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Isolation of Genomic DNA from *Chlamydomonas reinhardtii*

Tyler M Wittkopp*

The Salk Institute for Biological Studies, La Jolla, CA, USA
*For correspondence: tylerwittkopp@gmail.com

[Abstract] *Chlamydomonas reinhardtii* is a soil-dwelling eukaryotic green alga that is widely used as a laboratory model organism for research on photosynthesis, ciliary biology, lipid metabolism and many other aspects of cell biology and physiology. With sequenced nuclear, chloroplast and mitochondrial genomes, *Chlamydomonas* is also an excellent organism for genetics and genomics research. This protocol describes the isolation of genomic DNA from *Chlamydomonas* using a standard phenol:chloroform extraction method followed by ethanol precipitation. The protocol requires minimal lab materials, takes approximately 4 h to complete, and can also be used for isolation of genomic DNA from other eukaryotic green algae.
Keywords: Algae, Genomic DNA, Phenol/chloroform extraction, Nucleic acid, DNA purification

[Background] Isolating nucleic acid is a critical first step for cloning and sequencing genetic material and provides the basis for diverse molecular biological studies ranging from gene expression to gene evolution. A number of protocols exist for isolating DNA from algae (Weeks *et al.*, 1986; Fawley and Fawley, 2004; HwangBo *et al.*, 2010). Generally, cells are pelleted by centrifugation and lysed in buffer containing detergents such as SDS to solubilize membranes. This is followed by at least one extraction in phenol:chloroform and at least one extraction in chloroform. In some cases, an RNase treatment step is performed to degrade RNA. DNA is then precipitated by addition of ethanol or isopropanol and incubating on ice or in the freezer. After pelleting and washing the precipitated DNA, it is usually resuspended in water or buffer (*e.g.*, Tris-EDTA) and quantified spectrophotometrically at 260 nm.

The protocol described herein makes use of two phenol/chloroform/isoamyl alcohol extractions and two chloroform/isoamyl extractions, with an RNase treatment step in between. The addition of isoamyl alcohol to the organic solvents prevents foaming and stabilizes the interphase, which contains a high concentration of coagulated proteins. Importantly, this protocol results in high quality genomic DNA that is suitable for downstream applications such as cloning and sequencing.

Materials and Reagents

1. Pipette tips
2. 1.5 ml microcentrifuge tubes
3. 50 ml centrifuge tubes
4. Kimwipe
5. RNase A (50 mg/ml) (Sigma-Aldrich, catalog number: R6513)

6. Phenol/chloroform/isoamyl alcohol pH 8.0 (25:24:1) (Sigma-Aldrich, catalog number: P2069)
   Notes:
   a. *Separates into two phases–use the bottom organic phase.*
   b. *Use caution when handling this solution; work in a chemical hood and wear gloves, eye protection.*
7. Chloroform/isoamyl alcohol (24:1) (Sigma-Aldrich, catalog number: 25666)
   Note: Use caution when handling this solution; work in a chemical hood and wear gloves, eye protection.
8. Absolute ethanol (Sigma-Aldrich, catalog number: 792780)
9. 70% ethanol
10. Nuclease-free water
11. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
12. Sodium dodecyl sulfate (SDS) (Sigma-Aldrich, catalog number: L5750)
13. Ethylenediaminetetraacetic acid, disodium salt, dihydrate ($EDTA{\cdot}Na_2{\cdot}2H_2O$) (Sigma-Aldrich, catalog number: E5134)
14. Ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}{\cdot}7H_2O$] (Sigma-Aldrich, catalog number: A7302)
15. Sodium selenite (Na_2SeO_3) (Sigma-Aldrich, catalog number: S5261)
16. Zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$) (Sigma-Aldrich, catalog number: Z4750)
17. Manganese(II) chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$) (Sigma-Aldrich, catalog number: M3634)
18. Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) (Sigma-Aldrich, catalog number: F2877)
19. Sodium carbonate (Na_2CO_3) (Sigma-Aldrich, catalog number: S7795)
20. Copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$) (Sigma-Aldrich, catalog number: C3279)
21. Tris base (Biopioneer, catalog number: C0060)
22. Ammonium chloride (NH_4Cl) (Sigma-Aldrich, catalog number: A4514)
23. Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) (Sigma-Aldrich, catalog number: C3306)
24. Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$) (Sigma-Aldrich, catalog number: 230391)
25. Potassium hydroxide (KOH) (Sigma-Aldrich, catalog number: 221473)
26. Potassium phosphate dibasic (K_2HPO_4) (Sigma-Aldrich, catalog number: P3786)
27. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P0662)
28. Glacial acetic acid (Fisher Scientific, catalog number: A38-212)
29. Hydrochloric acid (HCl) (Fisher Scientific, catalog number: A144-212)
30. DNA Extraction buffer (see Recipes)
31. Micronutrient stock solutions (see Recipes)
32. Stock solutions (see Recipes)
33. Tris-Acetate-Phosphate (TAP) growth medium (see Recipes)

Equipment

1. 250 ml culture flasks (*e.g.*, Corning, PYREX®, catalog number: 70980-250)
2. Pipettes
3. Hemacytometer (*e.g.*, Sigma-Aldrich, catalog number: Z359629) or automated cell counter (*e.g.*, Bio-Rad Laboratories, model: TC20™)
4. Basic light microscope (*e.g.*, Amscope, catalog number: B100B-MS)
5. Vortex mixer (*e.g.*, Scientific Industries, model: Vortex-Genie 2, catalog number: SI-0236)
6. Centrifuge (*e.g.*, Eppendorf, model: 5810 R) and microcentrifuge (*e.g.*, Eppendorf, model: 5424)
7. Freezer
8. Nano-Drop 1000/2000 spectrophotometer or Qubit 2.0 fluorometer
9. Optional: PCR thermal cycler
10. Autoclave

Procedure

1. Grow algae in 50-100 ml liquid medium (*e.g.*, TAP) until cells reach exponential or early stationary growth phase (typically 3-4 days from plate in TAP) (see Figure 1A).
   Notes:
   a. *For growth in TAP medium, a typical cell concentration for exponential or early stationary phase is 2-10 x 10^6 cells/ml. Cells can be counted using a hemacytometer or automated cell counter.*
   b. *Optional: Dilute into fresh medium the night before to ensure cells are in exponential phase.*
2. Pellet 25-50 ml cells in a 50 ml centrifuge tube by centrifugation (2,000-4,000 *x g*, 20 °C, 10 min) (see Figure 1B).
3. Discard supernatant (*i.e.*, growth medium) using a pipette.
   Note: The cell pellet can be stored at -80 °C if the experimenter wants to continue later.
4. Resuspend cell pellet in 400 µl DNA Extraction buffer by pipetting gently and transfer to a pre-labeled 1.5 ml microcentrifuge tube.
5. Into the same microcentrifuge tube, add 400 µl phenol/chloroform/isoamyl alcohol solution (from lower, organic phase) and vortex at the highest speed (setting 10 on the Vortex-Genie 2) for 1-2 min (see Figure 1C, Figures 2A-2B).
   Note: Make sure to mix the aqueous (cell) and organic (phenol/chloroform/isoamyl alcohol) phases very well.

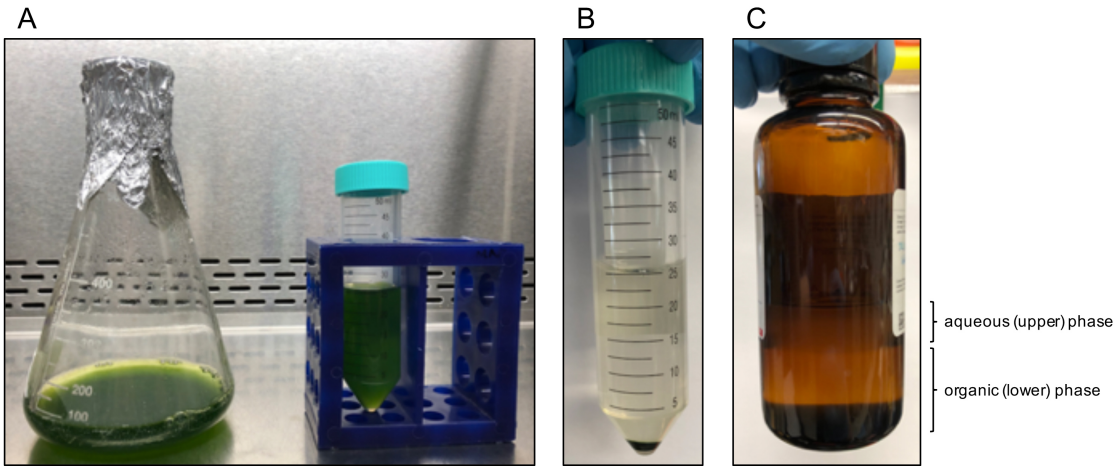

Figure 1. *Chlamydomonas* cells and phenol:chloroform:isoamyl alcohol solution. A. Flask of *Chlamydomonas reinhardtii* cells (left) and 50 ml centrifuge tube with 25 ml cells [Step 1]. B. Cell pellet at the bottom of the centrifuge tube after centrifugation [Step 2]. C. Phenol/chloroform/isoamyl alcohol solution with aqueous (upper) and organic (lower) phases highlighted. Only the organic (lower) phase was used [Steps 4 and 8].

6. Separate phases using a microcentrifuge (max speed, room temperature, 5 min).
   Note: The maximum setting on the Eppendorf 5424 microcentrifuge is 21,130 x g.
7. Transfer the aqueous (upper) phase to a new, pre-labeled 1.5 ml microcentrifuge tube (see Figures 2C-2D).
   Notes:
   a. Avoid disrupting or transferring any of the white interphase material.
   b. The organic (lower) phase should look very green from the extracted chlorophyll.
8. Into the microcentrifuge tube containing the aqueous phase from Step 7, add 400 µl phenol/chloroform/isoamyl alcohol solution (from lower, organic phase) and vortex on highest setting for 1-2 min (see Figure 1C, Figures 2E-2F).
9. Separate phases using a microcentrifuge (max speed, room temperature, 5 min) (see Figure 2G).
10. Transfer the aqueous (upper) phase to a new, pre-labeled 1.5 ml microcentrifuge tube (see Figures 2H-2I).
    Note: Avoid disrupting or transferring any of the white interphase material.
11. Treat the aqueous phase with 2 µl RNase A for 1 h at room temp.
    Note: After the first 30 min, mix by inverting the tube.
12. Into the microcentrifuge tube containing the aqueous phase from Step 10, add 400 µl chloroform/isoamyl alcohol solution and vortex on highest setting for 1-2 min.
13. Separate phases using a microcentrifuge (max speed, room temperature, 5 min) (see Figure 2J).
14. Transfer the aqueous (upper) phase to a new, pre-labeled 1.5 ml microcentrifuge tube (see Figure 2K).

Note: Avoid disrupting or transferring any of the interphase material.

15. Into the microcentrifuge tube containing the aqueous phase from Step 4, add 400 µl chloroform/isoamyl alcohol solution and vortex on highest setting for 1-2 min.
16. Separate phases using a microcentrifuge (max speed, room temperature, 5 min) (see Figure 2L).
17. Transfer the aqueous (upper) phase to a new, pre-labeled 1.5 ml microcentrifuge tube.
    Note: Here, it is very important not to transfer any of the organic (lower) phase or interphase material. Slow and careful pipetting can be helpful.
18. To precipitate the genomic DNA, add 1 ml of absolute ethanol, mix by inverting and incubate on ice for at least 30 min (see Figure 2M).
    Notes:
    a. *If desired, it is okay to incubate overnight at -20 °C.*
    b. *It is important to avoid vortexing the DNA and ethanol mixture at this stage to avoid shearing of the genomic DNA.*
19. Pellet the precipitated genomic DNA by centrifugation (max speed, 4 °C, 20 min) (see Figure 2N).

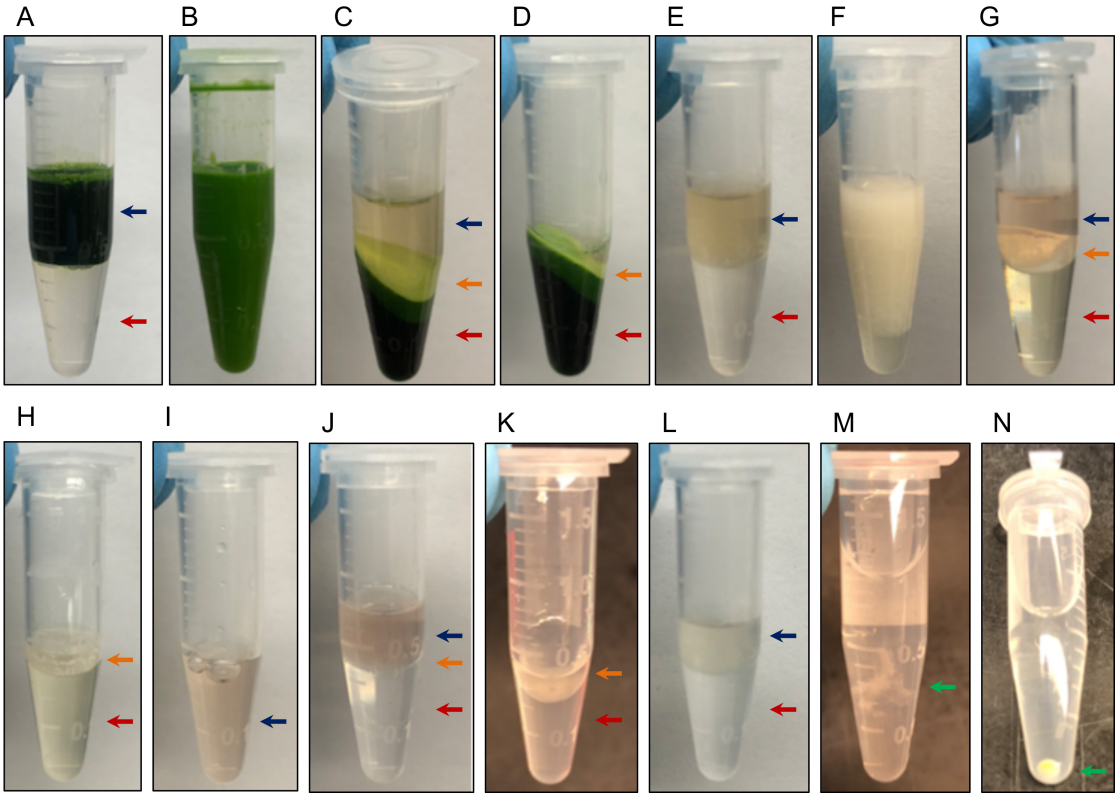

Figure 2. Images of microcentrifuge tubes used in genomic DNA isolation from *Chlamydomonas*. In all images, blue arrows denote aqueous (upper) phase, while red arrows denote organic (lower) phase. Orange arrows denote interphase material between aqueous and organic phases. In all steps, only the aqueous phase was saved or used (*i.e.*, the organic phase and interphase material were discarded in each step). A. *Chlamydomonas* cells resuspended

in DNA Extraction buffer (blue arrow) mixed with phenol/chloroform/isoamyl alcohol solution (red arrow) [Step 5]. B. Mixture of *Chlamydomonas* cells in DNA Extraction buffer and phenol/chloroform/isoamyl alcohol solution after vortexing [Step 5]. C. Separation of aqueous and organic phases after centrifugation. Note the white interphase (orange arrow) between the aqueous (blue arrow) and organic (red arrow) phases [Step 6]. D. The aqueous phase from Step 6 was transferred to a new tube, leaving behind the white interphase (orange arrow) and organic phase (red arrow) [Step 7]. E. Mixture of aqueous phase (blue arrow) from Step 6 and phenol/chloroform/isoamyl alcohol solution before vortexing [Step 8]. F. Mixture of aqueous phase (blue arrow) from Step 6 and phenol/chloroform/isoamyl alcohol solution after vortexing [Step 8]. G. Separation of aqueous and organic phases after centrifugation. Note the white interphase (orange arrow) between the aqueous (blue arrow) and organic (red arrow) phases [Step 9]. H. The aqueous phase from Step 9 was transferred to a new tube, leaving behind the white interphase (orange arrow) and organic phase (red arrow) [Step 10]. I. The aqueous phase from Step 9 was treated with RNase A for 1 h at room temperature [Step 11]. J. Separation of aqueous and organic (chloroform/isoamyl alcohol) phases after centrifugation. Note the white interphase (orange arrow) between the aqueous (blue arrow) and organic (red arrow) phases [Step 13]. K. The aqueous phase from Step 13 was transferred to a new tube, leaving behind the white interphase (orange arrow) and organic phase (red arrow) [Step 14]. L. Separation of aqueous and organic phases after centrifugation. Here, there is little or no obvious interphase material [Step 6]. M. The aqueous phase from Step 16 was transferred to a new tube. After addition of absolute ethanol, a conspicuous white precipitate is visible (green arrow) [Step 18]. N. Precipitated genomic DNA pellet after centrifugation (green arrow) [Steps 19 and 21].

20. Discard the supernatant by pipetting. The remaining pellet should be white or slightly yellow in color and should strongly adhere to the microcentrifuge tube. Wash the pellet once by adding 1 ml 70% ethanol and gently pipetting up and down several times.
    Note: At this point, the genomic DNA pellet will not go into solution.
21. Pellet the genomic DNA by centrifugation (max speed, 4 °C, 10 min).
22. Carefully discard the supernatant using a pipette and allow the genomic DNA pellet to dry at room temperature for at least 30 min. This can be done by inverting the microcentrifuge tube on a Kimwipe or by placing it in an exhaust hood.
23. Resuspend the dried genomic DNA pellet in 50 µl nuclease-free water by gently pipetting up and down several times. If it seems viscous or doesn't dissolve well, add another 50 µl nuclease-free water. Repeat until the pellet is fully dissolved.
    Note: The genomic DNA will typically go into solution in ~100 µl water.
24. Quantify genomic DNA using a Nano-Drop spectrophotometer or a Qubit fluorometer.
    Notes:
    a. *Quantification of DNA by NanoDrop may be inaccurate at low concentrations. Be sure to check the 260 nm/280 nm ratio to assess the purity of the DNA since potential contaminants*

(e.g., phenol or protein) absorb strongly in the UV region.

b. *Typically, the amount of genomic DNA purified from 50 ml of cells in exponential phase (2-4 x 10^6 cells/ml) will range from 50-300 µg.*

25. Optional: Dilute genomic DNA to 50 ng/µl in sterilized Milli-Q water.
26. Optional: Perform PCR to check the quality of genomic DNA.

Notes

1. Be careful when working with phenol/chloroform/isoamyl alcohol and chloroform/isoamyl alcohol solutions, both of which can cause burns to skin and eyes.
2. Be careful when discarding used with phenol/chloroform/isoamyl alcohol and chloroform/isoamyl alcohol solutions. Check with your Environment, health and safety (EHS) group for how to properly discard organic waste.
3. Some algae strains have tough cell walls, so it may be necessary to lyse such cells by chemical or physical means (*e.g.*, gametolysin treatment, glass beads, sonication) prior to genomic DNA isolation.
4. A protocol for using a hemacytometer to count cells can be found at https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/counting-cells-in-a-hemacytometer.html.

Recipes

1. DNA Extraction buffer
   50 mM Tris-HCl, pH 8.0
   20 mM EDTA
   200 mM NaCl
   1% SDS
2. Stock solutions (all prepared in Milli-Q water)
   a. 1 M Tris Base (1 L, 50x)
      121.14 g Tris Base
   b. Phosphate Buffer II for TAP (100 ml, 1,000x)
      10.8 g K_2HPO_4
      5.6 g KH_2PO_4
   c. Solution A for TAP (500 ml, 100x)
      20 g NH_4Cl
      5 g $MgSO_4 \cdot 7H_2O$
      2.5 g $CaCl_2 \cdot 2H_2O$
   d. 125 mM EDTA·Na_2 pH 8.0 (300 ml)
      13.959 g EDTA·Na_2

Dissolve in ~250 ml water, titrate pH to 8.0 with KOH, adjust volume to 300 ml

e. 285 µM $(NH_4)_6Mo_7O_{24}$ (250 ml)
   0.088 g $(NH_4)_6Mo_7O_{24}$
f. 1 mM Na_2SeO_3 (250 ml)
   0.043 g Na_2SeO_3

3. Micronutrient stock solutions (all prepared in Milli-Q water)
   Note: Based on Kropat et al., 2011.
   a. 25 mM EDTA·Na_2 (250 ml)
      50 ml 125 mM EDTA·Na_2 pH 8.0
   b. 28.5 µM $(NH_4)_6Mo_7O_{24}$ (250 ml)
      25 ml 285 µM $(NH_4)_6Mo_7O_{24}$
   c. 0.1 mM Na_2SeO_3 (250 ml)
      25 ml 1 mM Na_2SeO_3
   d. Zn-EDTA (250 ml)
      0.18 g $ZnSO_4·7H_2O$
      5.5 ml 125 mM EDTA·Na_2 pH 8.0
   e. Mn-EDTA (250 ml)
      0.297 g $MnCl_2·4H_2O$
      12 ml 125 mM EDTA·Na_2 pH 8.0
   f. Fe-EDTA (250 ml)
      1.35 g $FeCl_3·6H_2O$
      2.05 g EDTA·Na_2
      0.58 g Na_2CO_3
      Combine EDTA·Na_2 and Na_2CO_3 in water and mix; add $FeCl_3·6H_2O$ after first two components are fully dissolved (do not use 125 mM EDTA·Na_2, pH 8.0 stock)
   g. Cu-EDTA (250 ml)
      0.085 g $CuCl_2·2H_2O$
      4 ml 125 mM EDTA·Na_2 pH 8.0

4. Tris-Acetate-Phosphate (TAP) medium (for 1 L)

Stock solution	Volume	Conc.
1 M Tris base	20 ml	50x
Solution A	10 ml	100x
Phosphate Buffer II	1 ml	1,000x
Glacial acetic acid	1 ml	1,000x
25 mM EDTA·Na_2	1 ml	1,000x
28.5 µM $(NH_4)_6Mo_7O_{24}$	1 ml	1,000x
0.1 mM Na_2SeO_3	1 ml	1,000x
Zn-EDTA	1 ml	1,000x
Mn-EDTA	1 ml	1,000x

Fe-EDTA	1 ml	1,000x
Cu-EDTA	1 ml	1,000x

Adjust pH to 7.3 with HCl

Sterilize by autoclaving

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