# 08. (August) 2019

Project: iGEM\_Munich2019 Shared Project

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SATURDAY, 24/8/2019

#### Alejandro:

#### reuptake assay - fluc, recipient cells

- Time: 10:30 a.m.; 24 h after transfection
- medium was discarded and cells were resuspended in 1 mL cold PBS (16 times up and down)
- 52 μL suspension were used for the fluc assay and a 0, 10, 20, 30, 40, 50 fmol HiBit standard curve in PBS was used
- 5 min incubation time at 300 min<sup>-1</sup>

Fluc assay for reuptake assay, 24/08/19												
	1	2	3	4	5	6	7	8	9	10	11	12
Α	HiBit sta	andard c	urve 0-50									
В												
С												
D					1							
Ε	1: only Gag *		2: only		3: MCP VLPs *		4:					
F			fluc *			L7Ae VLPs *						
G												
Н			1									

• \* 2 wells with supernatant form origin cells 1, 2 wells with supernatant from origin cells 2, 2 wells with supernatant from origin cells 3

### cell culture

- T75 flasks transfection for qPCR and Purification
  - Important guidelines

Make DNA-Lipofectamine™ 3000 complexes in serum-free medium such as Opti-MEM™ Reduced Serum Medium and add directly to cells in culture medium, in the presence or absence of serum/antibiotic.

It is not necessary to remove complexes or change/add medium after transfection.

The amount of Lipofectamine™ 3000 Reagent for successful transfection varies. Start any new transfection by testing the recommended two concentrations of Lipofectamine™ 3000 Reagent to determine an optimum amount

- o materials:
  - Plasmid DNA (0.5-5µg/µL stock)
  - Opti-MEM Reduced Serum Medium
  - Microcentrifuge tubes
  - Lipofectamine 3000 reagent
  - Cells (e.g. HEK 293 T)
- both flasks were at about 50-60 % confluent
- o medium over the cells was removed and 15 mL new medium was added
- o time transfection finished: 13:30 p.m.
- o T75 flask VLPs-MCP

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T75 fl	ask VLPs-MCP				^
	V8	V11	V14	V27	
1	8 µg	4 µg	4 µg	4 μg	

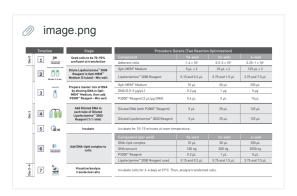
## o T75 flask Exosomes-L7Ae

T75 f	ask Exosomes-L	.7Ae	
	E10	V15	
1	12 µg	8 µg	

## o Transfectionscheme

transfectionmix T75					
	Α	В			
1	DNA per flask	20 μg			
2	P3000 Reagent per flask	40 μL			
3	Lipofectamine 3000 reagent per flask	30 µL			
4	OptiMEM per flask	2 x 750 μL			

■ Transfect cells according to the following table. Use the indicated volume of DNA and P3000<sup>™</sup> Reagent with each of the two volumes of Lipofectamine<sup>™</sup> 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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