

09. (September) 2019

Project: iGEM_Munich2019 Shared Project

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Johanna

Purification

- cells transfected on tuesday -> prepare SN (2000 g, 10 min)
- prepare 2 x 4 gravity flow columns (o/n and 1 hour):
 - 2 x Biotin: 500 µL Biotin-PBS-Agarose/column -> x2
 - 2 x His: 500 µL NiNTA-Agarose/column -> x2
 - control for both: MOck (without PSGL-1)
- load 2 mL sample (SN) onto the beads (for Mock only 1 mL)
- incubate for 1 h RT / o/n 4 °C
- 2x wash: Biotin with 1 mL PBS, His with 1 mL 10 mM Imidazole
- 2 x elution: Biotin with 1 mL 50 mM Biotin, His with 1 mL 50 mM Imidazole
- HiBit
- result: Biotin seems not to work, His semmes better for 1 h
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results purification 06/09/2019

	A	B	C	D
1	His	His Ctrl	Biotin	Biotin Ctrl
2	171000	21300	89100	21300
3	103000	28800	46900	28800
4	351000	208000	57400	140000
5	171000	89100	21300	103000
6	46900	28800	4180	22300
7	8520	1190	160	160
8	58700	31800	1380	3380
9	34200	9800	691	2030
10				
11	351000	208000	57400	140000
12	217900	117900	25480	125300
13	92900	41600	2071	5410
14	661800	367500	84951	270710
15				
16	53,03717	56,59864	67,56836	51,71586
17	32,92536	32,08163	29,99376	46,28569
18	14,03747	11,31973	2,437876	1,998449
19	100	100	100	100

Alejandro

Cell culture

- medium over the MIN6-K8 cells was exchanged with new one (12 mL + β -Mercaptoethanol)
- lots of dead cells were swimming around and the flask was very thin populated with cells