

Protocol: Purification of DNA from Plant Tissue Using the BioSprint 96

This protocol is for purification of genomic, chloroplast, and mitochondrial DNA from plant material using the BioSprint 96 workstation and the BioSprint 96 DNA Plant Kit. We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material.

Important points before starting

- The optimal amount of starting material depends on the plant type and its state (fresh or lyophilized). We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material. In some cases, up to 100 mg fresh plant material can be used. We strongly recommend performing a preliminary experiment with different amounts of starting material.
- Ensure that you are familiar with operating the TissueLyser II and the BioSprint 96. Refer to the *TissueLyser II Handbook* and the *BioSprint 96 User Manual* for operating instructions.

Things to do before starting

- Ensure that Buffer RPW has been prepared as described on page 12.
- 96-rod covers are supplied either in packs of two, or as a pack of one inserted into an S-Block. If using a new pack of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Disruption of plant tissue using the TissueLyser II

1. Place a plant tissue sample into each tube of two collection microtube racks.

Keep the clear covers from the collection microtube racks for use in step 4. If processing fresh or frozen plant tissue, 30 mg of starting material is sufficient. Do not use more than 50 mg (wet weight) unless preliminary experiments suggest that the optimal amount is higher. Up to 30 mg of lyophilized material may be used.

2. Add one 3 mm tungsten carbide bead to each collection microtube, and seal the tubes with the caps supplied. If using fresh or frozen plant material, proceed to step 3. If using lyophilized plant material, proceed directly to step 4.

Note: Buffer RLT may corrode tungsten carbide beads if samples are stored for more than 6 hours.

For some samples, such as small seeds, one 5 mm stainless steel bead can be used or two 3 mm tungsten carbide beads.

3. Cool the collection microtubes in liquid nitrogen for 30 s. Ensure that the collection microtubes remain tightly closed.
4. Place a clear cover (saved from step 1) over each rack of collection microtubes, and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the collection microtubes. If processing frozen plant material, ensure that no liquid nitrogen remains, but do not allow the plant material to thaw. Remove the clear cover.
5. Place each rack of collection microtubes between the adapter plates of the TissueLyser Adapter Set 2 x 96, and fix into the TissueLyser II clamps as described in the *TissueLyser II Handbook*. Ensure that the collection microtubes are properly sealed with caps.

Important: Two plate sandwiches must be clamped to the TissueLyser II to provide balance. To process 96 or fewer samples, assemble a second plate sandwich using a rack of collection microtubes containing tungsten carbide beads but no samples or buffers, and fix it into the empty clamp.

6. Homogenize the samples for 1 min at 30 Hz.
7. Remove and dismantle the plate sandwiches. Ensure that the collection microtubes are tightly closed. If using lyophilized plant material, proceed directly to step 8. If using fresh or frozen plant material, cool the collection microtubes again in liquid nitrogen for 30 s.

8. Knock the racks against the bench 5 times to ensure that the tungsten carbide beads can move freely within the collection microtubes. Reassemble the plate sandwiches so that the collection microtubes nearest the TissueLyser II in steps 5 and 6 are now outermost. Reinsert the plate sandwiches into the TissueLyser II clamps.

Rotating the racks of collection microtubes ensures that all samples are thoroughly disrupted.

Important: Merely rotating the entire plate sandwich so that the QIAGEN logos are upside down when reinserted into the TissueLyser II is not sufficient since the samples that were outermost during the initial disruption will remain outermost in the second disruption step.

9. Homogenize the samples for 1 min at 30 Hz.
10. Remove and dismantle the plate sandwiches. Knock the racks against the bench 5 times to ensure that no tissue powder remains in the caps. Carefully remove the caps and immediately pipet 300 μ l Buffer RLT into each collection microtube.
Note: Buffer RLT may corrode tungsten carbide beads if samples are stored for more than 6 h.
11. Reseal the tubes with the caps and manually shake the entire rack in an upright position 20 times back and forth. Ensure that the collection microtubes remain tightly closed.
Vortex the rack of collection microtubes upside down at full speed for 20 s.
12. Centrifuge the rack at 6000 $\times g$ for 5 min at room temperature (15–25°C).
13. Proceed with "Purification of DNA using the BioSprint 96", below.

Purification of DNA using the BioSprint 96

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

Note: For elution of DNA, use distilled water that does not contain Tween 20.

Table 2. Preparation of S-Blocks and microplates

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

* Contains 0.02% (v/v) Tween 20.

† Added in steps 2, 3 and 4: includes volume of cleared plant lysate, isopropanol and MagAttract Suspension G.

- Transfer 200 µl cleared plant lysate into each well of an S-Block.
- Add 200 µl isopropanol to each sample in the S-Block.
- 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 first use and for 1 min before subsequent uses.

- Switch on the BioSprint 96 at the power switch.
- Slide open the front door of the protective cover.
- Select the protocol “BS96 DNA Plant” using the ▲ and ▼ keys on the BioSprint 96 workstation. Press “Start” to start the protocol run.
- The LCD displays a message asking you to load slot 7 of the worktable with the 96-rod cover (Table 2, page 23). 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.

Warning: Avoid contact with moving parts during operation of the BioSprint 96. 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 (see 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 your local safety regulations.

Note: See page 4 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.

Note: Do not use bleach as disinfectant. See page 4 for safety information.