QIAGEN Supplementary Protocol:

Purification of PCR products using the BioSprint 15 workstation

This protocol is for purification of single- or double-stranded PCR products (100 bp – 10 kb) from 1–15 amplification reactions (100 µl) per run using the BioSprint 15 workstation.

Introduction

The BioSprint 15 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 15 workstations purchased before 31 March 2005 will need to have the protocol "BS15 PCR Cleanup" installed. For more information, please contact one of the QIAGEN Technical Service Departments or local distributors.

IMPORTANT: Please read the *BioSprint 15 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 15 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 15 workstation, cat. no. 9000850
- BioSprint 15 Plasticware (130), cat. no. 1030058
- 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
- Ethanol (96–100%)[§]
- Distilled water

* 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.

[§] Do not use denatured alcohol, which contains other substances such as methanol or methyethylketone.

- Tween® 20
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Multidispenser (e.g., Finnpipette® Stepper from Thermo Electron)*
- Tubes for storage of purified DNA
- 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 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

* 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 6 µl Tween 20 to 30 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.

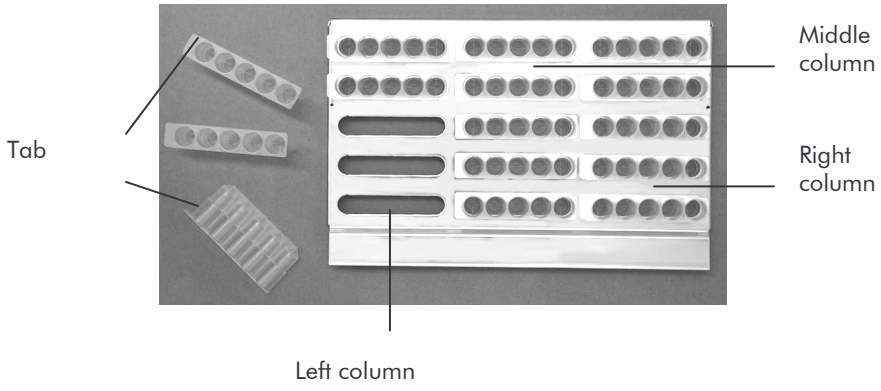
Procedure

1. **Switch on the BioSprint 15 at the power switch.**
2. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
3. **Load up to fifteen 5-tube strips into the tube strip tray. One 5-tube strip is used per sample.**

   If loading five 5-tube strips or fewer, we recommend loading them as a single column. If loading ten 5-tube strips or fewer, we recommend loading them as 2 columns.

   Load the 5-tube strips in the tube strip tray so that the tab of each 5-tube strip faces to the left. Make sure that the 5-tube strips are fully inserted into the tray and are not skewed.

Correct Loading of 5-Tube Strips in the Tube Strip Tray



4. **Add reagents into each 5-tube strip according to the table on the next page.**

   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.

Well	Reagent	Volume of reagents (µl)
1	Buffer PM	180
1	MagAttract Suspension G	20
2	Buffer PE	500
3	Buffer PE	500
4	Distilled water*	500
5	Buffer EB	100

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

Note: Well 1 is at the left of the 5-tube strip, well 5 is at the right.

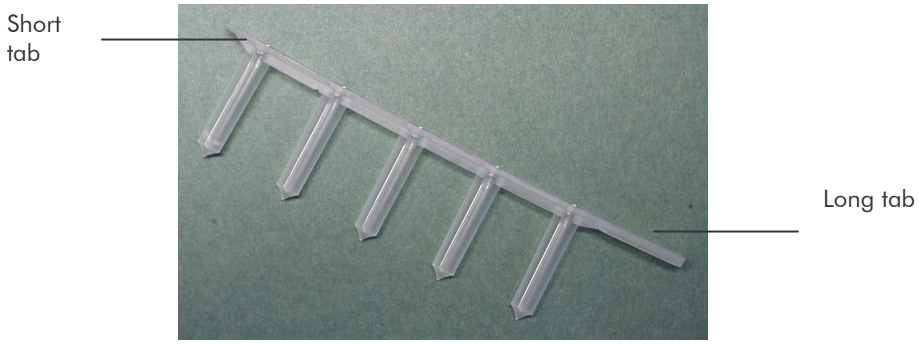
5. **Pipet 100 µl sample into well 1 of the 5-tube strip.**
6. **Load up to three 5-rod covers into the rod cover slots. There must always be a 5-rod cover above a column of 5-tube strips.**

   Note: If necessary, remove the tube strip tray to allow easier loading of the 5-rod covers.

Rod Cover Slot

Insert a 5-rod cover into a rod cover slot so that the short tab faces inward and the long tab faces outward. 5-rod covers must be inserted so that they click into place.

Tabs of the 5-Rod Cover



IMPORTANT: Do not push 5-rod covers further after they click into place; otherwise an instrument crash will occur.

7. **Slide the tube strip tray fully into the BioSprint 15.**
8. **Close the front door of the BioSprint 15.**
   Closing the front and top doors protects the samples from contamination.
9. **Select the protocol "BS15 PCR Cleanup" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**
   Warning: Avoid contact with moving parts during operation of the BioSprint 15. See the *BioSprint 15 User Manual* for safety information.
10. **After the protocol run ends, press "STOP" and slide out the tube strip tray. Transfer the eluted DNA from well 5 of each 5-tube strip to other tubes for long-term storage.**
    Note: Well 5 is at the right of the 5-tube strip.
11. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**
    Note: See page 1 for safety information.
12. **Switch off the BioSprint 15 at the power switch.**
13. **Wipe the surface of the tube strip tray and adjacent surfaces with a soft cloth or tissue moistened with distilled water or a mild detergent solution. If infectious agents are spilt onto the tube strip tray, clean using 70% ethanol or other disinfectant.**
    Note: Do not use bleach as disinfectant. See page 1 for safety information.

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 15 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 .