

5/11/2015

1 2AB, 2BC, 2CA Ligation Test

1.1 BsaI digestion of 2AB, 2BC, 2CA

Used 5 ug of each DNA.

	2AB (112 ng/uL)	2BC (205 ng/uL)	2CA (218.7 ng/uL)
BsaI	4 uL	4 uL	4 uL
Template	44.6 uL	24.39 uL	22.86 uL
Cutsmart	5 uL	5 uL	5 uL
ddH2O	0 uL	16.61 uL	18.14 uL
Total	53.6 uL	50 uL	50 uL

50 C 2 hr

65 C 20 min

We are conducting ligation tests between all possible combinations using T4 and T7 ligase

2 NotI Concatemers

Sri suggested using NotI to digest the plasmids and create a super plasmid that contains multiple copies of the monomers.

Testing NotI digestion today using 2CA with the protocol below.

	2CA (280 ng/uL)
NotI-HF	2 uL
DNA	7.2 uL
Cutsmart	5 uL
ddH2O	35.8 uL
Total	50 uL

3 Results

Cast 200 mL of a 1.5% TAE agarose gel to visualize results of both BsaI and NotI digestion.

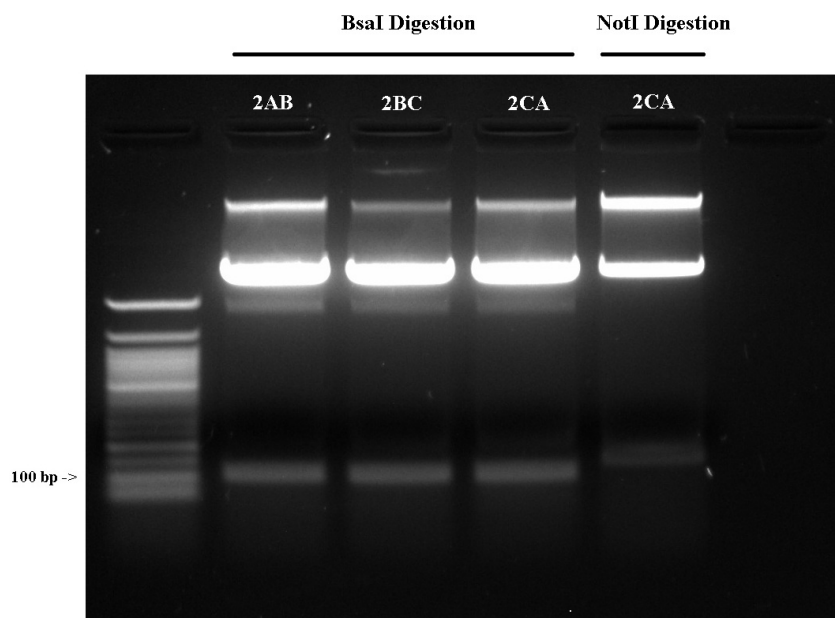


Figure 1: Digestion Results: The 100 bp bands were excised as well as the second band in the NotI digestion.