PROLUNG

BIOCONTAINMENT

CUMATE REGULATORY SYSTEM

LAB BOOK 2

iGEM Stockholm

Ligation of cumate and colicin

Objective

Remove BFP from the cumate system and replace it with colicin.

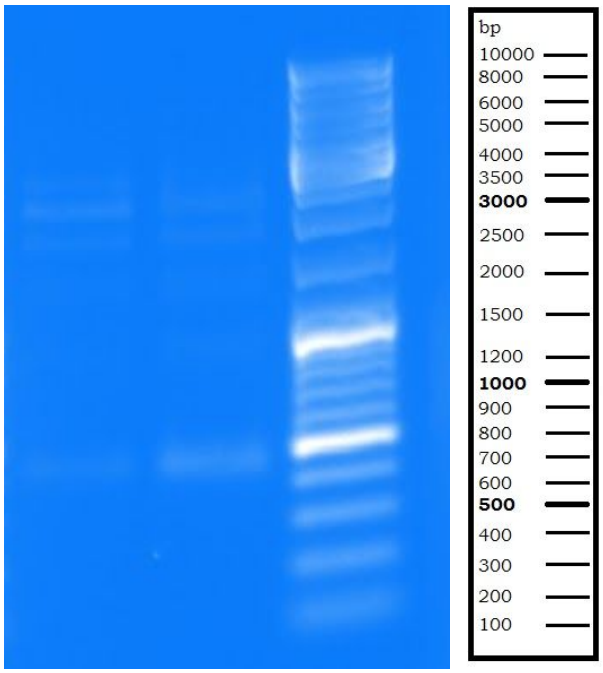
Procedure

Digestion

Keep reaction on ice. Add enzymes last.

	Cumate	Colicin
10XCutSmart buffer	5	5
XmaI	1	1
MfeI	1	1
DNA	1ug	1ug
RNase-free water	To 50ul	To 50ul

Incubation: 37C/1h
Gel: 110V/45mins



Ladder: M. Very faint band at approx. 1600bp

Stab PCR

The faint band at 1600bp was stabbed, because the cumate+colicin has the length of 1601bp.

Stab with a syringe the band at the right size and swirl it in the master mix.

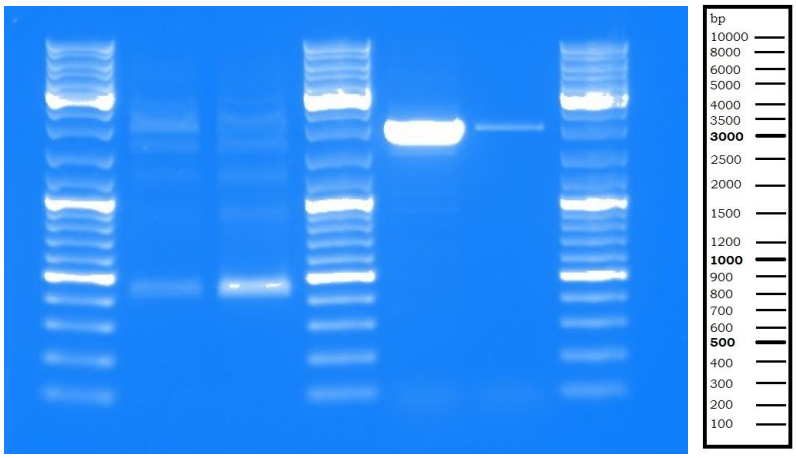
Component	Volume (ul)
Q5 Hi-Fi Master Mix	25
Primer mix	1.2
RNase-free water	24
Total	50ul

Table 2. PCR conditions

Step	Temperature (℃)	Time
Initial denaturation	98	30s
25 cycles:		
Denaturation	98	15s
Annealing	69	10s
Extension	72	1.10min
Final extension	72	5min
Hold	4	-

Gel: 110V/30min

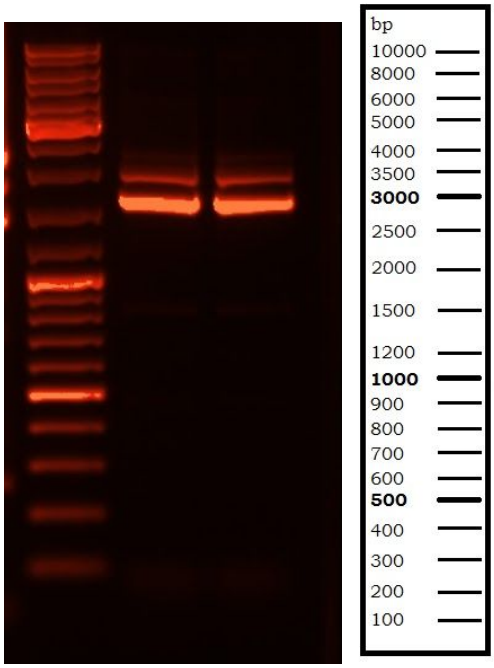
The gel contains the ligated cumate+colicin product and the stab PCR product from this reaction.



Ladder: M
It was unclear whether the right band had been stabbed, so a new stab PCR was run, this time with the correct stabbed band.

Stab PCR

Reaction setup and conditions as above.
New gel: 110V/1h



Ladder: M
Strong band at ~1600bp is the correct fragment of cumate+colicin.