Plant DNA Extraction Protocol for DArT

BUFFER STOCK SOLUTIONS

EXTRACTION BUFFER STOCK

	To make 500 ml:
0.35 M sorbitol	31.9 g sorbitol
0.1 M TrisHCl pH 8.0	50 ml 1M TrisHCl pH 8.0
5 mM EDTA pH 8.0	5 ml 0.5 M EDTA pH 8.0
	fill up to 500 ml MiliQ H_2O

LYSIS BUFFER STOCK

	To make 500 ml:
0.2 M Tris HCl pH 8.0	100 ml 1M Tri HCl pH 8.0
0.05 M EDTA pH 8.0	50 ml 0.5 M EDTA pH 8.0
2M NaCl	200 ml 5 M NaCl
2% CTAB	10 g CTAB
	fill up to 500 ml with MilliQ H_20

SARCOSYL STOCK 5% (w/v)

FRESH BUFFER WORKING SOLUTION*:

0.5 % (w/v) sodiumdisulfite (= sodium metabisulfite)
2 % (w/v) PVP-40 (K29-32) Sigma

dissolve in required volume of extraction buffer stock; add same volume of lysis buffer stock and 0.4 volume of extraction (=lysis) buffer stock of sarcosyl stock.

For example to make 120 ml:

Add 0.6 g sodiumdisulfite (= sodium metabisulfite) and 2.4 g PVP-40 (K29-32) to 50 ml extraction buffer stock and dissolve; add 50 ml lysis buffer stock and 20 ml sarcosyl stock

For example to make 30 ml:

Add 0.15 g sodiumdisulfite (= sodium metabisulfite) and 0.6 g PVP-40 (K29-32) to 12.5 ml extraction buffer stock and dissolve; add 12.5 ml lysis buffer stock and 5 ml sarcosyl stock

*This buffer may settle into two layers on standing. Heat to 65ºC and shake immediately before adding to extraction tubes.

PROTOCOL

For 15 ml Sarsted tubes:

- aliquot 6 ml of freshly prepared preheated to 65ºC well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3, 4 days old “fresh buffer solution” works fine),
- grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,

- suspend powder in 6 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary),
- incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking,
- cool down for 5 min and add 6 ml of chloroform : isoamyl alcohol (24 : 1) mixture,
- mix well for 30 min,
- spin 20 min, 3000 x g, RT,
- transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible,
- spin 30 min, 3000 x g, RT,
- discard supernatant, wash pellet with 2 ml 70 % EtOH,
- discard EtOH, dry pellet and dissolve in 200 µl – 500 µl 1 x TE (10 mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0),
- check DNA quality and quantity on 0.8 % agarose gel. (If RNA quantity is several fold less than DNA, RNase treatment is not necessary for DArT applications).

For 2 ml Eppendorf tubes:

- aliquot 1 ml of freshly prepared preheated to 65ºC, well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3, 4 days old “fresh buffer solution” works fine),
- grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,
- suspend powder in 1 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary),
- incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking,
- cool down for 5 min and add 1 ml of chloroform : isoamyl alcohol (24 : 1) mixture,
- mix well for 30 min,
- spin 20 min, 10000 x g, RT,
- transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible,
- spin 30 min, 10000 x g, RT,
- discard supernatant, wash pellet with 2 ml 70 % EtOH,
- discard EtOH, dry pellet and dissolve in 250 µl of 1 x TE (10 mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0),
- check DNA quality and quantity on 0.8 % agarose gel. (If RNA quantity is several fold less than DNA, RNase treatment is not necessary for DArT applications).

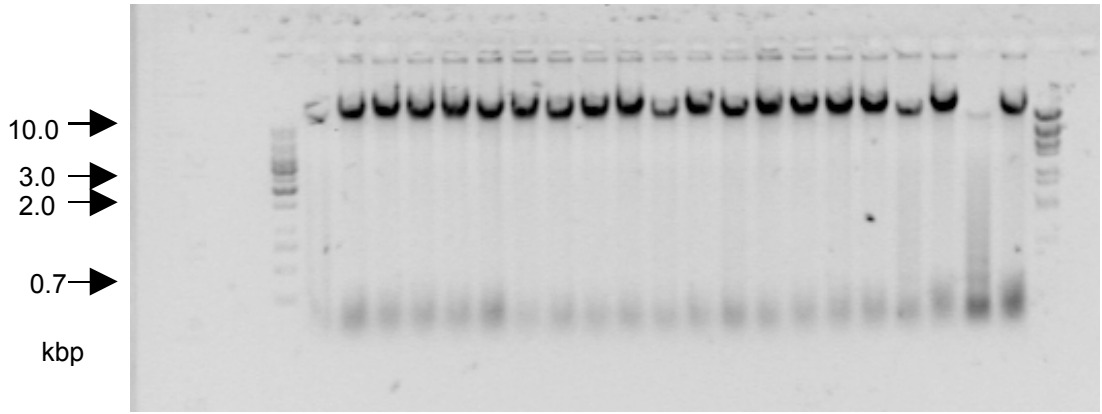

FIGURE 1. An example of sugarcane DNA extracted with the described method.