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Seminavis robusta protocol collection V.2

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Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

This is a collection of media, reagents, and protocols for the cultivation and manipulation of the diatom *Seminavis robusta*. Our hope is that these documents will help future researchers develop genetic tools for studying this organism.

GUIDELINES

In our (unsuccessful) attempts to transform *S. robusta* to confer resistance to puromycin or glyphosate, we used the attached plasmid sequences: pBS_ActAgroCP4GlyphR (an *Agrobacterium tumefaciens* derived glyphosate resistance cassette flanked by *S. robusta* sequences upstream and downstream of its Actin 1 homolog), pBS_ActEF_AgroCP4GlyphR (an *Agrobacterium tumefaciens* derived glyphosate resistance cassette flanked by *S. robusta* Actin 1 5' region upstream and Elongation Factor 1 3' downstream), and pBS_Act_PurR (a puromycin acetyltransferase glanked by *S. robusta* sequences upstream and downstream of Actin 1). All the plasmids used a pBluescript II SK (+) backbone and the resistance gene cassettes were codon optimized for the *S. robusta* genome.

☐ **pBS_Act_AgroCP4GlyphR.gb**   ☐ **pBS_Act_PurR.gb**   ☐ **pBS_ActEF_AgroCP4GlyphR.gb**

Collection protocols