08. (August) 2019

Project: iGEM_Munich2019 Shared Project
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FRIDAY, 23/8/2019

Johanna and Sarah:

VLP-Purification:

- start: 8:30 (--> 40 h after medium-exchange)
- prepare columns XXXX
  - cut tips and add filter
  - add VE-water, check if water runs through
  - columns should not be dry -> cover with water and close the tip
- load Beads
  - add 500 µL Biotin-Agarose in PBS (≙250 µL Biotin-Beads) or 500 µL Ni-NTA
  - add 10 mL PBS for equilibration -> let it run through; close the tips and add 500 µL PBS
- sample preparation
  - centrifuge medium: 2000 g, 10 min, 4 °C
  - transfer the supernatant to a new falcon
- prepare incubation
  - transfer the Biotin-Agarose/Ni-NTA to a new falcon with 1 mL PBS
  - add 2 mL supernatant to each falcon
  - incubate 2 h (biotin) or 1 h (His) at 4 °C, shaking
- cover the empty columns with PBS and close the tips
- His-Purification
  - see Exosome Lab Book
  - wash sith 15 mM Imidazole
  - elute with 250 mM imidazole
  - readout: HiBit
- Biotin-Purification
  - open tip from the columns & let the PBS run through
  - close tips
  - add Biotin-samples -> 5 min incubation
    - collect FT in a falcon
  - close tips
  - apply 1 mL Buffer W (Wash Buffer: 100 mM TRIS ph 8.0, 150 mM NaCl) = W1
    - incubate
    - collect in a eppi
    - close tip
  - apply 1 mL Buffer W = W2
    - incubate
    - collect in an eppi
    - close tip
  - apply 1 mL BXT (Elution Buffer: 100 mM TRIS pH 8.0, 150 mM NaCl, 1 mM EDTA, 50 mM Imidazole) = E1
    - incubate
    - collect in an eppi
    - close tip
  - apply 1 mL BXT = E2
    - incubate
    - collect in an eppi
- HiBit assay

- lysed: SN, FT, W1, W2, E1, E2 (with VLB: 40 µL sample + 40 µL VLB)
- unlysed: SN, E1 (with PBS4Mix: 40 µL sample + 40 µL PBS4Mix)
- dilute lysed and unlysed: 45 µL DIL-Buffer + 5 µL sample
- standard curve: 0, 5 fmol, 10 fmol, 20 fmol

HiBiT assay for VLP-Purification, 23/08/19

	A	B	C	D	E	F	G	H	I	J	K	L	M
1		1	2	3	4	5	6	7	8	9	10	11	12
2	A	6,18E+04	1,28E+05	1,06E+05	7,70E+04	1,45E+05	1,51E+05	2,94E+04	2,72E+04	3,00E+01	1,70E+01	1,20E+01	6,00E+00
3	B	1,03E+06	1,42E+06	9,04E+05	1,38E+06	1,72E+06	3,27E+06	1,64E+05	1,21E+05	3,60E+01	1,20E+01	7,00E+00	6,00E+00
4	C	8,45E+05	9,00E+05	6,08E+05	8,38E+05	1,11E+06	2,30E+06	1,04E+05	3,11E+04	9,11E+04	1,30E+01	1,50E+01	2,10E+01
5	D	2,75E+05	2,97E+05	2,04E+05	1,76E+05	2,83E+05	5,50E+05	2,62E+04	9,68E+03	6,38E+04	1,70E+01	1,20E+01	1,50E+01
6	E	6,60E+04	6,09E+04	5,07E+04	4,41E+04	7,15E+04	1,05E+05	1,31E+04	4,35E+03	1,93E+05	1,70E+01	1,10E+01	1,80E+01
7	F	5,48E+02	4,12E+02	3,01E+02	1,49E+03	4,15E+03	3,48E+03	1,58E+04	1,14E+04	1,72E+05	1,20E+01	1,20E+01	6,00E+00
8	G	1,74E+04	1,68E+04	1,25E+04	5,32E+04	6,49E+04	9,91E+04	3,86E+04	5,33E+04	3,23E+05	1,50E+01	1,60E+01	1,20E+01
9	H	4,52E+03	7,97E+03	8,44E+03	1,72E+04	2,52E+04	4,63E+04	7,61E+03	2,50E+04	3,16E+05	2,10E+01	5,00E+00	7,00E+00

- results
  - single chain avidin on L7Ae:

single chain avidin on L7Ae - 23/0…

	A	B
1	50 mM Biotin	Percentage
2	Flowthrough	69 %
3	Wash	29 %
4	Elution	2 %

  - single chain avidin on MCP

single chain avidin on MCP - 23/0…

	A	B
1	50 mM Biotin	Percentage
2	Flowthrough	70 %
3	Wash	28 %
4	Elution	2 %

Johanna:

Cell culture: reuptake assay

- 9:30 a.m. - 10:30 a.m.
- take out 600 µL supernatant of origin cells --> centrifugation: 2000 g, 10 min
- recipient cells:
  - take out 600 µL
  - add 200 µL of the centrifugated supernatant and 400 µL of new medium --> 2 wells with recipient cells per 1 well of supernatant from origin cells
- 100 µL of the centrifugated supernatant were transfered in a new eppi to carry out a fluc assay

Alejandro:

reuptake assay: fluc assay, origin cells and supernatant:

- standard curve with Promega's HiBit protein
- for all samples the remaining medium was taken out and the cells were resuspended in 1 mL PBS (16 times up and down)
- 52 µL were used for the assay (1/20; 5 %)
- the supernatant samples were 52 µL from Johanna's centrifugated supernatnat (1/16, 6.25 %)

Fluc assay for reuptake assay, 23/08/29

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z1					SN1						
B												
C	Z2					SN2						
D	Z3					SN3						
E	Z4					SN4						
F												
G	HiBit Standard curve 0-50											
H												

- Z=cells, SN=supernatant
- standard curve: 0, 10, 20, 30, 40, 50 fmol HiBit in PBS, duplicates
- integration time 0.5 sec, 5 min shaking at 300 min^{-1}

reuptake assay: HiBit assay, origin cells and supernatant

- with the same saples as the fluc assay to check if undamaged VLPs had been transfered to the recipient cells
- 10 µL samples were mixed with 10 µL buffer:
  - Z + VLB and PI -> 10 min, 60 °C
  - SN + VLP and PI -> 10 min, 60 °C (lysed)
  - SN + PBS and PI -> no heat treatment (unlysed)
- all samples were then diluted 1:10 in PBS and PI (45 µL + 5 µL sample) and 42 µL from these were used for a HiBit assay

HiBit assay for reuptake assay, 23/08/2019

	1	2	3	4	5	6	7	8	9	10	11	12
A	HiBit standard curve 0-50											
B												
C	Z1			L1			U1					
D												
E	unlysed 2-4											
F												
G	lysed 2-4											
H	cells 2-4											

- 10 min shaking at 300 min^{-1}; 0.5 sec integration time

Cryostock:

- Cryostock of BBa_K2170002 was made