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Ligation Protocol (better concentrated) for 20 uL of mixture

Note	For both pcr and plasmid heat inactivation after Double Digest wasn't used !!!
	column purification was done instead(pcr purif kit and gel purif kit respectively)

1	pCM184 digested with ApaI and SacI (from gel purification)	5	uL
2	PCR 4 digested with Apa and Sac (PCR purification, H2O eluted)	15	uL
3	10xT4 DNA ligase buffer (Always thaw on ice!!! Contains ATP)	4	uL
4	H2O	12	uL
5	T4 DNA ligase (0.2 uL = 1 U)	4	uL
	Total:	40	uL

1 Ligation set overnight at 15 C (sometimes at 4 C overnight or overweekend)

2 The next day, ligation mix kept RT for 3 hours, and frozen at -20 C.

3 Ligation set overnight at 15 C (sometimes at 4 C overnight or overweekend)

4 Ligation was transformed into E.coli DH5a using 100 uL of Ca2+ competent cells.

https://www.addgene.org/plasmid-protocols/dna-ligation/
recommends

25ng Vector DNA	125
75ng Insert DNA	375
Ligase Buffer (1µL/10µL reaction for 10X buffer, and 2µL/10µL reaction for 5X buffer)	
0.5-1µL T4 DNA Ligase	
H20 to a total of 10µL	

plasmid conc was negative: -0.5 (More plasmid should be used for digest)

	conc	mass(ng)	100 ng of total DNA	lengths		Molar Ratio	% Ratio
plasmid	30 ug/ml	150		6700	0.022388	0.447761	30.92784
pcr	3 ug/ml	45		900	0.05	1	69.07216
				max	0.05		