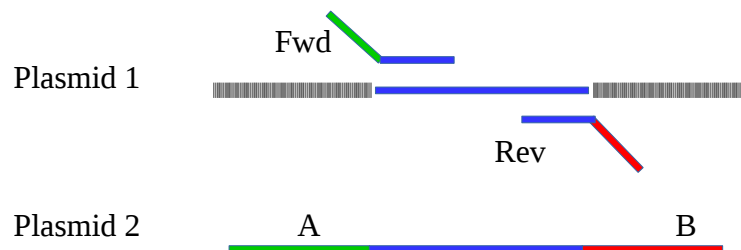


## Cloning and mutagenesis (TPCR)

Transfer a DNA fragment from plasmid 1 to plasmid 2

### 1) Design primers:

- Fwd = ~20-30 bp overlap with plasmid 2(A) + ~20-30 bp overlap with 5'-fragment (from plasmid 1)
- Rev = ~20-30 bp overlap with 3'-fragment (from plasmid 1) + ~20-30 bp overlap with plasmid 2(B) and reverse complement.
- If possible, avoid A or T at the extremes of the overlapping regions of the primers. Each of these regions (2 per primer) should have a  $T_m \sim 60-70$  ( $T_m = (\sum GC)*4 + (\sum AT)*2$ )



### 2) Prepare reactions (25ul reaction is best):

Reagent	Cc	Volume (ul)
plasmid 1	10 ng/ul	1
plasmid 2	10 ng/ul	1
primer Fwd	1 uM	1
primer Rev	1 uM	1
dNTPs	10 mM	1
Buffer	5x	10
H <sub>2</sub> O		34.3
Phusion	2U/ul	0.65
TOTAL		~50

### 3) run PCR

temp	time	
95	60"	
95	30"	x35
60	60"	x35
72	4' *	x35
72	7'	
7	∞	

\* → 5 min or longer times for larger plasmids.

### 4) DpnI digest (100% efficiency of DpnI in Phusion buffer):

10-25ul reaction + DpnI for 90min to O.N. @37°C

### 5) Transform into E.coli