

05. (May) 2019

Project: iGEM_Munich2019 Shared Project

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Thess

Cell culture: **HEK293T Splitting** of the mother-plate according to protocol cell splitting

Joshi

Colony PCR:

Colony PCR was done for Plasmid E6 according to colony PCR Protocol

Colony PCR for E6			^
	A	B	
1	MiliQ-Water	10 µL	
2	Primer #12 (forward)	1.25 µL	
3	Primer #13 (reverse)	1.25 µL	
4	Dream Taq Master Mix	12.5 µL	

Thermocycler Colony PCR for E6				^
	A	B	C	
1	Temperature	Duration	Cycle	
2	95°C	10 min		
3	95°C	30 s	x 35	
4	51.2 °C	20 s		
5	72°C	1 min		
6	72°C	10 min		
7	4°C	hold		

Gel electrophoresis: see protocol

1 % Agarose gel

160V, 400A, 20 min

The gel was viewed under UV light and colonies 1-2 were determined as positive. Colonies 2, 6 and 10 were picked.

Miniprep preparation : see protocol

9 x 5 ml (LB + 1:1000 Amp) bacterial overnight cultures were inoculated with transformed bacteria

Restriction Digest: see protocol

Double Digestion of gBlock lamp2b

Restriction Digest of gBlock lamp2b			^
	A	B	
1	Component	100 µl Reaction	
2	DNA	47 µl	
3	10X CutSmart Buffer	10 µl	
4	PcoI	2 µl	
5	MluI	2 µl	
6	ddH2O	39 µl	

Incubate at 37°C for 15min instead of 1 hour.

DNA/PCR NEB purification kit: see protocol