TagMaster Site-Directed Mutagenesis Kit
TROUBLESHOOTING

Variations in the DNA sequence composition, the length of the plasmid template, the mutation type, and the PCR machine performance may contribute to differences in the mutagenesis efficiency. The following guidelines are provided for troubleshooting these variations.

Observation	Suggestions
Absence of PCR product on agrose gel	Although it is not part of the standard protocol, some researchers choose to verify amplification by gel electrophoresis (typically, 10 µl of the PCR product is analyzed on a 1% agarose gel). While a positive result verifies successful synthesis, a negative result does not indicate failure of the reaction. Successful mutagenesis may or may not result from a visible band of mutagenesis reaction product. In some cases, even multiple bands or faint bands or smear bands may give successful mutagenesis. We recommend proceeding transformation when gel electrophoresis analysis yields negative results. Multiple Sites Mutation will give a strong shorter band that is the amplified fragment between the two primers. It is not necessary to remove it before performing transformation.
No colony obtained for sample reaction	If possible, try not to do difficult mutations (Long-Range Mutation, Deletion of Long Fragment, and Multiple Site Mutation) in a large plasmid (>8kb). Difficult mutations in a large plasmid would decrease the efficiency synergistically. Do difficult mutation in a short plasmid (less than 8kb), or do a Point Mutation in a large plasmid.
	For Point Mutation, increase the annealing temperature from 60°C to 68°C. Increase the amount of the plasmid template to 100ng would also be helpful in some cases.
	Some plasmid template (vector backbone and/or the gene insert) forms strong secondary structure, which would prevent successful mutagenesis reaction. Try to use a different vector backbone.
	Ensure the plasmid template in good purity and quantity. Too many nicked or linearized plasmid DNA will lead to failure of the mutagenesis reaction. Verify that the template DNA is at least 80% supercoiled by run the plasmid on a gel.
	If using a mineral oil overlay during thermal cycling, ensure that excess mineral oil is not transferred into the transformation reaction. Using the smallest pipet tips available, insert the pipet tip completely below the mineral layer overlay to collect the sample.
	Confirm the LB plate with the correct antibiotics.
Low colony number for sample reaction	When performing difficult mutations (Long-Range Mutation, Deletion of Long Fragment, and Multiple Site Mutation) or performing reaction for a large plasmid, it is common to observe low numbers of colonies. Most of the colonies, however, will contain desired mutagenized plasmid.
Low colony number with control reaction	Three control reactions are suggested in the kit: negative control, 2bp mutation control, 6His insertion control. The negative control is expected to give only a few colonies. The 2bp mutation control and 6His insertion control are expected to give hundreds of colonies.
	Ensure the TagMaster Competent cells are stored at -80°C immediately upon arrival. Thaw the competent cell only one time, prepare single-use aliquote and store the aliquots at -80°C. Aoid multiple freeze-thaw cycles.
	Avoid multiple freeze-thaw cycles for the dNTP mixture. Thaw the dNTP only one time, prepare single-use aliquote and store the aliquots at -20°C.

	Different PCR machine may contribute to variations in amplification efficiencies. Optimize the cycling parameters for the control reaction and then repeat the protocol for the sample reactions using the adjusted parameters.
Erroneous mutation in the primer region	Poor quality primers can lead to erroneous mutations. Pick and screen more colonies for the desired mutation.