AlgaeCeuticals

Development of microalgae-based natural UV Sunscreens and Proteins as cosmeceuticals and nutraceuticals

ALGAENET4AV

DNA EXTRACTION FROM MICROALGAE

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European Commission

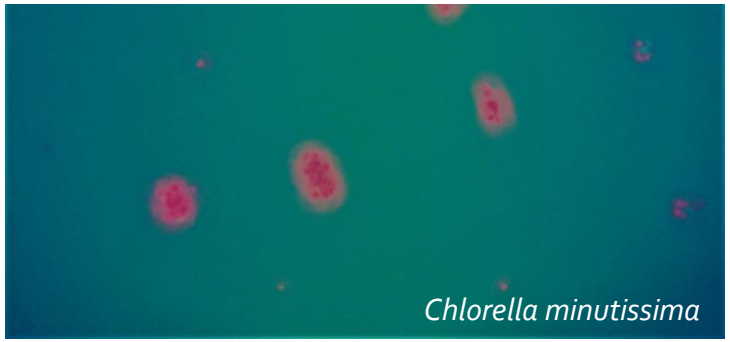
MARIE CURIE ACTIONS

CONTENT

- AlgaeCeuticals project
- Microalgae
- DNA extraction
  - Materials
  - Buffers
  - Method

- DNA quantitation and quality assessment

MICROALGAE



Genomic information/genetic code → DNA → gene → specific enzyme → specific reaction in the cell

- For several microalgae this genetic code, the genome, has become available
- However, we do not yet know the function of many genes. In **AlgaeCeuticals** we want to shed light into the genes activated under various different environmental conditions such as low temperature, low pH, nitrogen starvation etc
- We are using strains, such as *Nannochloropsis* sp, *Chlorella* sp., *Chlamydomonas* sp. and more

But before all that we need to grow our algae!

WHAT IS DNA EXTRACTION?

- Isolating DNA can be done by disrupting (breaking) cell wall/cell membrane and a nuclear membrane
- It is done with the use of chemicals, enzyme or physical disruptions
- Isolating nucleic acid is a critical first step for conducting further diverse molecular biological studies ranging from gene expression to gene evolution

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A number of protocols exist for isolating DNA from algae

(HwangBo *et al.*, 2010; Mosa *et al.*, 2018)

Homemade protocol

Commercial Kits

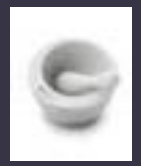
DNA EXTRACTION FROM ALGAE

The modified CTAB protocol by Doyle and Doyle (1987) requires:

Minimal lab materials

Fast (1 or 2 days) and effective

Ideal for isolation of genomic DNA from plant material and eukaryotic green algae



→ Grind into fine powder using Liquid Nitrogen

→ Grind with plastic pestle or add silica beads in lysis buffer

Due to the rigid nature of the algae cell wall

MATERIALS & REAGENTS

CTAB buffer
β- mercaptoethanol
Microcentrifuge tubes/pipets and tips
Mortar and Pestle/ plastic pestels
Liquid Nitrogen (if needed to be ground in fine powder)
Microcentrifuge
70 % Ethanol
3 M Sodium Acetate
65^{o} C water bath
Chloroform : Iso Amyl Alcohol (24:1)
1x TE
Agarose
10x Loading Buffer
1x TAE solution
Agarose gel electrophoresis system
Ethidium Bromide solution

STOCK BUFFERS

2xCTAB buffer 1000 ml
20 g CTAB (Hexadecyl trimethyl-ammonium bromide)
81.82 g NaCl
10 g PVP (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer))
100 ml 1 M Tris pH 8.0
40 ml 0.5 M EDTA pH 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)
Adjust the volume up to 1000 ml with H_2O

50x TAE buffer 1 L
242 g Tris base
750 ml distilled H_2O
57.1 g glacial acetic acid
100 ml of 0.5M EDTA (pH 8.0)
Top up to 1L with water
To make a 1x working solution, do a 1:50 dilution of the concentrated stock

1 M Tris pH 8.0
Dissolve 60.55 g of Tris base in 200 ml of H_2O
Adjust pH to 8.0 by adding approximately 40 ml of concentrated HCL
Adjust the volume to 500 ml with H_2O
Sterilize using an autoclave

PROCEDURE- DAY 1

Separating DNA from the other organelles

1. Homogenize 100 – 200 mg tissue in 800μL CTAB + 10 μL b-mercaptoethanol per sample, add 2-4 μL RNase of 10 μL/μL and vortex
2. Place samples in a waterbath at 65°C for 30 to 45 min
3. Add 800 μL chloroform/ isoamyl alcohol and shake up and down
4. Spin at 7,000 rpm for 20 min

2X

5. Take upper phase in new 2 mL tube
6. Transfer the upper phase into a 1.5 mL Eppendorf tubes

Precipitation of the DNA

7. Add 2:3 of the volume isopropanol and 1/10 Na acetate and mix well
8. Place at -20°C for 1 hour (*it is preferable to leave the samples overnight*)

PROCEDURE-DAY 2

Purification of extracted DNA

9. Spin at 10,000 rpm for 10min
10. Remove upper phase and wash with 1mL of 70% ethanol (+ shake)

} 2X

11. Spin at 16.000 rpm for 10 min
12. Remove upper phase and spin shortly
13. Remove remaining liquid if any and leave tube open for 5 min

Elute the DNA

14. Add required volume of 1x TE (usually 100μL), RNase 2-4μL of 10μg/μL stock and place at 37°C for 30 min to 1 hour
15. Dissolve the pellet (if any) by pipetting up and down

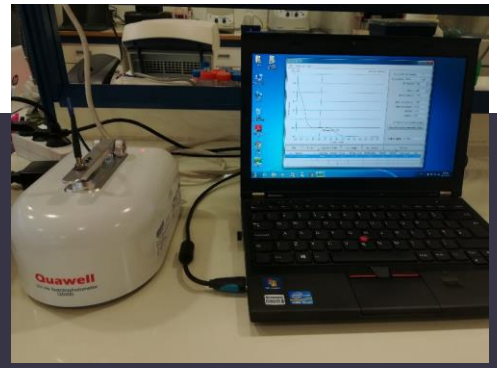
QUALITY & QUANTITY

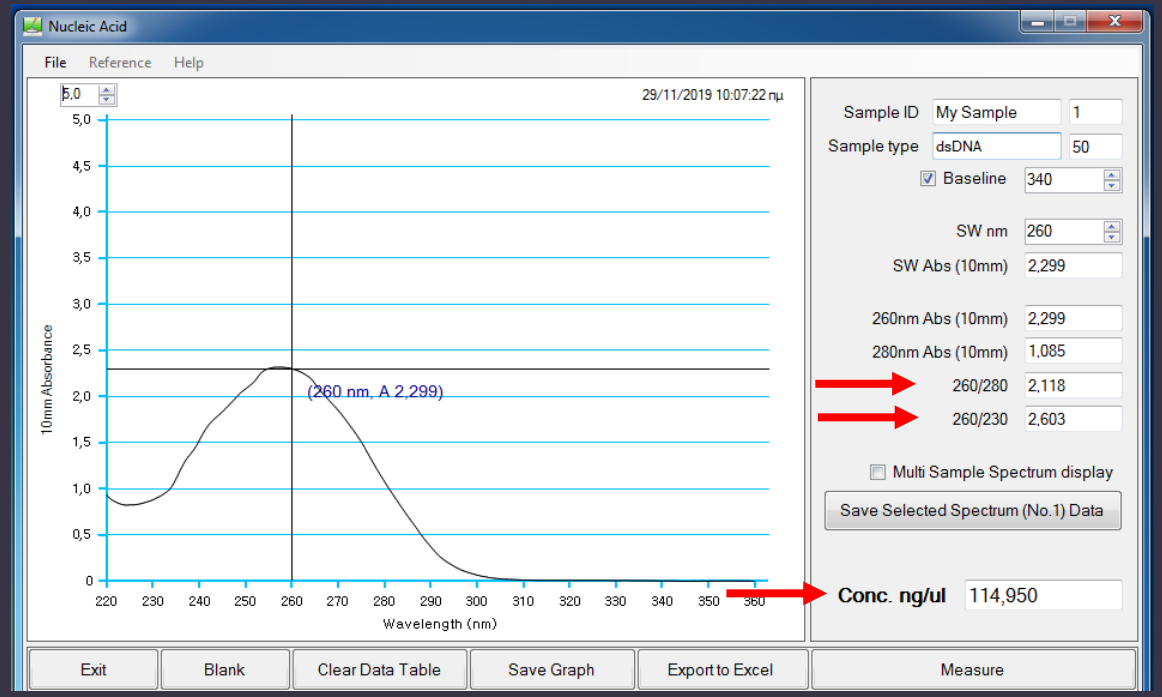
Quantitation with spectrophotometer

It is common for nucleic acid samples to be contaminated with other molecules (i.e. proteins, organic compounds, other)

- Absorbance ratio $A_{260/280}$ → determines sample purity from protein contamination (purity at ~1.8)

- Absorbance ratio $A_{260/230}$ → determines sample purity from contaminants which absorb at 230 nm (purity at ~ 2.0)





The concentration is calculated in ng/ μl

QUALITY & QUANTITY

Perform 1% gel electrophoresis (TAE) + 2 μL EtBr (in 80 mL –in respected proportions)

- Load 2 μL of DNA + 2 μL of loading buffer (mix on parafilm) + ladder (1 μL)
- Run at 100 V for 20 to 25 min