Plasmid DNAs designed for expression in Micromonas CCMP1545

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Abstract

We have available several plasmids designed for expression of Cas9, guide RNA, chloramphenicol acetyl-transferase, GFP, and beta-lactamase in Micromonas CCMP1545. For protein expression we used the promotor and 3' end elements from the endogenous RPS9 gene, and codon optimized the coding region. For expression of guide RNAs we used the Micromonas U6 snRNA promoter. DNA is available by contacting M. Ares <ares@ucsc.edu>.

We have constructed and sequence verified 9 plasmids which we would like to make available to others attempting to detect transformation of DNA into Micromonas. Using the CCMP1545 genome as a source for the U6 promoter sequence, and for the promoter and 3' UTR sequences of ribosomal protein RPS9, we built the following plasmids:

CRISPR/Cas9 plasmids for Micromonas
1. Mp U6 promoter driving Bae cassette for guide RNA expression in pUC13
2. Mp RPS9-Cas9SV40-RPS9 in pUC13
3. Both Mp U6 promoter driving Bae cassette for guide RNA expression and Mp RPS9-Cas9SV40-RPS9 in pUC13

These first three plasmids were anticipated to enable stable incorporation of transgenes at specific genomic locations. BaeI is a type IIS restriction enzyme that leaves noncompatible sticky ends. In the context of the gRNA cassette, a pair of 24 nt oligos designed to have the sticky ends compatible with BaeI cleaved plasmid are annealed and cloned into the plasmid, replacing the BaeI cassette while adding the 20 nt target complementary sequence of the desired guide RNA.

Selectable/Detectable Marker genes
4. Mp RPS9-codon optimized GFPsv40-RPS9 in pUC13
5. Mp RPS9-codon optimized chloramphenicol acetyltransferase-RPS9 in pUC13
6. Mp RPS9-codon optimized beta-lactamase-RPS9 in pUC13

These three plasmids have the indicated coding regions codon optimized for Micromonas flanked by RPS9 promoter and 3' end sequences. In the case of GFP, a nuclear localization signal from SV40 has been added to the C-terminus.

Plasmids for Agrobacterium-mediated gene transfer
7. Mp RPS9-codon optimized GFPsv40-RPS9 in pOSCAR
8. Mp RPS9-codon optimized chloramphenicol acetyltransferase-RPS9 in pOSCAR
9. Mp RPS9-codon optimized beta-lactamase-RPS9 in pOSCAR

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