I have found that this works much better when introduced via conjugation rather than natural transformation. I am not sure why. Transformation should still work though.

Below is a sample timeline for conjugation into Synechococcus 2973. Obviously the timeline will change depending on growth rate of your strain.

**Day 1:** Conjugate editing plasmid into cyanobacteria. We use a triparental mating system with HB101 pRL443 and HB101 pRL623. Filters are transferred onto antibiotics after 6 hours. We use at least 150uE light for the whole process.

**Day 3:** Pick colonies and patch onto BG11 Km10.

**Day 5:** At this point 10-20% of the colonies are edited. It is easy to identify edited colonies with PCR between a region on the chromosome outside of what is on the editing template and the insertion, or upstream of the deletion. Point mutations must be sequenced.

Alternatively, restreak onto BG11Km10 to allow editing of more colonies to occur. I restreak 3 times so that most of the colonies become edited and I do not have to do much sequencing.

**Day 7:** Restreak onto BG11Km10 to allow more colonies to achieve editing.

**Day 9:** Restreak onto BG11Km10 to allow more colonies to achieve editing.

**Day 11:** Check more colonies with PCR or sequencing as required. By this point most of the patches are usually edited.

**Day 12:** Start 25mL of a verified colony in BG11 w/o antibiotics to cure it of the editing plasmid. Grow to stationary phase.

**Day 15:** Plate dilutions on BG11 to obtain single colonies.

**Day 16**: Pick 100 colonies and patch onto BG11 Km10 THEN to BG11. This will identify kanamycin sensitive colonies that have been cured of the editing plasmid for a markerless mutation. I usually sequence one last time for a final verification.