EXTRACTING DNA FROM PLANTS FOR DArT *KEJ 1 MAY 2005*

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- Hand-grind plant material together with equal volume of acid-washed sand and liquid nitrogen using an acid-washed and autoclaved mortar & pestle (or 1.5-mL tube and micro-pestle) until the material reaches a chalky consistency. Transfer to 1.5-mL tube if necessary.
- Add 500ul CTAB buffer and 50ul of SARKOSYL buffer, both pre-warmed to 60 degrees
- Add 10ul 10mg/mL PROTEINASE K
- Vortex vigorously & incubate for 60 minutes @ 60ºC with occasional vortexing
- SEVAC extraction:
  - Add an equal volume of SEVAC and vortex vigorously
  - centrifuge top speed 3 minutes
  - transfer supernatant into a new tube without disturbing the interface
- Repeat the SEVAC extraction
- add 2/3 volume (approx. 400ul) ice-cold isopropanol. Mix by gentle inversion and leave on ice for 30 min.
- centrifuge 3 min and remove isopropanol to leave DNA pellet in bottom of tube
- air dry pellet at 60 degrees or room temp (can stop here overnight or longer @ -20ºC)
- re-suspend pellet in 32ul 0.1M T.E. buffer, run out 2uL on a 1% agarose gel
- transfer 10ul into a different tube and store at –20 for safe keeping
- bring vol of remaining 20uL DNA up to 200UL by adding 180uL 0.1X TE
- Phenol-chloroform purification (in fume hood):
  - add 100uL each of PHENOL and SEVAC and invert gently 1 minute
  - centrifuge top speed 10 minutes
  - transfer aqueous (top) layer into a new tube without disturbing the interface
  - Add 200uL SEVAC and invert gently for 1 minute.
  - Centrifuge at top speed for 10 minutes
  - transfer aqueous (top) layer into a new tube without disturbing the interface
- Add 20uL 3M SODIUM ACETATE pH 5.5 then 2X volumes (400ul) ice-cold 100% ETHANOL, mix gently by inversion and incubate at -20ºC overnight or one hour @ –80ºC
- Centrifuge @ 4ºC for 15 minutes then remove ethanol carefully with a pipette
- Wash with 500ul 70% ETHANOL by gentle inversion and centrifuge @ 4ºC for 5 minutes full speed
- Carefully remove ethanol with a pipette and air dry pellet in 60 degree heat block
- Re-suspend pellet in 30ul 0.1X TE (at 60C if necessary to dissolve difficult pellets)
- Run out 2uL on a 1% agarose gel
- Test DNA quality by restriction digest. Add the following, *IN ORDER,* to each restriction digest tube (keep reagents, *especially the enzyme* on ice at all times). Also set up a restriction digest of the concentration standard pGEM (20ng/uL), or some other comparable standard. Be sure to set up a no-enzyme control digest for each DNA sample to run out alongside your digest.

RESTRICTION DIGEST	NO-ENZYME CONTROL
5.8uL H20	6uL H20
1uL 10X buffer (buffer "2" from NEB)	1uL 10x buffer (buffer "2" from NEB)
2uL DNA*	2uL DNA*
1uL 10X BSA	1uL 10X BSA
…mix well, then add: 0.2uL 50u/uL MseI (from NEB)	…mix well

**How much DNA to use is a matter of judgement. 2uL is about right if you can see your high molecular weight DNA as a discrete band on your gel. If you have a very bright smear of DNA which masks your high molecular weight band, try reducing your DNA to 1uL; if you can barely see your high molecular weight band, increase your DNA volume. For any adjustment in DNA volume, adjust water accordingly so that reaction volume is always 10uL*

  - mix gently by inverting and tapping tube on bench-top, then gently spin down contents of tubes and incubate for 2 hours at 37ºC. Add 2uL loading dye to each tube and run whole reaction out on 1% Agarose gel for at least 1 hour at 40 Volts.

- Estimate DNA concentration by comparing un-digested DNA to un-digested pGem on the gel. DArT requires at least 1ug DNA at approx 100ng/uL concentration.

Solutions:

- CTAB buffer: 2% CTAB in 100Mm TRIS-HCL pH 8.0, 1.4M NaCL, 20mM EDTA
- SARKOSYL buffer: 10% N-LAURYL SARCOSINE, 100mM TRIS-HCL pH8.0, 20mM EDTA
- SEVAC: CHLOROFORM:ISOAMYL ALCOHOL 24:1
- 0.1M T.E. BUFFER: 100Mm TRIS-HCL pH 8.0, 20mM EDTA