PCR mutagenesis

11/10/2004 Amy at Fred's lab

10xTaq buffer

0.166M (NH4)2SO4

0.67M Tris-Hcl pH. 8.8

61mM MgCl2

67microM EDTA pH 8.0 in 0.7mg/ml BSA

1M BME

260microlitter water, add 20microlitter BME

#reactions	10xtaq	1M BME	DMSO	10mM dNTP	Oligo (each)	H2O	Ampli Taq	Total
4	50	5	50	50	4	334	4	500
6	70	7	70	70	5	468	5	700
8	90	9	90	90	6	602	6	900

Add 100 microlitter mix to 1microlitter template, run program 10

Use 10-30 ng/microlitter template

Try 1:3 diltion 4-5 times to see which template concentration is the best.

Control: No template

Program10:

1min 95 degree

1min 55 degree (annealing)

4min (plus 5" cycle) extend at 72 degree

After the PCR reaction, DNA should be purified using DNA purification kit.

** You also need cut plasmid for transformation.

Digest your plasmid (which you used as a template), and add EDTA at 20mM final concentraion to stop the reaction.

You can store this sample at 4degree or –20 degree.

You transform purified PCR product and cut-plasmid at 4:1 ratio. Do not forget to make negative control which is cut-plasmid only. It is not bad idea to include PCR product only, and on DNA control.