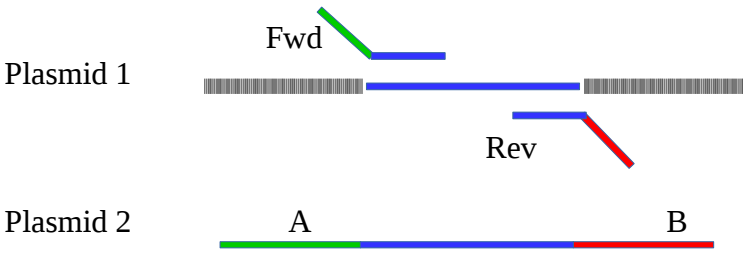
Cloning and mutagenesis (TPCR)

Transfer a DNA fragment from plasmid 1 to plasmid 2

1) Design primers:

- Fwd = ~20-30 bp overlap with pasmid 2(A) + ~20-30 bp overlap with 5'-fragment (from plasmid 1)
- Rev = ~20-30 overlap with 3'-fragment (from plasmid 1) + ~20-30 bp overlap with pasmid 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 Tm ~ 60-70 (*Tm = (∑GC)*4 + (∑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_2O		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. @37ºC

5) Transform into E.coli