# Supplemental Material for "Positional Cloning in *Arabidopsis*"

**(http://carnegiedpb.stanford.edu/methods/ppsuppl.html)**

## PREPARATION OF *ARABIDOPSIS* DNA FOR PCR PURPOSES

### Protocol 1: CTAB extraction

Modified after **Murray MG, Thompson WF** (1980) Rapid isolation of high-molecular-weight plant DNA. Nucl Acid Res **8:** 4321–4325.

Reagents:

- 2X CTAB buffer (2% (w/v) cetyl-trimethyl-ammonium bromide (CTAB), 1.4 M NaCl, 100 mM Tris HCl pH 8.0, 20 mM EDTA)
- Chloroform
- 2-Propanol
- 70% Ethanol
- TE buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA)

Procedure:

1. Mash or grind a small amount of plant tissue in a 1.5 ml reaction tube
2. Add 300 µl 2X CTAB buffer and incubate at 65° C for at least 10 minutes (up to several hours)
3. Allow to cool
4. Add 300 µl chloroform and vortex thoroughly
5. Spin briefly in a microfuge to separate phases
6. Transfer the upper, aqueous phase to a fresh reaction tube
7. Add 300 µl 2-Propanol and mix well
8. Spin in a microfuge for 5 minutes to pellet the DNA
9. Remove supernatant and wash pellet with 500 µl 70% ethanol
10. Spin briefly in a microfuge
11. Carefully remove the ethanol and air dry pellet
12. Add 100 µl TE buffer and allow the pellet to dissolve
13. Mix or vortex before use. Use 1-2 µl in a PCR reaction

Notes:

A. This protocol yields relatively clean, high molecular weight DNA (see Figure S1). We have successfully used this DNA for Southern blotting and PCR amplification of long DNA fragments (up to 10 kilobase pairs).

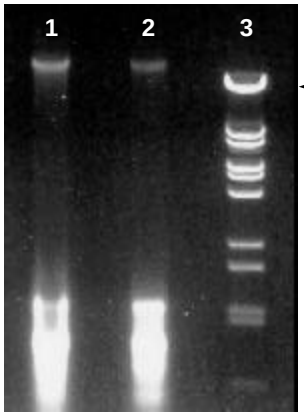


**Figure S1**. Agarose gel electrophoresis of CTAB DNA preparations from a single *Arabidopsis* inflorescence (lane 1) and a single rosette leaf (lane 2). One tenth of each DNA preparation was loaded. A standard (λ DNA / *Bst* E II digest, 500 ng total DNA) is shown in lane 3.

B. A single rosette leaf or an inflorescence provide sufficient starting material. The tissue can be used fresh or stored at –20° C prior to processing. If more than 100 mg of tissue is used, the amount of buffer should be adjusted such that it equals roughly four times the amount of tissue.

C. Plastic pestles that fit 1.5 ml reaction tubes can be used to grind the tissue (step 1). Appropriate pestles can be purchased from the Kimble-Kontes Glass Company (Vineland, NJ; www.kimble-kontes.com; catalog# 749521-1500). In this case, the tissue should be ground with the 2X CTAB buffer added. The pestles can be mounted in a power drill for easier grinding. If only co-dominant markers are analyzed, it is not necessary to use a fresh pestle for each tissue sample – briefly rinsing the pestle in water will be sufficient to avoid visible cross contamination. As a cheap alternative to pestles, 1 ml pipettor tips can be used to mash the tissue. In this case, the tissue should be mashed without buffer by pressing against the walls of the tube until only small fragments remain.

D. When transferring the aqueous phase (step 6), it is often difficult to avoid carry-over of some material accumulating at the interphase between chloroform and buffer. In our experience, this contamination will not affect PCR reactions.

E. Upon precipitation, a pellet should be visible (step 8).

F. The DNA can be stored at 4 °C for several weeks or at -20° C for a prolonged time.

### Protocol 2: Alkaline lysis

After Klimyuk et al. (1993).

Reagents:

- 0.25 N sodium hydroxide
- 0.25 N hydrochloric acid
- 0.5 M Tris HCl pH 8.0 / 0.25% (v/v) IGEPAL CA-630

Procedure:

1. Mash tissue in 50 µl 0.25 N sodium hydroxide
2. Incubate for 30 seconds at 96° C
3. Add 50 µl 0.25 N hydrochloric acid and 25 µl 0.5 M Tris HCl pH 8.0 / 0.25% (v/v) IGEPAL CA-630
4. Incubate for 2 minutes at 96° C
5. Use 1-2 µl of the crude lysate in a PCR reaction

Notes:

A. The crude lysate will allow PCR amplification of small DNA fragments, such as SSLP markers. However, we have occasionally experienced problems with the amplification of larger fragments (500 base pairs and longer). It is advisable to determine before-hand if a given marker can reliably be amplified from this kind of template.

B. Part of a young rosette leaf or a small inflorescence (about 5 buds) provides sufficient starting material. The tissue material can be used fresh or stored at -20° C prior to processing. Processing significantly larger amounts of tissue may lead to problems with PCR amplification due to the presence of inhibiting substances in the lysate.

C. Typically, this protocol is used to process a large number of tissue samples in a microtiter plate format. A 96-well plate for use in a thermocycler is ideal for the purpose. The plate can be placed on ice in a rack for 10µl pipettor tips for better support (see Figure S2). Using the round end of flexible polyethylene stirring rods that exactly fit into the wells of the microtiter plate, several tissue samples can be mashed simultaneously (step 1; see Figure S2). Appropriate stirring rods can be purchased from Sarstedt (Nümbrecht, Germany; www.sarstedt.com; catalog# 81-970). The tissue is

sufficiently mashed if the sodium hydroxide solution turns bright green or yellow-green from the extracted pigments. Heating can conveniently be done in a thermocycler (steps 2 and 4). To avoid cross-contamination of neighboring samples, microtiter plates *should not be covered* with lids or tape during the heating steps.

D. The crude lysate can be stored at -20° C for several weeks.

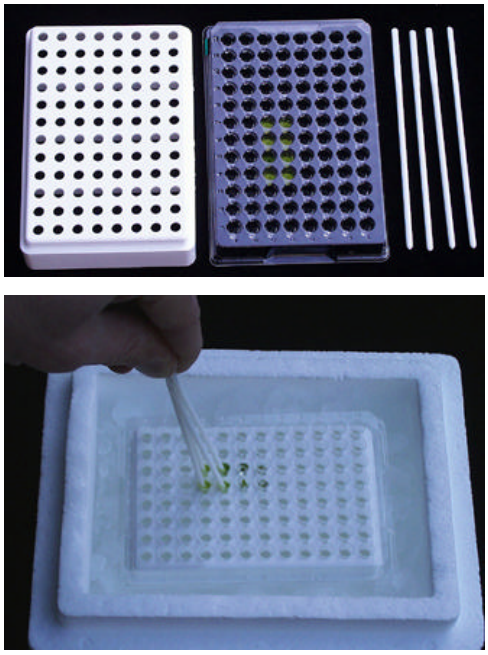

**Figure S2**.

## A COLLECTION OF 22 SSLP MARKERS FOR BULKED SEGREGANT ANALYSIS

Table S1 contains technical information on 22 SSLP markers that are spaced at a distance of 10 to 30 % recombination over the entire Arabidopsis genome and can be used for bulked segregant mapping. The nga markers, F21M12, CTR1 and PHYC have been described previously (Bell and Ecker, 1994; http://genome.bio.upenn.edu/SSLP_info/SSLP.html), the ciw markers were created by C. S. G.

All markers can be amplified using the following PCR profile: 1 minute @ 94°C, 40 cycles: 30 seconds @ 94°C, 30 seconds @ 55°C, 30 seconds @ 72°C. The concentration of reagents in the PCR reaction is: 50 mM potasium chloride, 10 mM Tris-HCl pH 9.0 @ room temperature, 0.1% Triton X-100), 200 μM dNTPs (each), 1 μM primers (each), 1.0 to 2.5 mM $MgCl_2$ (see Table S1), 2 μl template DNA (per 20 μl reaction), 0.5 U Taq polymerase (per 20 μl reaction).

A convenient way to set up the PCR reactions is as follows: For each of the 22 markers, 4X stock solutions containing both primers and magnesium are prepared (4 μM of each primer and, dependent on the primer pair, 4, 6, 8 or 10 mM MgCl2). For both DNA samples, the pooled mutant DNA and the heterozygous control DNA, a master mix containing all the other reagents (buffer, dNTPs, template DNA and enzyme) is prepared. Aliquots of the primer / $MgCl_2$ stocks are distributed on a microtiter plate and the master mix is added.

The PCR products are analyzed on high-resolution agarose gels in order to resolve the small size differences. We use 4% gels prepared with a high-resolution agarose blend (agarose 3:1) from Amresco (Solon, OH; www.amresco-inc.com; catalog# E776) and 1X TBE as running buffer (3 $Vcm^{-1}$ for 3-5 hours).

**Table S I**. *SSLP markers for bulked segregant analysis*

| Chr. | (cM) | Marker | (BAC) | Forward Primer (5'→3') | Reverse Primer (5'→3') | Size of PCR Product (bp) | | | [$MgCl_2$] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | COL | LER | WS | (mM) |
| I | (10) | **F21M12** | (F21M12) | GGCTTTCTCGAAATCTGTCC | TTACTTTTTGCCTCTTGTCATTG | 200 | ~160 | ~215 | 2.0 |
| | (39) | **ciw 12** | (T22C5) | AGGTTTTATTGCTTTTCACA | CTTTCAAAAGCACATCACA | 128 | ~115 | ~115 | 1.5 |
| | (72) | **ciw 1** | (F14J22) | ACATTTTCTCAATCCTTACTC | GAGAGCTTCTTTATTTGTGAT | 159 | ~135 | ~159 | 2.0 |
| | (81) | **nga 280** | (F14J16) | CTGATCTCACGGACAATAGTGC | GGCTCCATAAAAAGTGCACC | 105 | 85 | 85 | 1.5 |
| | (113) | **nga 111** | (F28P22) | CTCCAGTTGGAAGCTAAAGGG | TGTTTTTTAGGACAAATGGCG | 128 | 162 | 146 | 1.5 |
| II | (11) | **ciw 2** | (T18C20) | CCCAAAAGTTAATTATACTGT | CCGGGTTAATAATAAATGT | 105 | ~90 | ~105 | 2.5 |
| | (30) | **ciw 3** | (T26I20) | GAAACTCAATGAAATCCACTT | TGAACTTGTTGTGAGCTTTGA | 230 | ~200 | ~230 | 2.5 |
| | (50) | **nga 1126** | (F10A12) | CGCTACGCTTTTCGGTAAAG | GCACAGTCCAAGTCACAACC | 191 | 199 | 199 | 2.0 |
| | (73) | **nga 168** | (T7F6) | TCGTCTACTGCACTGCCG | GAGGACATGTATAGGAGCCTCG | 151 | 135 | 135 | 2.0 |
| III | (20) | **nga 162** | (MDC16) | CATGCAATTTGCATCTGAGG | CTCTGTCACTCTTTTCCTCTGG | 107 | 89 | 85 | 1.0 |
| | (43) | **ciw 11** | (MFE16) | CCCCGAGTTGAGGTATT | GAAGAAATTCCTAAAGCATTC | 179 | ~230 | ~240 | 2.5 |
| | (70) | **ciw 4** | (F18B3) | GTTCATTAAACTTGCGTGTGT | TACGGTCAGATTGAGTGATTC | 190 | ~215 | ~190 | 2.5 |
| | (86) | **nga 6** | (T17J13) | TGGATTTCTTCCTCTCTTCAC | ATGGAGAAGCTTACACTGATC | 143 | 123 | 131 | 1.0 |
| IV | (10) | **ciw 5** | (T15B16) | GGTTAAAAATTAGGGTTACGA | AGATTTACGTGGAAGCAAT | 164 | ~144 | ~164 | 2.0 |
| | (47) | **ciw 6** | (T6G15) | CTCGTAGTGCACTTTCATCA | CACATGGTTAGGGAAACAATA | 162 | ~148 | ~135 | 2.0 |
| | (65) | **ciw 7** | (F17L22) | AATTTGGAGATTAGCTGGAAT | CCATGTTGATGATAAGCACAA | 130 | ~123 | ~150 | 2.0 |
| | (104) | **nga 1107** | (T9A14) | GCGAAAAAACAAAAAAATCCA | CGACGAATCGACAGAATTAGG | 150 | 140 | 140 | 1.5 |
| V | (10) | **CTR1** | (F7P15) | CCACTTGTTTCTCTCTCTAG | TATCAACAGAAACGCACCGAG | 159 | 143 | 145 | 2.5 |
| | (42) | **ciw 8** | (MQJ16) | TAGTGAAACCTTTCTCAGAT | TTATGTTTTCTTCAATCAGTT | 100 | ~135 | ~100 | 2.0 |
| | (71) | **PHYC** | (MIK22) | CTCAGAGAATTCCCAGAAAAATCT | AAACTCGAGAGTTTTGTCTAGATC | 207 | 222 | 222 | 2.0 |
| | (88) | **ciw 9** | (MFO20) | CAGACGTATCAAATGACAAATG | GACTACTGCTCAAACTATTCGG | 165 | ~145 | ~140 | 1.0 |
| | (115) | **ciw 10** | (MSL3) | CCACATTTTCCTTCTTTCATA | CAACATTTAGCAAATCAACTT | 140 | ~130 | ~135 | 2.0 |