**Frequently Asked Questions**

**Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit [PN 78480]**

1. **What types of mutations can be created?**
   The Change-IT Multiple Mutation Site Directed Mutagenesis Kit can be used to create single or multiple base changes, insertions, and deletions.

2. **Why do 5’-phosphorylated oligonucleotides need to be used?**
   The PCR amplification reaction includes a ligation step after each cycle. The DNA ends must be phosphorylated for ligation to occur.

3. **Can more than one mutation be created at a time with a single oligonucleotide?**
   A single oligonucleotide can code for more than one base change as long as both ends of the oligonucleotide are sufficiently extended to anneal to the plasmid template during PCR amplification.

4. **How are multiple mutations created at points in my template that are too distant to be spanned by a single oligonucleotide?**
   Design a single oligonucleotide for each mutation desired and perform PCR as usual using all of the mutagenic oligonucleotides as well as the common primer. If creating two mutations, it is recommended that the mutagenic oligonucleotides anneal to opposite strands of the plasmid and that the common primer be omitted. For more than two mutagenic primers, the additional primers can anneal to either strand of the plasmid.

5. **Can a mutation be created in a 15 kb plasmid?**
   Yes, but it may be necessary to double the volume of Change-IT enzyme in the reaction and/or to increase the mass of template in the reaction, and/or to perform two sequential Dpn I digests to reduce the background colonies.

6. **Will the PCR reaction work with the low mass of plasmid template recommended for the reaction?**
   For plasmid sizes from 3 to 10 kb, the masses suggested have been optimized for amplification yield and for efficacy of Dpn I digestion. Increasing the template mass will increase the yield but will also increase the background colonies.

7. **Does a DNA column cleanup procedure need to be performed, as some other mutagenesis kits require?**
   No. DNA cleanup is neither required nor recommended at any point in the procedure.

8. **Will this kit generate a linear or an exponential amplification of the mutated product?**
   Unlike some kits, the Change-IT Kit generates an exponentially amplified mass of mutated product, allowing 75% to 98% of the colonies to harbor plasmid bearing the desired mutations.

9. **Will the Change-IT Kit generate a linear product that then must be circularized by the bacteria after transformation, like some mutagenesis kits?**
   No, the product of the Change-IT Kit reaction is a circular, replication-competent plasmid. This allows a much greater transformation efficiency, and consequently, more bacterial colonies harboring plasmid with the desired mutations(s) than would be obtained by transforming linear DNA.
10. Why are there no primer-dimers in my reaction?

Unlike some kits, the primers in the Change-IT Kit do not overlap, so primer-dimers do not form.

11. I ran my reaction on an agarose gel but was unable to see a product band. Should I continue?

Yes. The product is a circular dsDNA and so does not run as expected for a linear dsDNA. Also, the circular dsDNA product can be obscured by side reaction products and template DNA, neither of which will survive the complete mutagenesis procedure.