Calcium phosphate transfection of Oxyrrhis marina with an Alexa488-labelled DNA fragment

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Abstract

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Protocol

Materials used:

Step 1.

-O marina cultures in F/2 medium supplemented with heat inactivated bacteria. The cultures were maintained with the heat inactivated bacteria supplanted every week with an extra addition an hour prior to the transfection. (It has not been tested yet if this addition one hour before the transfection is critical or not).

-ULYISIS Nucleic acid labeling kit (Molecular Probes, Cat # U21650). This kit is used to prepare the DNA and label it with a fluorophore. In our case, we used the ULYSIS Alexa 488 labeling kit.

-Transfection reagents

2.5 M CaCl2

2XHEBS (274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, 15 mM D-glucose, 42 mM HEPES (free acid), pH 7.10

HBS0/2: 144 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES pH 6.7 (alternatively other protocols use PBS pH 7.4). It was also possible to see the transfection with no washes.

1. Prepare DNA for labelling.

Step 2.

Use only fragments that are 100 bp to 1000 bp

Long DNA can be degraged with DNAse into fragments 100 to 1000 bp. The ULYSIS kit includes DNAse

and a protocol to produce fragments in that range. Alternative PCR products can be used. The labelling kit suggests not to use longer fragments to avoid precipitation of the labelled fragments.

For labeling 1 ug of DNA, cleaned PCR products or DNA fragments should be precipitated in 3M sodium acetate (pH5.2) and two volumes of absolute ethanol, freeze at -70 °C for 30 min and then for 15 min at 12,000 g. Wash the pellet with 70 % ethanol and allow it to air dry. Resuspend the pellet in 20 ul of the labeling buffer from the ULYSIS kit. Alternatively I have eluted the PCR products from the mini column in 30 ul of the labelling buffer form the ULYISIS kit.

2. Label the DNA

Step 3.

The following two steps were performed exactly as indicated in the instruction manual of the kit. It was up scaled to label 5 ug of DNA

- Denature the DNA at 95 °C for 5 min and then snap cool on ice. Centrifuge the tube briefly to redeposit the sample to the bottom of the tube
- Add 1 ul of Alexa fluo Ulysis reagent per ug of DNA
- Incubate at 80 °C for 15 min. Stop the reaction by placing the tube on ice. Centrifuge briefly

3. Purify the labelled DNA fr the free ULS labelling reagent

Step 4.

- Purify the product from the excess of free ULS labelling reagent using a gel filtration-based spin column. The instructions recommend Bio-Rad Micro Bio-Spin ®P-30 or Princeton Separations Centri-Sep. I used Zeba spin desalting columns (Zeba, Thermo Scientific, cat # 89882)

3.Transfection

Step 5.

-Prepare DNA/$CaCl_2$ mix for transfection of 1 ml of dense culture:

DNA	1-8 ug
2.5 M $CaCl_2$	5 ul
Nuclease free H_2O	to 50 ul final volume

If there is a desire to increase the amounts of DNA to 3-24 ug , the $CaCl_2$ should be increased to 15 ul and the final volume to 150 ml.

-Mix the DNA/$CaCl_2$ with equal volumes of HeBS. **IMPORTANT:** add 1/10th of the volumes of the DNA/$CaCl_2$ mix at a time mixing well by vortexing 3 s after each addition. This gradual mixing avoids

local over concentrations of $CaCl_2$, which creates an unevenly sized precipitate. Supposedly, only very fine precipitate is efficiently taken up by the cells.

-Incubate 20 min at RT

-Add 800-1000 ul of O marina culture

-Incubate at RT. Image at different time points. I saw the fluorescence after about 4-5 and collected images 24 h later.

-The efficiency seems to be 30-40 % but a lot of them show fluorescence in the nucleus, suggesting that this could be potentially an appropriate method to generate stable transfected O marina.

-There is a wash step in which the cells are supposed to be rinsed with HBS0/2 three times and then resuspended in their medium but I noticed a lot of cell damage with centrifugation so I skipped the washes and did a dilution in F/2 medium in half the first day.

This protocol was adapted from Matz J, Gilyan A, Kolar A, McCarvill T, Krueger SR. Rapid structural alterations of the active zone lead to sustained changes in neurotransmitter release. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107(19):8836-8841. doi:10.1073/pnas.0906087107.