# 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 $min^{-1}$

**Fluc assay for reuptake assay, 24/08/19**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | HiBit standard curve 0-50 | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | 1: only Gag * | | 2: only fluc * | | 3: MCP VLPs * | | 4: L7Ae VLPs * | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

- * 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
  - 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
  - medium over the cells was removed and 15 mL new medium was added
  - time transfection finished: 13:30 p.m.
  - T75 flask VLPs-MCP

**T75 flask VLPs-MCP**

| | V8 | V11 | V14 | V27 |
|---|---|---|---|---|
| 1 | 8 µg | 4 µg | 4 µg | 4 µg |

- T75 flask Exosomes-L7Ae
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**T75 flask Exosomes-L7Ae**

| | E10 | V15 |
|---|---|---|
| 1 | 12 µg | 8 µg |

- Transfectionscheme

**transfectionmix T75**

| | A | B |
|---|---|---|
| 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™ 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