Site Directed Mutagenesis Protocol

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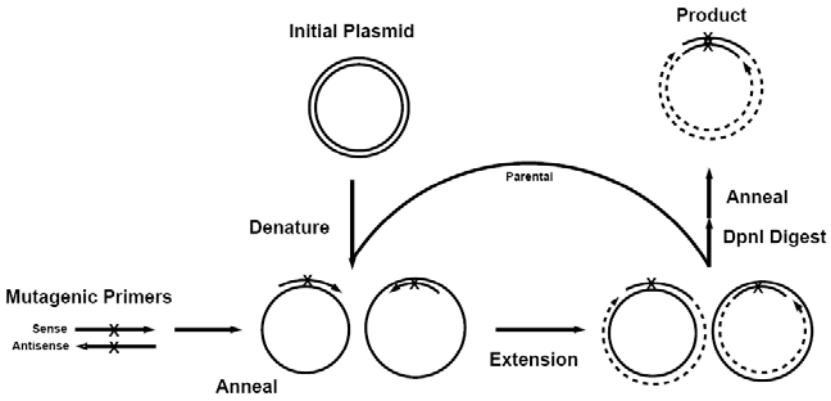
Background: This protocol is based on a combination of the Stratagene Quikchange protocol and information gleaned from the web. The important thing to keep in mind is that the concentration of primer versus template determines whether primer-primer or template-primer interaction dominates. If a reaction 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 means 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 colonies would be the parental. Note that this means that the template plasmid cannot come from a methylation deficient strain.

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, follow the protocol in the QuikChange Manual

Mix

10X pfx buffer: 5µl

2.5mM dNTPs mix: 7µl

50mM MgSO4: 1µl

Pfx Enzyme: 1µl

Primers: .125ng+125ng

Template DNA: 5-50ng

H2O: up to 50µl

PCR Program

1. 95C: 30 seconds

2. Repeat 15-18x

(a) 95C: 30 seconds

(b) 55C: 1 min

(c) 68C: 1min/kb of plasmid length

Gel Check: Run 5µl 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 37C for at least 1 hr.

Transformation: Transform the final reaction into competent cells.

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 5-10% 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.