Rapid Extraction Protocol

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1. Add coleoptiles or leaf tissue directly into a 96-well flat bottom tissue culture plate. Roll up tissue to fit into well, do not let tissue stick to the side of the plate. Freeze tissue at –20C until ready to extract. **Fresh tissue works best.
2. Add 40ul 0.25 NaOH directly into wells containing leaf tissue.
3. Heat microtiter plate in 95C water bath for 1 min.
4. Put stainless steel cylinders on top of tissue. Using the matrix mill, grind tissue for 7-10 min.
5. After grinding remove cylinders. Add 130ul of 0.1 Tris-HCl to each well.
6. Spin plate 3500rpm, 10min.
7. Remove 150ul supernatant and put into a 200uL 96-well conical bottom PCR plate
8. Add 15ul 3M NaOAc and 120ul 100% isopropanol
9. Freeze –80C 1hr. or –20C overnight
10. Centrifuge 3500rpm, 30min
11. Remove isopropanol and centrifuge upside down 600rpm, 1min
12. Add 200ul 70% EtOH
13. Centrifuge 3500rpm, 20min
14. Remove EtOH and centrifuge upside down 600rpm, 1min
15. Resuspend in 20ul TE buffer

Products we use:
Germination containers
8-well rectangular tissue culture dish (Nunc, Cat. No. 176600)

Extraction plastics
96-well flat bottom tissue culture dish (Nunc, Cat No. 167008)
Conical bottom 96-well PCR plate (Genemate®, Cat no. T-3031-21)

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