

Leaf Disk Template

Alkali Treatment of Plant Tissues for PCR Template

96 Well Microplates

The limiting step in MAS using PCR is the preparation of template DNA. Klimyuk *et al*, (1993) describe a method for the treatment of plant tissues with an alkaline solution, and the subsequent use of the tissue as template for PCR. This procedure has been modified;

The Alkali Treatment Protocol (with modifications from Klimyuk *et al* as follows)

1. Place a microplate on ice. (use a low-profile well plate) 
2. Harvest a small piece of plant tissue from coleoptiles of germinated seeds. We try to use a piece of the coleoptile as close to the seed as possible, the length of the harvested piece should be 3mm or less (more IS NOT better). Use tweezers to make sure the tissue is placed at the bottom of each well and slightly macerated (we keep several tweezers in a flask of 95% EtOH to clean the tweezer tips between use and avoid DNA contamination). The age of the leaf tissue does not seem to have a noticeable effect on the subsequent PCR reaction, but the leaf tissue should be as young as possible.
3. To each well, add 40mL of 0.25M NaOH. Make sure that the plant tissue is covered by the solution. Mix at 1650 RPM on MIXMATE 
4. Place the microplate in a boiling waterbath for exactly 30 seconds, or a heating block (PCR block) for 45 seconds. Remove and leave at room temperature.
5. To each well, add 60mL of 0.5M Tris-HCl (pH 8.0). Mix at 1250 RPM on MIXMATE.
6. Place in a heating block (PCR block) for 3 minutes. Remove and place on ice.
7. Seal the microplate with aluminum foil sealing film.



Klimyuk used the actual leaf tissue at this point as template for the PCR reactions. We have found that the solution (supernatant), may be used directly as template for the PCR and gives more consistent results. We have not studied the shelf life of the preparations, but have stored the preparations at 4° for up to 6 weeks with results identical to when the preparations were used fresh.

Add 0.5 to 3mL of the solution directly to the PCR reaction.

*** Klimyuk, V.I., Carroll, B.J., Thomas, C.M., and Jones, D.G. 1993. Alkali treatment for rapid preparation of plant material for reliable PCR analysis. *The Plant Journal* 3(3): 493-494.

The PCR Reaction.

The PCRs are performed under conditions that have been optimized for the particular set of primers in use. The annealing temperatures may need to be adjusted slightly depending on the primers used.

Microplates: Abgene Thermo-Fast 96 well Low-Profile
Catalog# AB0700 (Diamed Lab Supplies)

Sealing Film: Aluminum Foil Sheets
Catalog# DLAU658-5 (Diamed Lab Supplies)