DNA extraction: Modified CTAB method

There are two processes to prepare yam DNA for SSR analysis, I) Initial washing and II) Actual DNA extraction. For each process, buffers listed below should be prepared (stock solution) and mixed (working solution) as instructed.

Tips

Washing buffer (WB) in the washing process and Microprep Buffer (MB) in the DNA extraction process must be prepared JUST BEFORE USE (freshly prepared).

Process I. Initial washing of leaf samples

1) Prepare ca.~200 mg (0.2g) of sample (better to pre-cut into small pieces)
2) Transfer the sample into 2.0 mL tube with one zirconia bead (Φ5mm) and grind using mixer mill (e.g. QIAGEN MM300), 30/s for 1 min
3) Add 1mL of WB* and mix thoroughly
4) Centrifugation 1,000 rpm for 5 min at room temperature (r.t.)
5) Discard the supernatant and use the residue (washed tissue) for DNA extraction

>>> go to DNA extraction process (Process II)

*Washing buffer (WB): for ca. 20 samples, prepare just before use

0.1M HEPES-HCl (pH8.0)**	20mL
PVP (polyvinylpyrrolidone)	204mg
L-ascorbic acid	180mg
2-mercaptethanol	400μL

**0.1M HEPES-HCL (pH8.0):

12g of HEPES in 500mL of DW (final volume)
Adjust pH by NaOH, Sterilize by autoclave, Store in dark

Process II. DNA extraction from washed leaf samples

5') Grind and washed sample prepared in the initial washing process (Process I)
6) Add 750μL of MB* prepared just before use, and homogenize well
7) 65˚C for 1hr
8) Add 700μL of chloroform/isoamyl alcohol (24:1) and mix well
9) Centrifugation 12,000 rpm for 10 min at r.t.
10) Transfer aqueous phase (~600μL) into new tube
11) Add 300μLof isopropanol
12) Invert the tube until the DNA precipitate (invert gently, do not shake vigorously)
13) Centrifugation 12,000 rpm for 5 min at r.t.
14) Discard the supernatant
15) Add 500μL of cold 70% EtOH and tap the tube
16) Centrifugation 12,000 rpm for 10 min at r.t.
17) Discard the supernatant and dry the precipitate 5 min at r.t.
18) Add 150μL of TE and dissolve
>>> measure OD and use for further analyses

*Microprep Buffer (MB): for ca.10 samples, prepare just before use

Extraction Buffer (EB)**	3mL
Lysis Buffer (LB)***	3mL
5% Sarkosyl	1.2mL
Sodium Bisulfite	0.03g

**Extraction Buffer (EB) :500mL

Sorbitol	32g
Tris base	6g
EDTA-2Na	0.84g
Adjust to pH 7.5 with HCl	

***Lysis Buffer (LB) :500mL

Tris base	12.1g
EDTA-2Na	8.4g
NaCl	58.5g
CTAB	10g