

08. (August) 2019

Project: iGEM_Munich2019 Shared Project

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FRIDAY, 16/8/2019

Moritz

cell culture: splitting

4 x T75

Alejandro

HiBit Assay

- Integration time during fluorescence measurement was decreased to 0.5 sec
- possible mistakes:
 - possible cross-contaminations of rows D & E (happened while taking the 120 µL SN out)
 - Row H had cells without CLB for 5 min
- VLPs and exosomes harvesting

Protocol for harvesting from 96-well plates (160 µl medium)

Buffer preparation

Work on ice

- a. Add Protease Inhibitor Cocktail (SigmaAldrich, P8340) 1:50 to VLB (VLP lysis buffer: 1X PBS, 1 % Triton X-100), prepare 80 µl per well + 500 µl.
- b. Add Protease Inhibitor Cocktail 1:50 to PBS4MIX (PBS for mixing supernatant unlysed samples 1:1), prepare 10 µl per well + 500 µl.
- c. Add Protease Inhibitor Cocktail 1:100 to CLB (cell lysis buffer: 1X PBS, 0.5 % Triton X-100), prepare 160 µl per well + 500 µl.
- d. Add Protease Inhibitor Cocktail 1:100 to PBS4DIL (PBS for diluting all samples 1:10), prepare 114 µl per well + 500 µl.

Supernatant harvesting

- a. Harvest 120 µl supernatant containing the VLPs 72 h after transfection and 24 h after medium exchange into a black flatbottom 96-well plate (SN centrifugation plate).
- b. Centrifuge the plate at 2000 rcf for 10min to remove dead cells and bigger cell debris.
- c. Transfer 90 µl supernatant to a PCR 96-well plate (SN lysed plate) and work on ice from this step on. Discard the plate with the pelleted cells.
- d. Add 10 µl PBS4MIX to a new PCR 96-well plate (SN unlysed plate).
- e. Transfer 10 µl from the centrifuged supernatant to the SN unlysed plate and mix well (6 times up and down).
- f. Add 80 µl VLB to the SN lysed plate and mix well (6 times up and down). Put a plastic foil over the plate and make sure all the wells are well closed. Incubate then the plate at 60 °C for 15 min in a thermocycler and put it afterwards back on ice.
- g. Dilute samples from both the SN lysed and SN lysed plates 1:10 by transferring 4 µl to two new white flatbottom 96-well plates containing 38 µl PBS4DIL (SN lysed 4HiBit and SN unlysed 4HiBit plates). Push only until the pressure point when aliquoting the 38 µl PBS4DIL. Push all the way through when adding the 4 µl sample. Mixing is not necessary.
- h. Freeze the remaining 155 µl sample in the SN lysed plate by putting it in the -80 °C freezer (to do a qPCR with the same samples).
- i. Analyze the sample content with the HiBit Extracellular Detection Kit. Add 42 µl freshly prepared HiBit Reaction Mix (push only until the pressure point) to the 40 µl diluted samples (equivalent to 1.25 % or 1/80 total supernatant) and shake the plates at 300 min⁻¹ (black shaker on Jeff's bench area) for 10 min before measuring the luminescence.

Cell content analysis

- a. Carefully remove the remaining 40 µl medium in the wells.
- b. Add 160 µl CLB to each well and pipette up and down 16 times washing well the whole well area.
- c. Centrifuge the cell culture plate at 3.000 rcf for 10 min.

- d. Transfer 120 µl and into a new PCR 96-well plate (CC plate) and work on ice from this step on. Discard the plate with the pelleted cell debris.
 - e. Put a plastic foil over the plate and make sure all the wells are well closed. Incubate the plate at 60 °C for 15 min in a thermocycler and then put the plate back on ice.
 - f. Dilute the samples 1:10 by transferring 4 µl to a new white flatbottom 96-well plate containing 38 µl PBS4DIL (CC 4HiBit plate). Push only until the pressure point when aliquoting the 38 µl PBS4DIL. Push all the way through when adding the 4 µl sample. Mixing is not necessary.
 - g. Freeze the remaining 115 µl sample in the SN lysed supernatant plate by putting it in the –80 °C freezer (to do a qPCR with the same samples).
 - h. Analyze the sample content with the HiBit Extracellular Detection Kit. Add 42 µl freshly prepared HiBit Reaction Mix (push only until the pressure point) to the 40 µl diluted samples (equivalent to 2.5 % or 1/40 total supernatant) and shake the plate at 300 min⁻¹ (black shaker on Jeff's bench area) for 10 min before measuring the luminescence.
- new calibration curve with higher HiBit Protein concentrations: 0, 10 fmol, 20 fmol, 30 fmol, 40 fmol, 50 fmol HiBit in 40 µL PBS

results HiBit assay 96-well format, 8 replicates + purification via His and Biotin 16/08/19													
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	SN samples were switched during transfer. B3 corresponds to G10, B10 to G3, etc.												
2													
3		1	2	3	4	5	6	7	8	9	10	11	12
4	A	6.00E+01	5.60E+01	1.38E+05	1.42E+05	2.37E+05	2.71E+05	3.47E+05	4.16E+05	5.14E+05	6.14E+05	6.42E+05	7.50E+05
5	B	2.04E+05	1.56E+05	2.19E+05	2.17E+05	2.08E+05	1.93E+05	2.04E+05	2.20E+05	1.89E+05	2.23E+05	2.60E+01	1.30E+01
6	C	1.64E+05	3.00E+01	1.57E+05	1.46E+05	1.78E+05	1.31E+05	1.68E+05	1.60E+05	1.31E+05	1.78E+05	7.90E+03	8.51E+03
7	D	1.52E+05	1.40E+05	2.64E+05	2.75E+05	2.28E+05	2.49E+05	2.60E+05	2.69E+05	2.54E+05	2.67E+05	6.31E+03	6.82E+03
8	E	9.53E+04	8.92E+04	3.76E+05	3.79E+05	3.42E+05	2.86E+05	3.82E+05	3.87E+05	3.31E+05	3.39E+05	1.72E+04	1.65E+04
9	F	1.04E+05	8.80E+04	2.61E+05	2.52E+05	2.41E+05	2.50E+05	2.58E+05	2.73E+05	2.15E+05	2.19E+05	1.26E+04	1.21E+04
10	G	1.74E+04	1.69E+04	1.93E+02	2.10E+02	2.26E+02	2.03E+02	1.56E+02	2.03E+02	1.83E+02	2.23E+02		
11	H	1.01E+04	7.83E+03										
12													
13	Calibration curve parameters												
14	Slope	13941											
15	Y-intercept	-9255											
16													
17	Data standarized to 1 fmol HiBit protein												
18		1.64E+01	1.62E+01	1.56E+01	1.45E+01	1.53E+01	1.64E+01	1.42E+01	1.66E+01				
19		1.19E+01	1.11E+01	1.35E+01	1.01E+01	1.27E+01	1.21E+01	1.01E+01	1.34E+01				
20		1.96E+01	2.04E+01	1.70E+01	1.85E+01	1.93E+01	1.99E+01	1.89E+01	1.98E+01				
21		2.76E+01	2.79E+01	2.52E+01	2.12E+01	2.81E+01	2.84E+01	2.44E+01	2.50E+01				
22		1.94E+01	1.87E+01	1.79E+01	1.86E+01	1.92E+01	2.03E+01	1.61E+01	1.63E+01				
23		6.78E-01	6.79E-01	6.80E-01	6.78E-01	6.75E-01	6.78E-01	6.77E-01	6.80E-01				
24													
25	Punktspiegelung of values due to sample switch												
26		6.80E-01	6.77E-01	6.78E-01	6.75E-01	6.78E-01	6.80E-01	6.79E-01	6.78E-01				
27		1.63E+01	1.61E+01	2.03E+01	1.92E+01	1.86E+01	1.79E+01	1.87E+01	1.94E+01				
28		2.50E+01	2.44E+01	2.84E+01	2.81E+01	2.12E+01	2.52E+01	2.79E+01	2.76E+01				
29		1.98E+01	1.89E+01	1.99E+01	1.93E+01	1.85E+01	1.70E+01	2.04E+01	1.96E+01				
30		1.34E+01	1.01E+01	1.21E+01	1.27E+01	1.01E+01	1.35E+01	1.11E+01	1.19E+01				
31		1.66E+01	1.42E+01	1.64E+01	1.53E+01	1.45E+01	1.56E+01	1.62E+01	1.64E+01				
32													
33	Data standarized to the whole well												
34		5.44E+01	5.42E+01	5.43E+01	5.40E+01	5.43E+01	5.44E+01	5.43E+01	5.42E+01				
35		1.31E+03	1.29E+03	1.62E+03	1.53E+03	1.49E+03	1.44E+03	1.50E+03	1.55E+03				
36		2.00E+03	1.95E+03	2.27E+03	2.24E+03	1.69E+03	2.02E+03	2.23E+03	2.21E+03				
37		1.58E+03	1.51E+03	1.60E+03	1.54E+03	1.48E+03	1.36E+03	1.63E+03	1.57E+03				
38		1.07E+03	8.05E+02	9.70E+02	1.01E+03	8.06E+02	1.08E+03	8.90E+02	9.54E+02				
39		1.33E+03	1.14E+03	1.31E+03	1.22E+03	1.16E+03	1.25E+03	1.30E+03	1.31E+03				
40													
41	Unit change to nmol												
42		5.44E-02	5.42E-02	5.43E-02	5.40E-02	5.43E-02	5.44E-02	5.43E-02	5.42E-02				
43		1.31E+00	1.29E+00	1.62E+00	1.53E+00	1.49E+00	1.44E+00	1.50E+00	1.55E+00				
44		2.00E+00	1.95E+00	2.27E+00	2.24E+00	1.69E+00	2.02E+00	2.23E+00	2.21E+00				
45		1.58E+00	1.51E+00	1.60E+00	1.54E+00	1.48E+00	1.36E+00	1.63E+00	1.57E+00				
46		1.07E+00	8.05E-01	9.70E-01	1.01E+00	8.06E-01	1.08E+00	8.90E-01	9.54E-01				
47		1.33E+00	1.14E+00	1.31E+00	1.22E+00	1.16E+00	1.25E+00	1.30E+00	1.31E+00				
48													
49	Ordering												
50	1	0.054389	0.05416	0.054274	0.054005	0.054274	0.054406	0.054315	0.054217				
51	2	1.306965	1.288028	1.620859	1.533061	1.488875	1.435507	1.49863	1.550276				
52	3	2.000172	1.952543	2.272176	2.244057	1.693738	2.017961	2.228563	2.207905				
53	4	1.582412	1.508385	1.595036	1.542816	1.483136	1.362054	1.628893	1.569213				
54	5	1.073983	0.805423	0.969543	1.014303	0.805997	1.076279	0.889778	0.954049				
55	6	1.329919	1.13768	1.312704	1.224905	1.162356	1.247285	1.296636	1.309834				

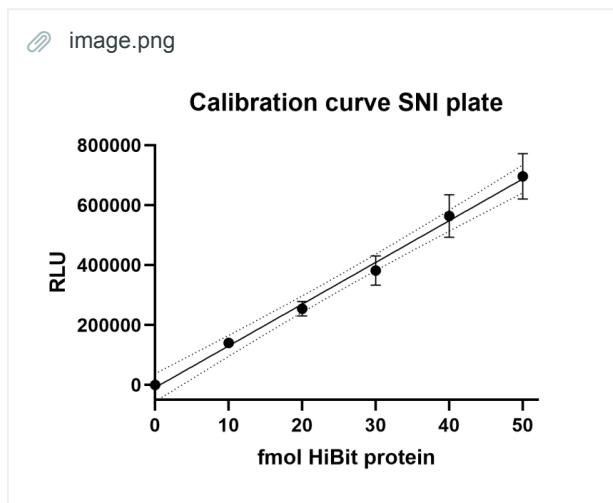
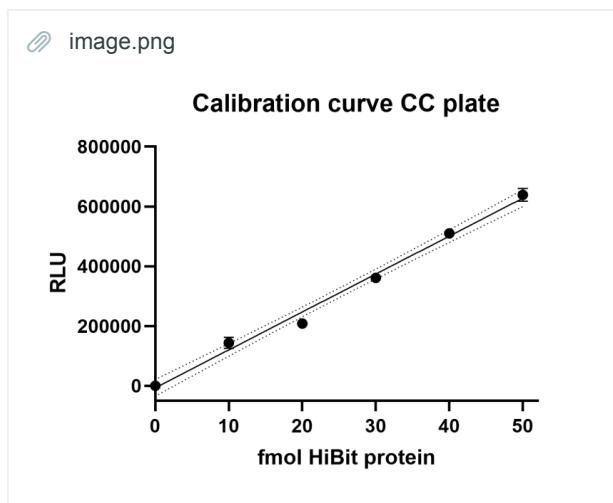
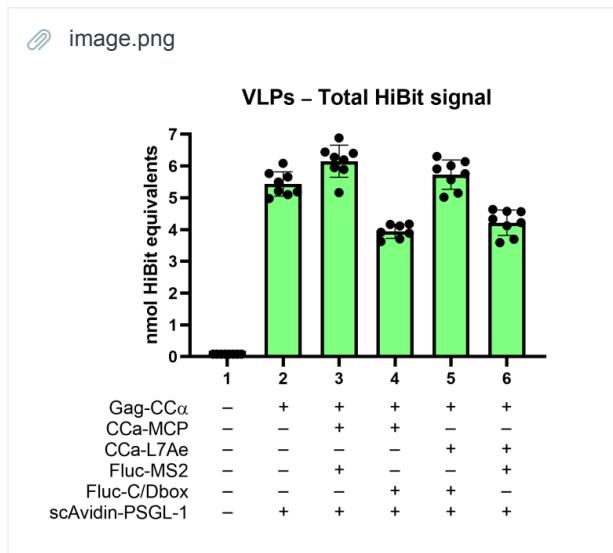
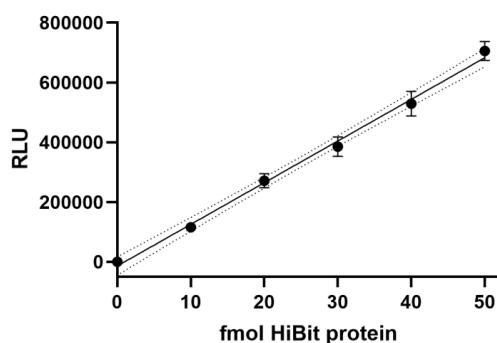
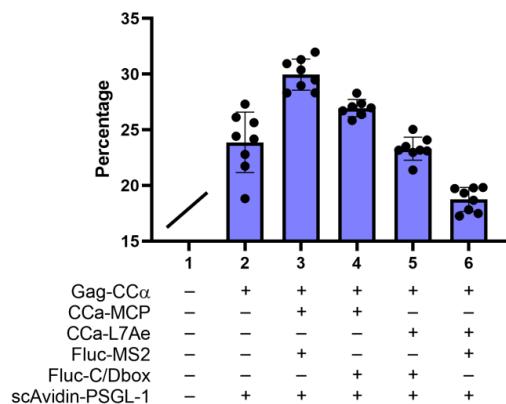


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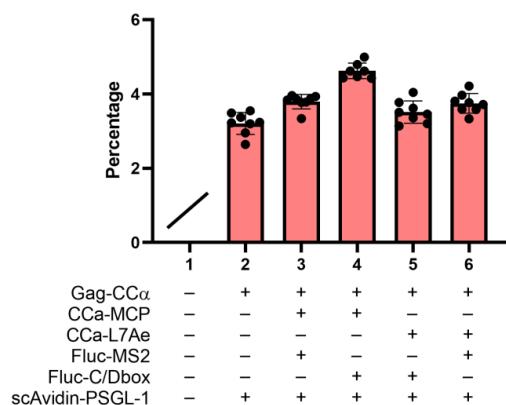
Calibration curve SNu plate

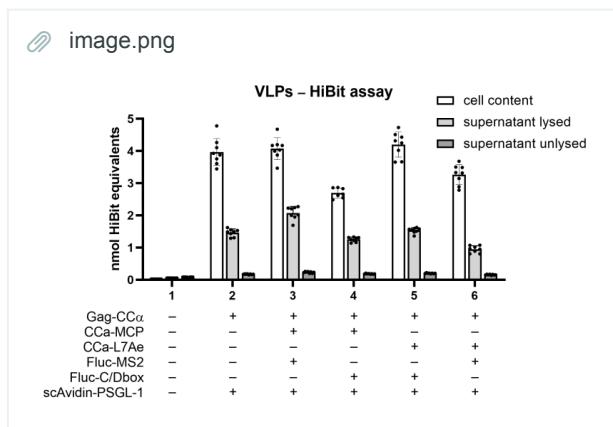

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VLPs – HiBit export


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VLPs – HiBit leakage





Sarah

Biotin- and His-Purification

- results: elution fraction contained 3x more VLPs in the case of the biotin purification
- determine if the eluted VLPs were whole or broken: HiBit assay: compare lysed and unlysed signals
- XXXXX