DNA extraction: SDS method

This method is effective for DNA extraction from tuber skin samples of yam. Before starting DNA extraction process, you must prepare the Extraction buffer as instructed below.

Process

1) Prepare c.a.~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 400 µL of Extraction buffer* (see below) and 4 µL of RNase A (e.g. QIAGEN) and vortex
4) 65°C for 10 min (invert the tube 2 to 3 times during this incubation time)
5) Centrifugation 14,000 rpm for 1 min at room temperature (r.t.)
6) Transfer the supernatant into new 2.0 mL tube
7) Add 130 µL of 3M potassium acetate (pH 5.5) and vortex
8) On ice 5 min
9) Centrifugation 14,000 rpm for 5 min at r.t.
10) Transfer the supernatant in to new 2.0 mL tube
11) Add 2/3 volume of isopropanol and invert the tube gently
12) Centrifugation 14,000 rpm for 10 min at r.t.
13) Discard the supernatant
14) Add 500 µL of 70% EtOH and tap the tube
15) Centrifugation 14,000 rpm for 2 min at r.t.
16) Discard the supernatant
17) Repeat the processes from 14) to 16)
18) Add 500 µL of 99.5% EtOH and stand for 5 min at r.t.
19) Centrifugation 14,000 rpm for 2 min at r.t.
20) Discard the supernatant and dry the precipitate 5 min at r.t.
21) Add 150 µL of TE and dissolve (>> measure OD if needed and use for further analyses)

Tips

If DNA solvent is not clear with impurities, in step 21) centrifugation 14,000 rpm for 5 min at r.t. and transfer the supernatant into new tube.

*Extraction Buffer for SDS method (100 mL, for ~200 samples)

SDS	0.3 g
1M Tris-HCL (pH 8.0)	2mL
0.5M EDTA (pH8.0)	1mL
NaCl	2.34 g
DW	up to 100 mL