# QIAGEN Supplementary Protocol:

# Purification of PCR products using the BioSprint 96 workstation

This protocol is for purification of single- or double-stranded PCR products (100 bp – 10 kb) from up to 96 amplification reactions (100 µl) per run using the BioSprint 96 workstation.

## Introduction

The BioSprint 96 workstation uses MagAttract® magnetic-particle technology for rapid purification of PCR products from amplification reactions. MagAttract technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA binds to the silica surface of the magnetic particles in the presence of a chaotropic salt. DNA bound to the magnetic particles is then efficiently washed. The magnetic particles are washed twice with Buffer PE, followed by a rapid rinse with distilled water, which considerably improves the purity of the DNA fragments. High-quality DNA is eluted in Buffer EB. The automated purification procedure completely removes enzymes, nucleotides, and other contaminants and inhibitors. Purified DNA is suitable for direct use in downstream applications, such as sequencing and microarray analysis.

**Note**: BioSprint 96 workstations purchased before 31 March 2005 will need to have the protocol "BS96 PCR Cleanup" installed. For more information, please contact one of the QIAGEN Technical Service Departments or local distributors.

**IMPORTANT**: Please read the *BioSprint 96 User Manual*, paying careful attention to the safety information, before beginning this procedure.

## Storage

All buffers and reagents can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

## Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

Buffer PM contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt

liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the BioSprint 96 workstation, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to components of the BioSprint PCR Purification procedure:

**Buffer PM**

Contains guanidine hydrochloride and isopropanol: harmful, flammable, irritant. Risk and safety phrases:* R10-22-36/38 S13-26-36-46

**24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

## Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioSprint 96 workstation, cat. no. 9000852
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- Large 96-Rod Cover (16), cat. no. 1031668
- 96-Well Microplates MP (20), cat. no. 1031656
- S-Blocks (24), cat. no. 19585
- MagAttract Suspension G† (1.6 ml), cat. no. 1026883 or MagAttract Suspension G† (13 ml), cat. no. 1026901
- Buffer PM (500 ml), cat. no. 19083‡
- Buffer PE (concentrate, 100 ml), cat. no. 19065
- Buffer EB (250 ml), cat. no. 19086

* R10: Flammable; R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; S13: Keep away from food, drink, and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show container or label.
† Contains sodium azide as a preservative.
‡ Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 1 for safety information.

- Ethanol (96–100%)*
- Distilled water
- Tween® 20
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable pipet tips with aerosol barriers (e.g., Finnpipette® Digital and Finntip® FIlters from Thermo Electron) †
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean worktable
- Disposable gloves

## Important notes

### Starting material and elution volumes

| | |
|---|---|
| Sample: | PCR product (100 bp – 10 kb) |
| Sample volume: | 10–100 µl (for sample volumes <100 µl, increase the volume to 100 µl using Buffer PM) ‡ |
| Elution volume: | 50–200 µl§ |
| Recovery of DNA: | >70% (depends on elution volume and fragment length) |

### Yield and concentration of purified DNA

DNA yields depend on the DNA content in the sample and the volume of buffer used for elution. Elution in smaller volumes increases the final DNA concentration in the eluate. The elution volume in the protocol can be adjusted within the range shown to give a yield and concentration of high-quality DNA appropriate for the intended downstream application.

## Important point before starting

- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.

* Do not use denatured alcohol, which contains other substances such as methanol or methyethylketone.
† This is not a complete list of suppliers and does not include many important vendors of biological supplies.
‡ The sample volume defined in the protocol can be increased to 200 µl using BioSprint software. If 200 µl samples are to be processed, increase the volume of Buffer PM and MagAttract Suspension G, accordingly.
§ The elution volume defined in the protocol can be changed using BioSprint Software. We do not recommend using an elution volume of less than 50 µl.

## Things to do before starting

- Add ethanol (96–100%) to the bottle containing Buffer PE before use (see bottle label for volume). Tick the check box on the bottle to indicate that ethanol has been added. Buffer PE should be stored tightly capped to prevent evaporation of ethanol.
- Add Tween 20 to the distilled water at a concentration of 0.02% (v/v) (e.g., add 50 µl Tween 20 to 250 ml distilled water).
- To ensure that the magnetic silica particles are fully resuspended, MagAttract Suspension G must be shaken and vortexed before use. Before the first use, shake the vial or bottle, and vortex for 3 minutes. Before subsequent uses, shake the bottle, and vortex for 1 minute.

## Procedure

1. **Prepare four S-Blocks (slots 1–4) and two 96-well microplates (slots 5 and 6) according to Table 1. 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).

   **Table 1. BioSprint 96 Workstable Setup and Reagent Volumes**

| Slot | Message when loading | Plate/block | To add | Volume to add per well (µl) |
|---|---|---|---|---|
| 6 | Load Rod Cover | 96-well microplate MP | Large 96-rod cover | — |
| 5 | Load Elution | 96-well microplate MP | Buffer EB | 100 |
| 4 | Load $H_2O$ Rinse | S-Block | Distilled water* | 500 |
| 3 | Load PE Wash II | S-Block | Buffer PE | 500 |
| 2 | Load PE Wash I | S-Block | Buffer PE | 500 |
| 1 | Load Sample | S-Block | Sample† | 300 |

   * Contains 0.02% (v/v) Tween 20.
   † Added at steps 2, 3, and 4; includes volume of amplification reaction, Buffer PM, and MagAttract Suspension G.

2. **Pipet 100 µl sample into each well of an S-Block.**
3. **Add 180 µl Buffer PM to each sample in the S-Block.**
4. **Add 20 µl MagAttract Suspension G to each sample in the S-Block. 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 PCR Cleanup" using the ^ and v keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.**
8. **The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see Table 1, page 4). After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 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.**
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 "Safety Information", page 1.
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 "Safety Information", page 1.

## Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information in this protocol or molecular biology applications (visit www.qiagen.com for contact information).

| | Comments and suggestions |
|---|---|
| **Low or no recovery** | |
| a) Buffer PE did not contain ethanol | Ethanol must be added to Buffer PE (concentrate) before use. Repeat procedure with correctly prepared Buffer PE. |
| b) Inappropriate elution buffer | DNA will only be eluted efficiently in the presence of low-salt buffer (e.g., Buffer EB: 10 mM Tris·Cl, pH 8.5.) or water. Maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water to elute, make sure that the pH is within this range. In addition, DNA must be stored at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent. We do not recommend using TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for elution since EDTA may inhibit subsequent enzymatic reactions. |
| c) MagAttract Suspension G was not completely resuspended | Before starting the procedure, ensure that the MagAttract Suspension G is fully resuspended. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses. |

### Minimizing magnetic particle carryover in the DNA

Carryover of magnetic particles in the eluates should not affect the performance of the DNA in downstream applications. However, for sensitive downstream applications, any trace amounts of magnetic particles should be minimized using a magnet.

Transfer the eluates to 1.5 ml microcentrifuge tubes. Apply the tubes to a suitable magnet (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) for 10 minutes, and carefully remove the supernatants. Alternatively, transfer the eluates to a flat-bottom microplate (e.g., QIAGEN 96-Well Microplate FB, cat. no. 36985). Apply the microplate to a suitable magnet (e.g., QIAGEN 96-Well Magnet Type A, cat. no. 36915) for 10 minutes, and carefully remove the supernatants.

If a suitable magnet is not available, transfer the eluates to microcentrifuge tubes, centrifuge for 1 minute at full speed to pellet any remaining magnetic particles, and carefully remove the supernatants.

The BioSprint 96 workstation is intended for life science research applications. No claim or representation is intended for its use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the BioSprint 96 workstation for any particular use, since its performance characteristics have not been validated for any specific organism. The BioSprint 96 workstation may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .