TAR – GFP

Goal:

The goal of this experiment is to observe if the cloned TAR and the “enhanced” TARs that we engineer reach the membrane and cross it in the correct matter leading to the chemotactic motion of the bacteria.

Fusing GFP to TAR might be complicated due to the size of TAR and GFP proteins. Moreover, the use of linker might be necessary to achieve right structure. A considerable amount and types of linkers is described in the literature providing different properties, for example rigid and fixable linkers. And each protein requires different properties form the linker.

Designed experiment:

Steps:

Open the plasmid containing the TAR insert at the C terminus of TAR and using Gibson assembly to introduce the GFP at that end.

In order to achieve a TAR fused to a GFP the following primers where designed to be used in a Gibson assembly:

Primers for the backbone.

Primer name: pOpen_P-R-T_for
Seq: tactagagccaggcatcaaataaaacg

This primer is designed to open the backbone of the pSB1A3 right after the TAR seq ends (3’ of TAR).

Primer name: TAR_REV_GFP_OP
Seq: ctcctttacgcatgctaccgctgccgcttccaaatgtttcccagtttgg

This primer is deigned to open the backbone of the pSB1A3 starting at the end of the TAR and going the reverse way while removing the stop codon and also introducing a new sequence that will act as a linker.

Both these primers are supposed to open the plasmid containing the TAR while adding the linker sequence at the 3’ of the TAR.

An illustration of where these primers bind can and the PCR product can be seen below in image 1 and 2 respectively.

The linker sequence will be: Ggaagcggcagcggtagc
Which translates to: GSGSGS

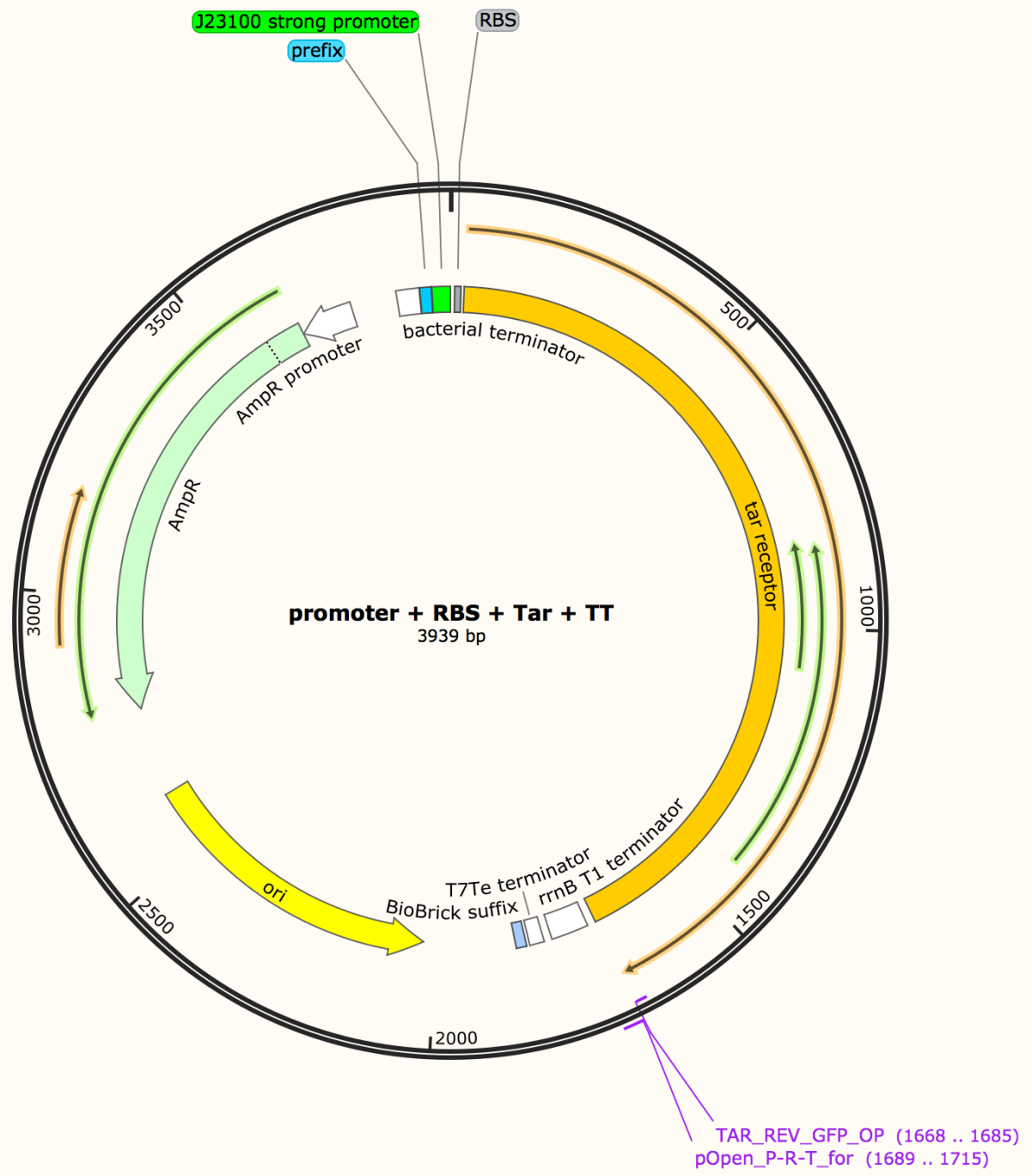

Image 1: binding sites for: pOpen_P-R-T_for and TAR_REV_GFP_OP

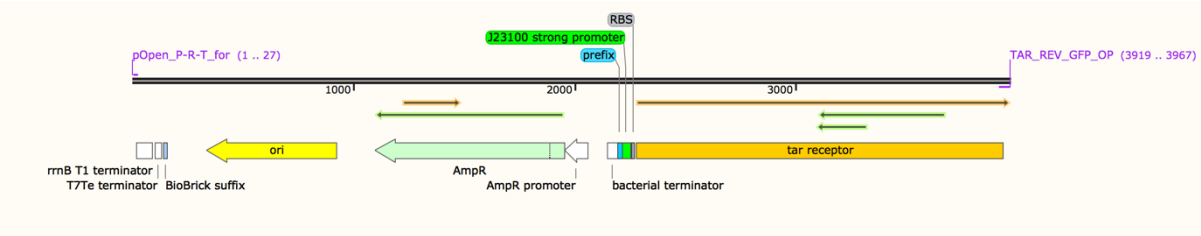

Image 2: PCR product for backbone.

Primers for the GFP insert.

Primer name: GFP_OVERHANG_FOR
Seq: gaagcggcagcggtagcatgcgtaaaggagaagaac

This primer is designed to amplify and extract the GFP (E0040 part) from the plasmid provided in the iGEM kit while also introducing an overlap to the C terminus and linker of the TAR.

Primer name: GFP_OVERHANG_REV
Seq: cgttttatttgatgcctggctctagtattatttgtatagttcatccatgc

This primer is designed to amplify and extract the GFP from the plasmid provided in the iGEM kit while also introducing an overlap plasmid.

An illustration of where these primers bind can and the PCR product can be seen below in image 3 and 4 respectively.

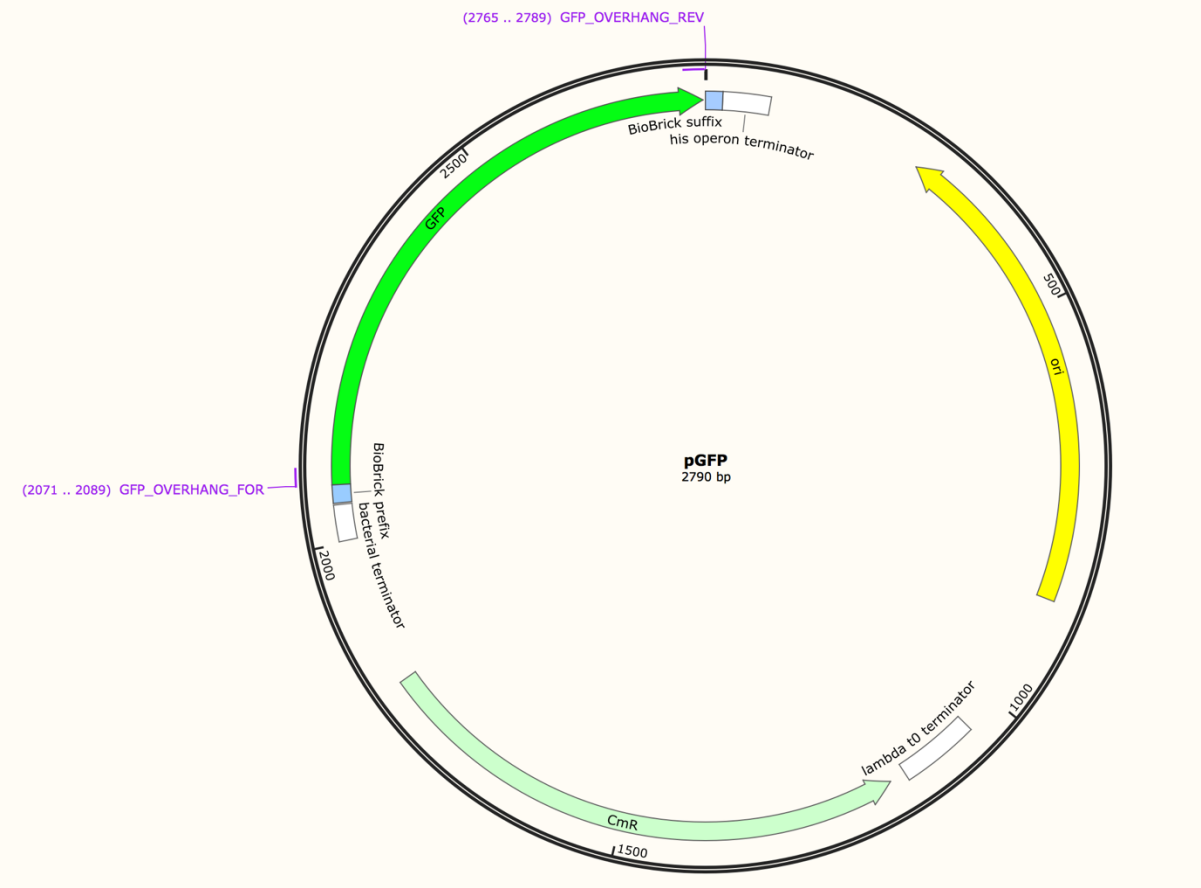

Image 3: binding sites for: GFP_OVERHANG_REV and GFP_OVERHANG_FOR

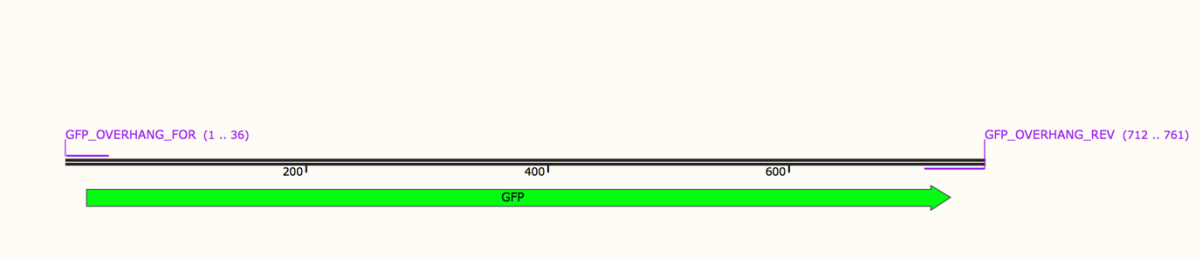

Image 4: PCR product for backbone.

Results:

After amplifying the insert (GFP) and the backbone (plasmid containing TAR) with the designed primers followed by a Gibson assembly the final product should be as described in image 5 as seen below.

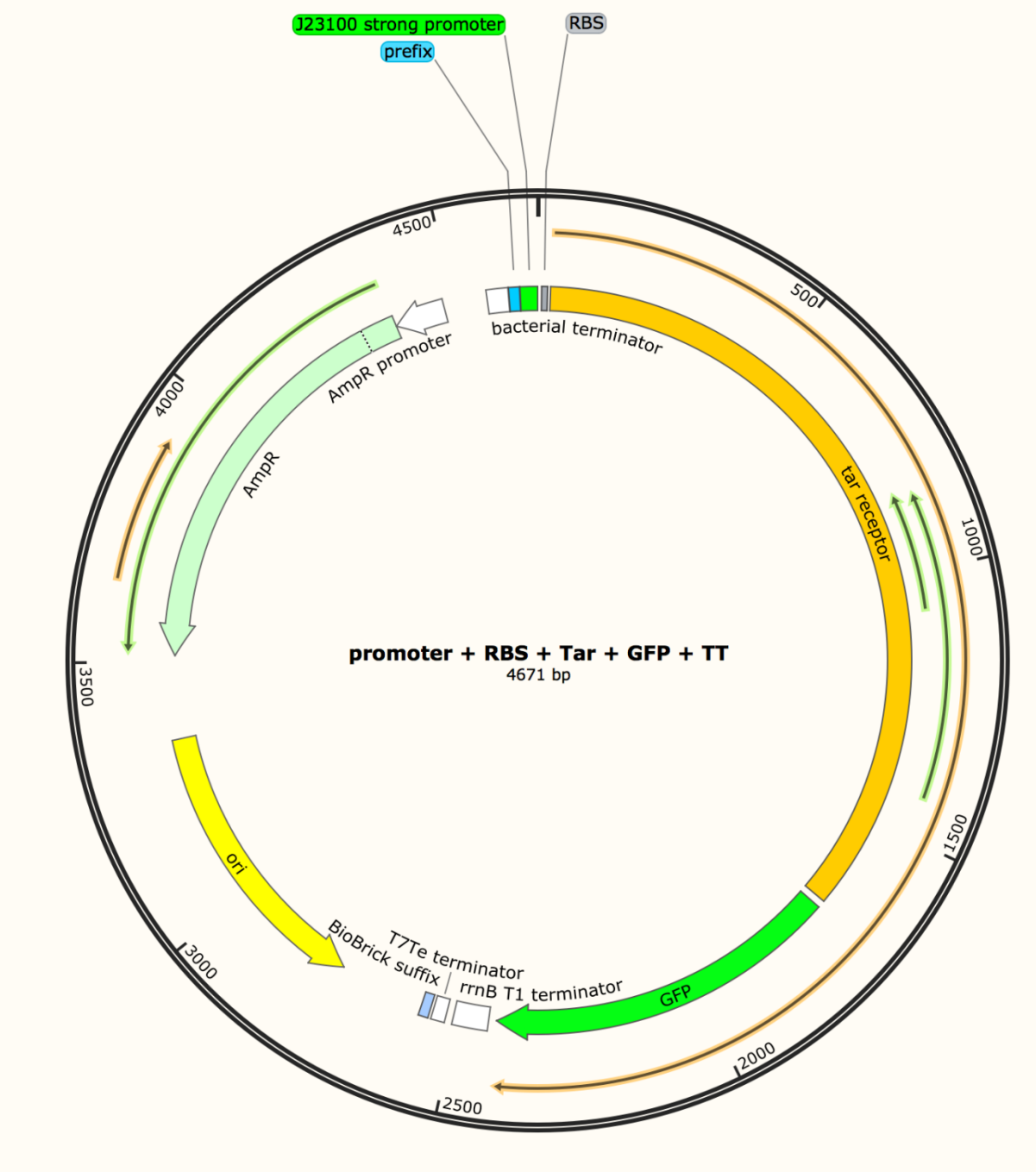

Image 5: Product of the Gibson assembly of the amplified parts.

Feel free to ask questions, the DNA files are attached to the mail so you can see it better for yourself.

Thanks!
Technion Team.