

Dutta, S. K. and N. Richman. The isolation of DNA from Neurospora crassa.

(Please note, more modern protocols for DNA isolation are available, eg [on the FGSC methods page](#))

The exercise given below is used in the laboratory portion of a general genetics course. Students work singly or in groups, depending upon the facilities available. If desired, the students can be assigned the duty of preparing reagents for this exercise during the preceding laboratory period.

Materials needed:

Equipment: table top centrifuges (capacity of at least 1500 rpm ) and centrifuge tubes incubator or waterbath adjustable to both 37 and 60C

125 ml Erlenmeyer flasks

test tubes and test tube racks

graduated cylinders

glass stirring rods

glass-stoppered bottles

Reagents: 1) 0.1 SCC - A stock solution of 10x SCC (sodium saline citrate) can be prepared by mixing 87.5 g NaCl and 44.1 g Na citrate and diluting to 1 liter.

2) Saline EDTA - Add 8.75 g NaCl to 37.23 g disodium EDTA and dilute to 1 liter. Adjust pH to 8.0 with NaOH.

3) Sodium lauryl sulfate - Dissolve 25 g in 75 ml distilled water. Note: solution solidifies below 25C.

4) Tris buffer - Mix 32.5 ml of 0.1 N HCl with 25 ml of 0.2M solution of tris (hydroxymethyl) amino methane and dilute to final volume of 100 ml.

5) Acetate EDTA - Mix 40.8 g Na acetate with 0.037 g EDTA and dilute to 100 ml.

6) RNase - Obtain commercial RNase (Calbiochem) and place water solution of this enzyme (2 mg/ml) into boiling water for 10 minutes to destroy DNase activity.

7) Phenol reagent - Saturate phenol with saline EDTA and adjust pH to 8.0 with 10N NaOH. Caution should be exercised in the preparation and use of this reagent.

8) Isopropanol.

Neurospora powder: Neurospora powder can be produced by harvesting mycelia grown in liquid medium (for a period of 14-18 hours) and then lyophilizing in a freeze dryer. The dry mycelia may be powdered by passage through a Wiley mill (mesh size 60). If facilities are unavailable for the production of Neurospora powder, it can be purchased. The powder may be stored in the freezer for long periods of time.

Isolation procedure:

- 1) Mix 1 g lyophilized Neurospora powder with 25 ml saline EDTA and 2 ml sodium lauryl sulfate in a 125 ml Erlenmeyer flask.
- 2) Stir by hand or by use of magnetic stirrer at low speed.
- 3) Incubate in 60C incubator for 30 minutes.
- 4) Remove from incubator and add 1-2 drops of chloroform.
- 5) Transfer to a stoppered bottle and add an equal volume of phenol reagent. Slowly rotate for 3-5 minutes.
- 6) Pour into centrifuge tubes and centrifuge at 1200-1500 rpm for 10 minutes.
- 7) Remove supernatant with pipette attached to aspirating tube.
- 8) Carefully layer two volumes of ethanol on the surface of the supernatant.
- 9) Carefully spool out DNA with a glass rod and redissolve in small quantity of 0.1 SCC.
- 10) Adjust pH to 7.8 with Tris buffer.
- 11) Add RNase to a concentration of 50 ug/ml DNA solution and incubate for 30-60 minutes at 37C.
- 12) Remove from incubator and add 1 ml acetate EDTA.
- 13) Carefully layer approximately 7 ml isopropanol on the surface and spool out the DNA.
- 14) Redissolve DNA in 0.1 SCC and purify by deproteinization with phenol as previously described.

Using these procedures, DNA of high molecular weight and good purity can be obtained. If desired, the identification and quantitation of the isolated substance can be made by means of UV-spectrophotometry or chemical tests, such as the diphenylamine reaction. For a 2-hour laboratory period, the procedure can be carried through step 8. The product at this stage is DNA with adhering protein and RNA. The experiment may be terminated at this point or else the DNA

can be dissolved in 0.1 SCC and stored in the refrigerator and the isolation can be continued during the next laboratory period.

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