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Media and reagents for *Seminavis robusta* cultivation and experiments

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

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ABSTRACT

This protocol describes the culturing conditions used to grow *S. robusta* in the lab for all experiments.

GUIDELINES

It matters whether you use NanoPure/MilliQ water or glass distilled water. In glass distilled water-based media, *S. robusta* tends to disadhere from the bottom of the culture flask and float more readily than in MilliQ-based media.

MATERIALS

NAME	CATALOG #	VENDOR
Bioactif artificial sea salt	10304	Tropic Marin
Guillard's (F/2) Marine Water Enrichment Solution	G9903-500ML	Sigma Aldrich
Sodium bicarbonate	S6014	Sigma Aldrich
Penicillin G sodium salt	P3032-25MU	Sigma Aldrich
Gentamycin sulfate	345814-1GM-M	Sigma Aldrich
Ampicillin sodium salt	BP1760-25	Fisher Scientific
Streptomycin sulfate	100556	MP Biologicals
Agar	BP1423-500	Fisher Scientific
175 cm2 flasks with sealing caps	156502	Thermo Scientific
25 cm2 flasks with sealing caps	CC7682-4325	USA Scientific
Stericup	SCGPT05RE	Millipore
Cell Scraper	08-100-241	Fisher Scientific
D-sorbitol	M4125	Sigma Aldrich
Mannitol	S1876	Sigma Aldrich

Preparation of F/2 medium

1 Dissolve **34.5 g Tropic-Marin Bioactif (TMB)** in **1 L MilliQ water**

2 Add 80 mg **sodium bicarbonate**

3 Autoclave

4 After cooling, add 20 ml **50x Guillard's supplement**

5 Filter using Stericup into pre-sterilized bottles

6 Store at 4C in the dark

Maintenance antibiotics (200x stock)

7 Dissolve 1 g **penicillin G sodium salt**, 1 g **ampicillin sodium salt**, 0.2 g **streptomycin sulfate**, and 0.1 g **gentamycin sulfate** in a final volume of 20 ml **F/2 medium**.

8 Aliquot and store at -20 C.

Agar plates

9 Dissolve 17.25 g **TMB** in 1 L **MilliQ**

10 Add 80 mg **sodium bicarbonate**

11 Add 0 µl 15 g **agar**, dissolve while stirring and heating (may require boiling)

12 Autoclave

13 After cooling below 60 C, add 20 ml **50x Guillard's supplement**, as well as selective antibiotics, if desired.

14 Pour approx. 19 ml per plate

15 Store in the dark at 4 C

Electroporation buffers

16 [M]1 Molarity (M) **sorbitol** in MilliQ, [M]0.77 Molarity (M) **mannitol** in MilliQ, or [M]0.77 Molarity (M) **mannitol** with [M]8 Volume Percent **F/2** in MilliQ.