Purification of Plant DNA using the BioSprint 96

1. Prepare four S-Blocks (slots 2-5) and two 96-well microplates (slots 6 and 7) according to the table below. The S-Blocks and microplates are loaded onto the worktable in step 8.

Note: In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1-H1 to A6-H6 of each plate or block).

2. Transfer 300* µl cleared plant lysate into each well of an S-Block, although this is an approximation one should transfer as much clear lysate as possible and will vary with the amount of RLT buffer added.

3. Add 200 µl isopropanol to each sample in the S-Block.

4. Add 20 µl MagAttract Suspension G to each sample in the S-Block.

Note: Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.

5. Switch on the BioSprint 96 at the power switch.

6. Slide open the front door of the protective cover.

7. Select the protocol "BS96 DNA Plant" using the A and Y keys on the

   BioSprint 96 workstation. Press "Start" to start the protocol run.

8. The LCD displays a message asking you to load slot 7 of the worktable with the 96-rod cover (see the table, page 22). After loading slot 7, press "Start". The worktable rotates and a new message appears, asking you to load slot 6 with the elution plate. Load slot 6 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.

Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

9. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.

   See the BioSprint 96 User Manual for safety information.

10. Press "Start" to start sample processing.

11. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate , or centrifuged. (see the Appendix, page 27.

12. Press "Stop" after all plates and blocks are removed.

13. Discard the used plates, blocks, and 96-rod cover according to EHS regulations.

Note: See page 6 of the Biosprint DNA Plant Handbook for safety information.

14. Switch off the BioSprint 96 at the power switch.

15. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

Slot	Message when loading	Plate/block	To add	Volume to add per well (µl)
7	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
6	Load Elution	96-well microplate MP	TE	200
5	Load Wash 4	S-Block	Wash Buffer*	500
4	Load Wash 3	S-Block	Ethanol (96–100%)	500
3	Load Wash 2	S-Block	Ethanol (96–100%)	500
2	Load Wash 1	S-Block	Buffer RPW	500
1	Load Lysate	S-Block	Lysate[t]	420**

* Contains 0.02% (v/v) Tween 20. To make 50 ml of wash buffer, enough for one plate add 10 ul of Tween 20 to 50 ml distilled water.

[t] Added in steps 2, 3, and 4; includes volume of cleared plant lysate, isopropanol, and MagAttract Suspension G.

** Total, but lysate volume will vary µl other volume is mag particles and isopropanol

Preparation notes:
Label plates before adding ethanol, wash buffer, RPW, etc. all are clear liquids and are indistinguishable. All plates can be prepared the day before to expedite extraction process.

Load isopropanol into S-block 1, add approximately 20 ml into reservoir and dispense with multi-channel pipette, 200 ul per well. Add votexed Mag Attract suspension to these wells, 20 ul per well.

Buffer RPW supplied as concentrated, add 125 ml of isopropanol and 1 vial of RNase(220ul) to each bottle of RPW follow label directions, store in fridge.

Load ethanol into S-blocks utilizing electronic multi-channel Finnpipette with 1.5 ml multistepper tips. Total ethanol volume for this procedure is 96 ml, load approximately 100 ml into reservoir and aliquot into S-blocks 3 and 4, 500 ul per well.