CTAB DNA Extraction Protocol of P. pruinosa Version 2

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Abstract

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Guidelines

- Gloves must be worn at all times. Change gloves frequently and decontaminate often with DNA Away.
- Use only unopened, sterile, aerosol-resistant pipette tips (filter tips) to minimize contamination of the pipette shaft and your samples. Clean the body of the pipettes regularly with DNA Away.
- Discard pipette tips after each use to avoid cross-contamination. Eject pipette tips carefully to prevent aerosol formation or other splashing.
- Be careful not to touch the inside lid of tubes as it will cross-contaminate samples.

Before start

There is the preparation of CTAB solution.

CTAB Buffer

100ml 1M Tris HCl pH 8.0

280ml 5 M NaCl

40ml of 0.5 M EDTA

20 g of CTAB (cetyltrimethyl ammonium bromide)

Bring total volume to 1 L with ddH_2O

TE Buffer

10ml 1 M Tris HCl pH 8.0

2ml 0.5 M EDTA

Bring total volume to 1 L with ddH_2O

1 M Tris HCl pH 8.0

121.1 g Tris Dissolve in about 700 ml of H_2O.

Bring pH down to 8.0 by adding concentrated HCl (you'll need about 50 ml).

Bring total volume to 1 L with ddH_2O.

0.5 M EDTA

186.12 g EDTA

Add about 700 ml H_2O

16-18 g of NaOH pellets

Adjust pH to 8.0 by with a few more pellets, EDTA won't dissolve until the pH is near 8.0

Bring total volume to 1 L with ddH_2O.

5 M NaCl

292.2 g of NaCl

700 ml H_2O

Dissolve (don't add NaCl all at once, it will never go into solution) and bring to 1 L

7.5 M Ammonium acetate

57.81g ammonium acetate

~50 ml of H_2O

Bring to 100 ml total volume

Filter sterilize all stock solutions.To do this use a nalgene filter (stockroom catalog # PL-365 – $39.00 per case) with the faucet vacuum attachment located in the drawer below the waterbath across from the sink.

Protocol

DNA EXTRACTION PREPARE

Step 1.

Prepare CTAB Buffer (recipe attached). Prior to starting extraction, add polyvinylpyrrolidone and β-mercaptoethanol. Once these have been added the shelf life of the buffer is only 2-3 days.

CTAB Buffer	PVP	β-mercaptoethanol
0.5mL	0.02g	2.5µL
5mL	0.2g	25µL
20mL	0.8g	100µL

Note: Double check your math if you are doubling, tripling, etc. the recipe**!** Put the solution in the water bath for 10-20 minutes to dissolve the PVP. Don’t shake the solution-the detergent will bubble up too much.

DNA EXTRACTION PREPARE

Step 2.

Collect the tissue that you need from the -80℃ freezer and keep them on dry ice. It' s very important that the tissue doesn't thaw! Label eppendorf tubes with a Lab Marker to transfer the tissue into (Sharpie markers will bleed if alcohol is spilled on them).

DNA EXTRACTION PREPARE

Step 3.

Using forceps, transfer 80-100 mg of frozen tissue in an eppendorf tube. It’s ok to guess on the mass because keeping the tissue frozen is more important than having exactly 90 mg. If it thaws a bit, quickly submerge the tube in liquid nitrogen to re-freeze. Dip forceps in bleach water, then water and dry between every sample. Record whether or not you use all of the tissue.

DNA EXTRACTION PREPARE

Step 4.

4. Grind tissue in liquid nitrogen with blue pestles, keeping tissue frozen the entire time.Use a new pestle for every sample. Soak pestles in bleach water for at least 20 minutes before rinsing and autoclaving.

DNA EXTRACTION

Step 5.

Add 1mL of CTAB buffer and mix the tubes.Make sure the leaf tissue is in solution and not in a clump at the bottom of the tube. Incubate at 65℃ for at least 30 minutes mixing once after 5 They can stay in the water bath for a few minutes if necessary.

Before beginning: turn on the water bath. Double check that it is set to 65℃ and there are a few inches of water in it and check that you have enough of all solutions: CTAB buffer, Isoporpanol,

70%ethanol, 95% ethanol, TE buffer.If you don’ t have enough of any see recipe below.

DNA EXTRACTION

Step 6.

While samples are in the water bath, label the top and sides of a regular 2mL tubes with a VWR marker. Use capital letters for the individual. Add the date to the side of the tube and wrap the tube in tape. The tape prevents the marker from rubbing off after frozen.

DNA EXTRACTION

Step 7.

After incubating for 30 minutes add 1.5µL RNase A. Incubate at 37℃ for 15 minutes.

Note: The following steps are best done in batches of 10-20, depending on how quickly you can work.

DNA EXTRACTION

Step 8.

Remove samples from waterbath and in fume hood, add 900uL of chloroform and mix by gently shaking tubes. Change gloves immediately if you spill chloroform on them. Be careful not to drip chloroform onto the tubes, it has a low viscosity and drips out of the tip-it will make the label bleed off of the tube.

DNA EXTRACTION

Step 9.

Centrifuge for 10minutes at 12000 r/min.

DNA EXTRACTION

Step 10.

Transfer the aqueous phase (top layer) into the new labeled tube. Be careful to avoid transferring any chloroform. You can tell if you get chloroform because it will be bright green.Chloroform waste should be disposed of in a glass bottle with a chemical waste label.

DNA EXTRACTION

Step 11.

Estimate the volume of the aqueous phase.

DNA EXTRACTION

Step 12.

Add 0.08 volumes cold 7.5 M ammonium acetate.

DNA EXTRACTION

Step 13.

Add 0.54 volumes of cold isopropanol. Mix by inverting tubes 20-30 times.

aqueous phase(µL)	ammonium acetate	isopropanol
350	28	204.12
360	28.8	209.952
370	29.6	215.784
380	30.4	221.616
390	31.2	227.448
400	32	233.28
410	32.8	239.112
420	33.6	244.944

Before beginning, move isoporanol and ethanol to freezer. Ammonium acetate is already cold in the refrigerator.

DNA EXTRACTION

Step 14.

Incubate on ice for 30-40 minutes.

DNA EXTRACTION

Step 15.

Centrifuge for 3 minutes at 12000 r/min.

DNA EXTRACTION

Step 16.

Discard supernatant into isopropanol chemical waste jar. Be careful not to dislodge pellet.

DNA EXTRACTION

Step 17.

Centrifuge for 1 minute at 12000 r/min.

DNA EXTRACTION

Step 18.

Add 700 uL 70% EtOH, invert tubes 5-10 times.