Back mutation using Gibson Assembly

We tried to reverse mutations using the Gibson Assembly technique.

1. First the majority of the plasmid must be PCR amplified. The mutation site, however, cannot be in this fragment.
2. Oligos (little strands of synthesised DNA: nearly 50 bp long) were designed. An oligo is made like a primer and is single stranded, so two oligos have to be melted together to become a double stranded DNA fragment (also done in a PCR machine: 5 minutes at 95°C and then at 50°C).
3. The melted oligo fragment has the correct, missing sequence of the amplified plasmid fragment. The plasmid fragment and the melted oligo fragment are added to the Gibson mix. The two fragments will overlap and form the right plasmid, devoid of the unwanted mutation.