
1	1	1250 ng	625 ng	-	-	625 ng	-
2	2	1250 ng	-	625 ng	625 ng	-	-
3	3	-	-	-	-	-	2
4	4	1250 ng	-	-	-	-	1

Alejandro

cell culture: Transfection

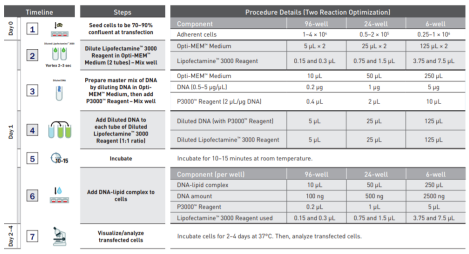
- 6-well plates

Transfectionmix 6-well plate 17/09…

	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 - 17/09/19 in ng per well

	condition	V8	V11	V14	V15	V26	V27	V30	V33	V36	V40	V41	
1	A	-	-	-	-	-	500 ng	500 ng	-	400 ng	200 ng	-	-
2	B	-	-	-	-	-	500 ng	1500 ng	-	-	200 ng	-	-
3	C	-	-	500 ng	-	-	500 ng	-	-	400 ng	-	-	-
4	D	-	-	-	500 ng	-	500 ng	-	-	400 ng	-	-	-
5	E	-	-	-	500 ng	-	500 ng	-	-	-	-	650 ng	-
6	F	-	-	500 ng	-	-	500 ng	-	-	-	-	650 ng	-
7	NC	1000 ng	500 ng	500 ng	-	-	-	500 ng	-	-	-	-	-
8	V26	1000 ng	500 ng	500 ng	-	500 ng	-	-	-	-	-	-	-
9	V27	1000 ng	500 ng	500 ng	-	-	500 ng	-	-	-	-	-	-
10	BB	1000 ng	500 ng	500 ng	-	-	-	-	-	-	-	-	5
11	V33	1000 ng	500 ng	500 ng	-	-	-	-	500 ng	-	-	-	-

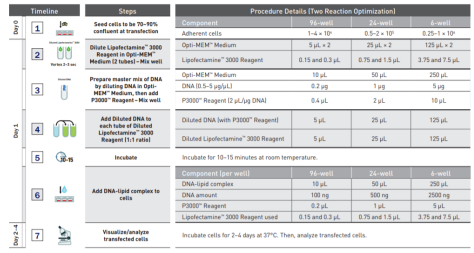
- 24-well plate with min6-K8 cells
  - time finished: 16:00

Transfectionmix 24-well plate 17/0…

	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.

image.png



- Transfection scheme

transfection scheme 17/09/2019 - MIN6-K8

	condition	V8	V10	V11	V14	V15	V27	V30	
1	2	400 ng	-	-	-	-	200 ng	400 ng	-
2	3	400 ng	-	200 ng	200 ng	-	200 ng	-	-
3	5	400 ng	200 ng	-	-	200 ng	200 ng	-	-
4	m2	-	-	-	-	-	200 ng	400 ng	4
5	m3	-	-	200 ng	200 ng	-	200 ng	-	4
6	m5	-	200 ng	-	-	200 ng	200 ng	-	4

- Problems:
  - not enough V36 in A
  - not enough V41 in F
  - Calculation error for the amounbt of OptiMEM in the 24-well plate. 57.2 µL per condition should have been used and not 29 µL