07. (July) 2019

Project: iGEM_Munich2019 Shared Project
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Mai Prep V11#4 :

- standard protocol
- elution with 300µL MilliQ

Colony PCR V15 + V17 :

- Primer 12 + 13 for both
- estimated bands :
  - V15 : 1800bp
  - V17 : 1600bp
- elongation time : 72°C, 70s
- annealing tmp : 51°C, 20s
- 12 colonies, each :
  - H_2O : 280µL
  - P12 : 35µL
  - P13 : 35µL
  - MM : 12.5µl each
- 1% agarose gel, 14µL sybre safe, 10µl sample, 3µL ladder
- V15#2,3,6 -> POSITIVE
- V17#4,8,9 -> POSITIVE

transfection for Hibit :

- cells looked healthy and were 70% confluent
- 500 from 600µL Medium were exchanged
- cells were transfected with 500ng of V8 according to the Lipofectamine 3000 protocol
- note : well D2 might be contaminated

harvest scheme Hibit Assay

	A	B	C	D
1	**24h**	**48h**	**72h**	**for pooling**
2	24h	48h	72h	for pooling
3	24h	48h	72h	for pooling

transformation :

- Plasmids V29 and V30 were obtained from Christoph
- NEB stable cells were transformed according to the NEB high efficiency transformation protocol
- these constructs will be used as mock DNA in mammalian cell transfections

Colony PCR for V25, V26, V27, V28 :

- Primer 12+13 for all
- 9 colonies/sample -> 36, MM for 40
  - P12 1.25µL -> 50µL

    - P13 1.25µL -> 50µL
    - H_2O 10µL -> 400µL
      -> 12.5µL per well + 12.5µL MM
- PCR :
    - elongation time : 80s, 72°C
    - annealing temp : 51.2°C, 20sec
- bands :
    - V25 #1 #8 #9
    - V26 #1 #8 #9
    - V27 #1 #8 #9
    - V28 #1 #8 #9
      -> overnight culture in 5ml LB Amp Medium

Glycerol stock :

- V26 : 850µL Glycerol, 750µL V26

Miniprep :

- V25#1,#8,#9
- V26
- V27#1,#8,#9
- V28#1,#8,#9
  -> centrifuge 15min at 4000g

Nanodrop measurements

	Plasmid	DNA concentration	sample	MilliQ	E
1	V25#1	486.4 ng/µL	3ml	12ml	
2	V25#8	515.8 ng/µL	3ml	12ml	
3	V25#9	490.4 ng/µL	3ml	12ml	
4	V26	493.0 ng/µL	3ml	12ml	
5	V27#1	499.7 ng/µL	3ml	12ml	
6	V27#8	419.7 ng/µL	4ml	11ml	
7	V27#9	540.1 ng/µL	3ml	12ml	
8	V28#1	474.4 ng/µL	3.5ml	11.5ml	
9	V28#8	390.2 ng/µL	3ml	12ml	
10	V28#9	374.1 ng/µL	3.5ml	11.5ml	