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Biolistic transformation of Emiliania huxleyi

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Preparing tungsten beads

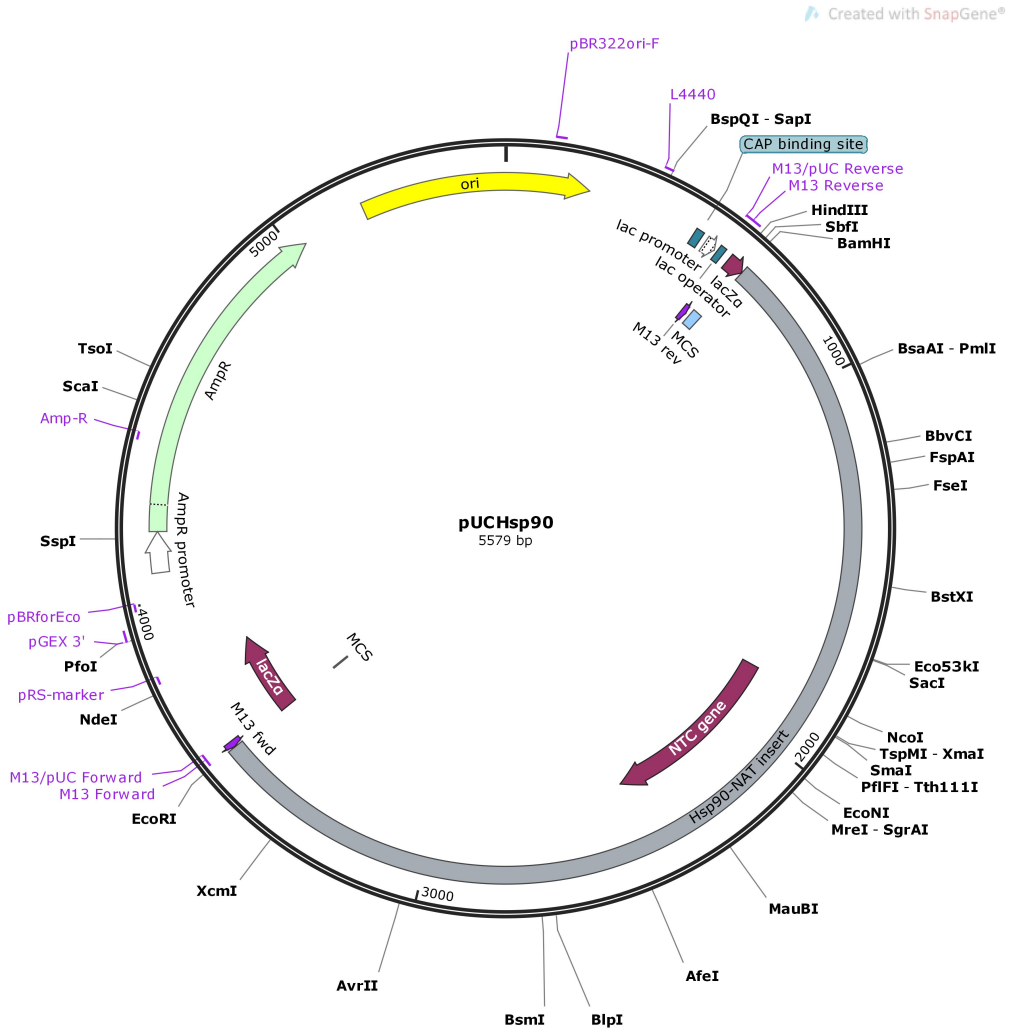
1 Weigh out 60 mg tungsten into a microfuge tube

2 Wash in 1 mL 100% ethanol. Vortex. Centrifugation at 13000rpm for 1 minute then remove ethanol

3 Wash four times in 1 mL molecular grade water. Centrifugation at 13000rpm for 1 minute. Remove the water.

4 Add 1mL of water to beads and transfer 50 µL aliquots (whilst vortexing frequently to avoid bead sedimentation) into microfuge tubes and freeze for later use.

5 Add 4 µg pUCHsp90 plasmid DNA (previously prepared using Qiagen miniprep) to a 50 µL aliquot of tungsten beads. The DNA volume added should not be more than 10 µL. pUCHsp90_plasmid.fa



pUCHsp90 contains a codon-optimised NAT gene for Nourseothricin resistance, flanked by promoter and terminator regions form the *E. huxleyi Hsp90* gene.

6 Add 50 µL of 2.5M CaCl2 and 20 µL of 0.1M spermidine to the tungsten and DNA and vortex gently

7 Leave the tube for 10 minutes to sediment the beads. Centrifuge briefly for 3 seconds at 2000rpm to sediment beads. Remove supernatant.

8 Wash beads in 250 µL of 100% molecular grade ethanol. Vortex and briefly centrifuge for 3 seconds at 2000rpm. Remove the supernatant.

9 Add 50 µL of 100% molecular grade ethanol. Whilst frequently vortexing to prevent sedimentation, add 10 µL aliquots to individual macrocarrier discs and leave to dry.

10 Store macrocarrier discs coated in DNA-tungsten beads inside a closed Petri dish lined with Whatmann filter paper at 4ºC until needed.

Biolistic transformation

11 Grow cultures of Emiliania for 4-7 days prior to transformation. Target cell density should be about 5 x10^5 - 1 x10^6 cells/mL

12 Clean biolistic PDS-1000/He particle delivery system (BIORAD, CA, USA). Wipe outside and inside of the biolistic chamber with ethanol. Immerse rupture discs, macrocarrier holders and stopping screens briefly in ethanol and leave to dry

13 Connect PDS-1000 apparatus to a helium supply and a vacuum pump

14 Set up biolistic apparatus according to manufacturers instructions.

14.1 Take out rupture disc retaining cap and place rupture disc inside and place assembly back inside

14.2 Assemble macrocarrier launch assembly. Place stopping screen onto shelf. Place macrocarrier disc in macrocarrier holder and position over stopping screen (with beads now facing downwards). Screw assembly together.

14.3 Place macrocarrier launch assembly onto the uppermost shelf of the biolistic chamber.

15 Prepare *E. huxleyi* cells immediately prior to loading to avoid drying out. Filter 150 mL of *E. huxleyi* (CCMP1516 from NCMA, USA) culture onto a 3 µm cellulose nitrate membrane filter (diameter 47 mm, Whatmann from GE Healthcare Life Sciences, UK)

16 Wash cells with 3-5 mL of biolistic loading buffer to remove excess salt. Leave filter damp so as not to dry the cells completely.

17 Transfer the filter containing cells to a small Petri dish (47 mm diameter).

18 Place the Petri dish onto the target plate shelf in the biolistic chamber 6cm below the macrocarrier launch assembly.

19 Perform biolistic transformation process

19.1 Turn on vacuum pump and biolistic chamber.

19.2 Remove the air in the biolistic chamber by setting the vacuum button to VAC until the pressure was 27 in.Hg.

19.3 Switch the vacuum button to hold.

19.4 Press and hold the FIRE button until the rupture disc bursts and beads have bombarded the cells.

19.5 Release the FIRE button and set the vacuum button to VENT to allow air into the chamber

20 Remove the Petri dish and replace its lid. Tungsten beads should be visible on the centre of the filter.

21 Wash the cells off the filter using 3 mL 50% salinity F/2 and place into a sterile cell culture dish.

22 Leave cells to recover overnight (16 h) in light chamber incubator

23 Count cells using a haemocytometer to determine the number of surviving cells post transformation (ideally should be similar to starting concentration). Subdivide cells into a 24 well plate, 10 µL of cells per well, and make the volume in each well up to 2 mL with 50% salinity F/2 media containing 80 µg/mL nourseothricin. Include control wells containing non-transformed cells in media with and without nourseothricin.

24 Observe cells each week to look at cell health (motility, cell integrity). Perform cell counts in each well.