# Site Directed Mutagenesis Protocol

Background This protocol is based on a combination of the Stratagene Quikchange protocol and information gleaned from the web. The important thing to keep in mind is that the concentration of primer versus template determines whether primer-primer or template-primer interaction dominate. If a reaction fails, sometimes dropping the primer concentration, increasing the template concentration, or decreasing the annealing temperature can help.

The basic reaction scheme is shown in the figure below. Keep in mind that the product of the reaction is never used as a template. This mean that this is a linear amplification technique, unlike standard PCR where you get exponential amplification of the product.
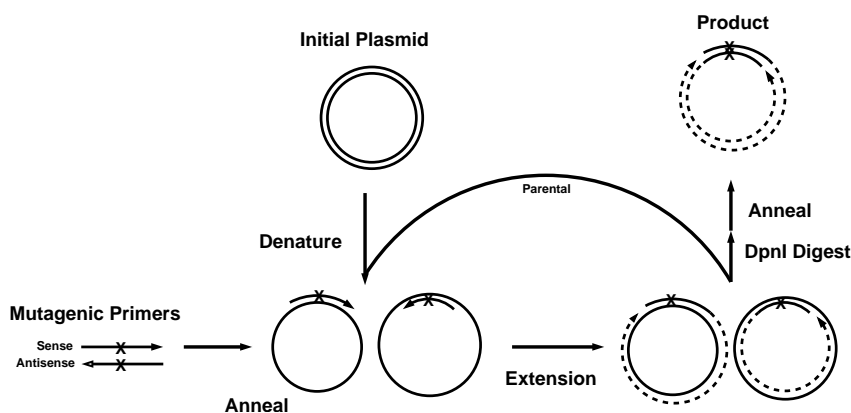
Following the reaction, the product is digested with DpnI. This digest is crucial. DpnI only cleaves at methylated sites, so it chews up the template plasmid but not the PCR product. Since the transformation efficiency of the circular template plasmid is several orders of magnitude better than the linear PCR product, without the DpnI digest a large number of your colonies would be the parental. Note that this means that the template plasmid cannot come from a methylation deficient strain (*e.g.* JM101).

A high fidelity polymerase is also crucial. Remember, the PCR reaction goes around the entire plasmid, so you need to minimize the chances of introducing unwanted mutations in both your gene and the backbone. A "hot start" formulation of the enzyme is desirable, as the proof-reading capability of most of these enzymes may otherwise degrade the primers during setup.

Oligos For oligo-design, you can follow the protocol in the QuikChange Manual, which basically says to design a primer with a T_m of at least 78 °C and center the mutation in the middle. This works well for single amino acid changes. An automated web site to help do this is available at: `http://bioinformatics.org/primerx`. For multiple amino acid changes and other more complicated primers, please see Zheng *et al.* [1].

The Stratagene protocol specifies that the oligos need to be PAGE purified, although most people use cartridge purified or even unpurified primers without any issues.

Mix

0.5 μl	forward primer, 2.5 pmoles/μl
0.5 μl	reverse primer, 2.5 pmoles/μl
0.25 μl	40 mM dNTP mix, (10 mM each)
1.25 μl	10x PfuUltra Buffer (contains Mg^{++})
1 μl	Template DNA, 2 ng/μl
0.25 μl	PfuUltra Hotstart (Stratagene)
8.75 μl	sterile H$_2$O
12.5 μl	total

PCR Program

1. 5 min @ 95°C
2. Repeat 18x
    (a) 50 s @ 95°C
    (b) 50 s @ 60°C
    (c) 1 min+1 min/1 kb template @ 68°C
3. 7 min @ 68°C

Gel Check Run 2.5 μl of the reaction on a gel. There should be a band corresponding to your product. Even if you don't see a reaction product, you can still try the rest of the protocol, but you may not get any colonies.

For those reactions that failed, if you see a strong primer dimer band it means that primer-primer annealing is favored over primer-template annealing. You can try reducing the concentration of primer next time, or redesign your primers.

DpnI Digest Add 0.25 μl of DpnI (20 U/μl, New England Biolabs) to the reaction. Incubate at 37°C for 1 hr.

Transformation Transform the final reaction into competent cells. I generally transform 1μl into 20μl of "XL-1 Blue Competent" cell's.

Final Pick a colony, miniprep, and sequence to check for your mutation and any PCR introduced errors.

Trouble Shooting

If no product is seen, try repeating the protocol with 5% DMSO in the reaction mix. DMSO disrupts base pairing, facilitating strand separation in GC rich regions of DNA and reducing the propensity of the DNA to form secondary structure. The end effect, is a little DMSO will often get you past issues with poor primer design and/or difficult templates.

References

[1] L Zheng, U Baumann, and JL Reymond. An efficient one-step site-directed and site-saturation mutagenesis protocol. *Nucl. Acids Res.*, 32:e115, 2004.