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Working

Chelex DNA isolation for quick plant genotyping

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ABSTRACT

Quick and dirty, but very very fast protocol for DNA isolation. It works beautifully if you have plenty of lines to genotype - example T-DNA insertion lines for validation of your favourite gene.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Chelex 100	C7901-100G	Sigma Aldrich

SAFETY WARNINGS

1 Grind tissue in liquid N2 (1 leaf should be enough)

If you collect the tissue in a tube containing glass beads (1-2 mm diameter) you can put the frozen tissue samples in the tissue grinder. This is by far the most efficient method to gind large quantities of various samples.

2 Add 200µl 10% Chelex (in MilliQ)

3 Vortex

4 Incubate 15min @ 95 degrees in shaker

5 Vortex

6 Spin 15min @ max RPM

7 Use (2µl) supernatant for PCR (optional: transfer supernatant to fresh tube for storage at -20C)

Genotyping PCR (DreamTaq)

8

step 1 94 degrees 4min
step 2 94 degrees 45s
step 3 50 degrees 45s (try higher temp for more specificity)
step 4 72 degrees 1:20min (depending on size insert)
step 5 repeat 35x step 3
step 6 72 degrees 10min
step 7 16 degrees 10min

Run the gel

9 Run 20uL of the PCR product on the 1% agarose gel