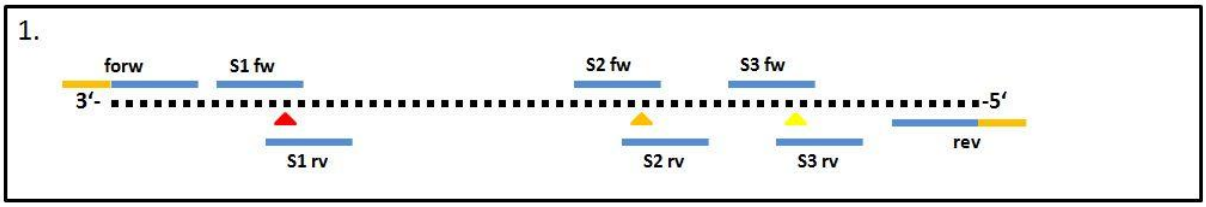
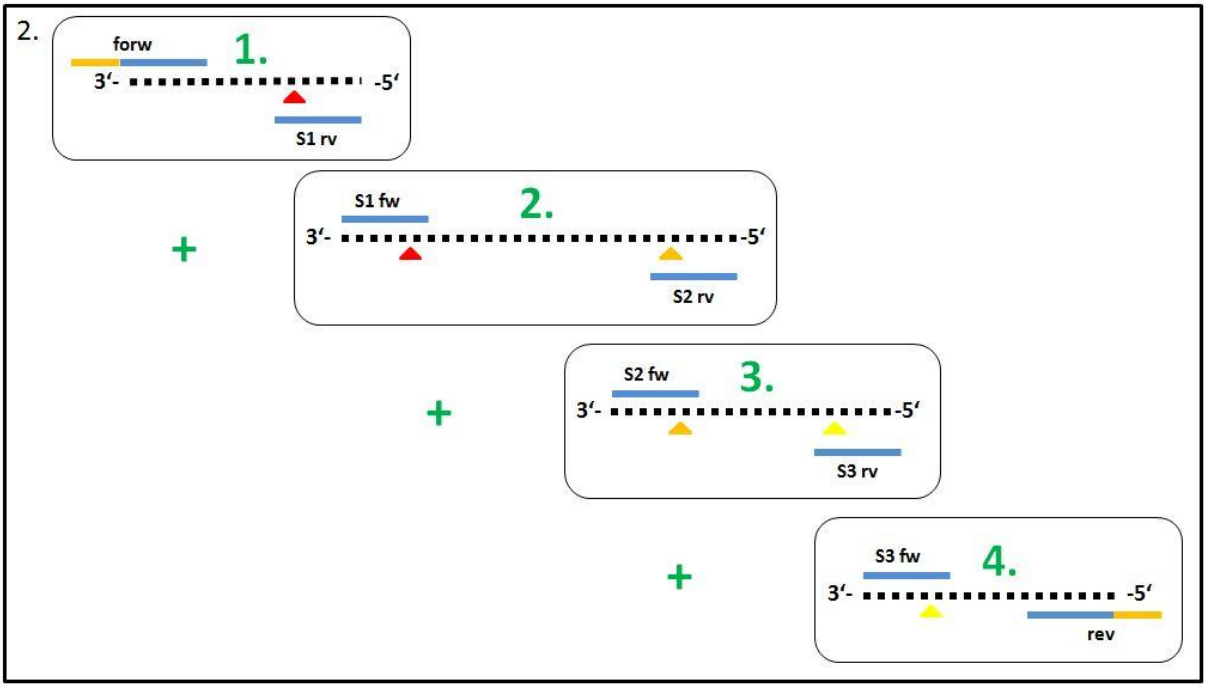
Overlap PCR for removal of several restriction sites

If you want to apply a gene for your iGEM project it might have several forbidden restriction sites like PstI, EcoRI, XbaI or SpeI. It can be very time-consuming to mutate all these in single steps. We applied overlap PCR to get rid of several restriction sites within one gene in just a few hours.

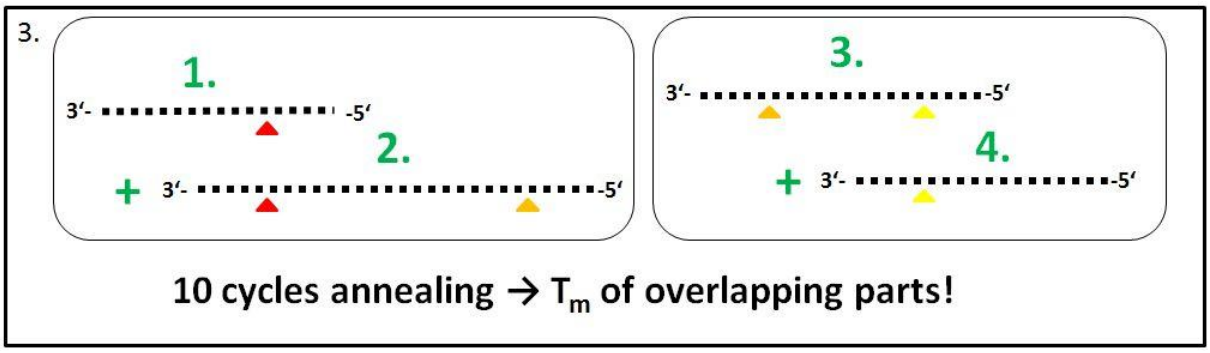


First, you will need two BioBrick-primers, the forward primer with a prefix) and the reverse primer with a suffix (Prefix and suffix are illustrated in orange Fig. 1). Furthermore, you need a forward and reverse primer with approximately 20 bases that overlap for each site to be mutated. In this example, the sites are marked with a red, orange and yellow triangle. **Be careful not to alter the codon usage in your gene!**

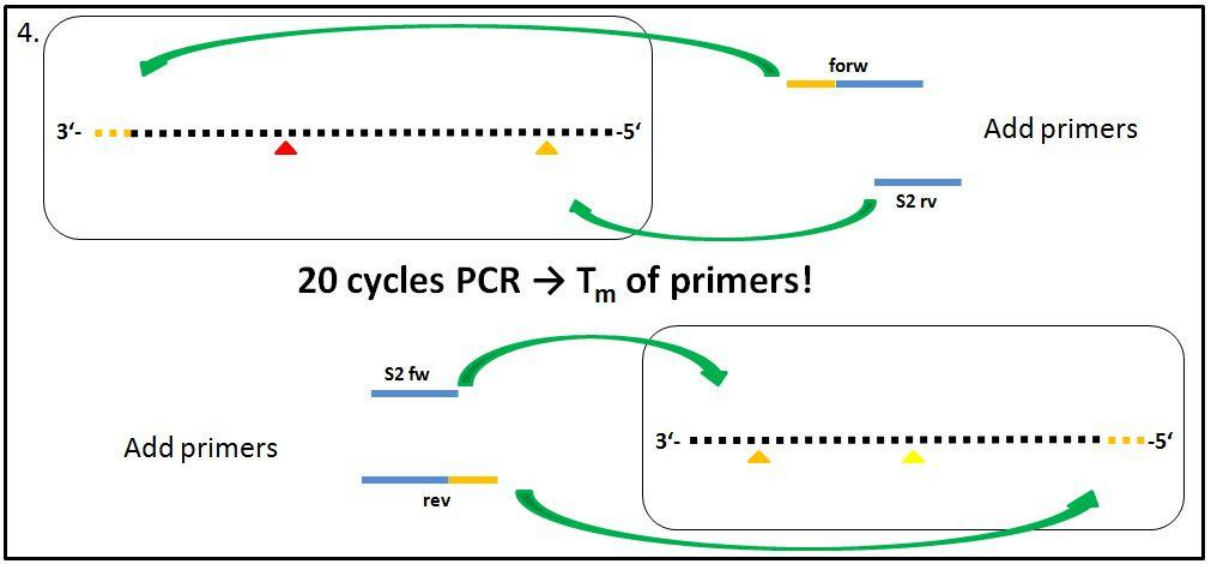
As a first step you amplify different parts of your gene via PCR using adjacent forward and reverse primers (Fig. 2). Of course the number of these fragments varies depending on how many sites you need to mutate. Purify your PCR products.



Your PCR products will now have overlapping ends. As a next step, you anneal two adjacent PCR-products to each other. You mix your PCR reaction as usual but exclude the primers. **It is crucial that you set the annealing temperature according to the T_m of the overlapping parts**. The rest of the PCR program is just as you would use it for amplification of the DNA that concludes the two fragments (In this case a PCR for 1+2 and a PCR for 3+4). Of course, the exact program depends on the polymerase (e.g. Phusion). Then you start the PCR and wait until ten cycles are completed.



Next, the two primers that flank your annealed gene part are added to the reaction (Fig. 4). These are in this example the forward primer of PCR product 1 and reverse primer of PCR product 2 and accordingly for 3 and 4. You may now change the annealing temperature according to the T_m of your primers. (Note: BioBrick prefix and suffix are now added to your PCR products so the T_m will be increased for the outermost primers). Then your PCR continues just like before for approximately 20 cycles. Purify the PCR products.



Now, in principle you just need to repeat the previous steps as shown in Fig. 5. In this case the adjacent PCR products from the previous PCR are annealed again and then amplified which yields the complete gene with mutated restriction sites and BioBrick prefix and suffix. This part can now be used for BioBrick construction!