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Primer Design Guide for Gene Integration in the Genome: by Daniel

We want two genes with the **same promoter and terminator,** we'll target both, and they will replace each other (antibiotics) as primers.

Daniel made a map of a specific location in the genome, the specific location is not that important, it just needs to not disrupt any gene, so between two genes, two antibiotic resistances (hyg and nat).

We'll work with two plasmids: Pag25 and Pag32. They are sourced from fungi, so there's no risk of homology.

1. We want to make a map of the source and what we want the destination to look like. So, the specific target sequence needs to be copied and pasted at the target
   location. Make sure the switch targets are identical.
2. So, there are two parts of the primer for both cases: 5' (tails) does not sit on the target, and 3' does fit perfectly to the gene. We add the homologous part of the target location (genome) to the tails, and that will make the recombination possible.
3. When looking at the primer, we need to make sure the GC content (50%, a bit flexible. In that case, we will delete the problematic tips, because they are probably not functional. If the sequences were deleted, make sure to delete them in all maps. DNA polymerase sits on the 3' of the primer, so the 3' needs to be with either G or C. We take about 20-25 bp.
4. Copy the sequence to the primer table. Add the length and the Tm of the complementary regions from Benchling. Add the homologs' part, 50+ bp, most companies will print a maximum of 80bp. This part needs to happen for the F and R primers.
5. For the R primer- take the complementary by copying: **right click-special-copy reverse complement**.
6. Add the length and the Tm of the whole primer from Benchling. The name should be appropriate to the target source location and direction.
7. Search the sequences from the table in the sources (plasmids) and the final target map (genome)
8. Make primers for PCR validation as well (in addition to antibiotic selection). The primers for validation need to target before (for the F primer), and after (for the R primer), so that in the validation PCR, we can see the whole length of the gene. The target primers should not be too close to the inserted gene (100-200bp away), preferably inside a gene, so it will be conserved. The sequence for the primers should end with C or G, about Tm 58°C, 20-25bp length, 50% GC, no repeat regions.
9. Copy these primers to the Primers Table, call them (val)idation, and their location in the target genome (after the insertion).