June 2012

BioSprint® 15 DNA Handbook

For purification of DNA from

- human whole blood
- animal whole blood
- buffy coat
- cultured cells
- tissues
- rodent tails
- buccal swabs
- dried blood spots

using the BioSprint 15 workstation

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

BioSprint 15 DNA Blood Kit	(45)	(360)
Catalog no.	**940014**	**940017**
Number of preps*	**45**	**360**
Buffer AL†	12 ml	3 x 33 ml
QIAGEN® Protease	1 vial‡	2 vials§
Protease Solvent¶	1.2 ml	2 x 4.4 ml
MagAttract® Suspension G¶	1.6 ml	13 ml
Buffer AW1† (concentrate)	19 ml	1 x 19 ml 1 x 98 ml
Buffer AW2 (concentrate)	17 ml	2 x 81 ml
Buffer AE	15 ml	128 ml
5-Rod Cover	10	72
5-Tube Strip (1 ml)	45	360
Quick-Start Protocol	1	1

* When each prep is from 200 µl blood.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

‡ Resuspension volume 1.2 ml.

§ Resuspension volume 4.4 ml.

¶ Contains sodium azide as a preservative.

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.

Lyophilized QIAGEN Protease can be stored dry at room temperature for up to 1 year without any decrease in performance. For storage longer than 1 year or if ambient temperatures constantly exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for up to 2 months when stored at 2–8°C. Storing reconstituted QIAGEN Protease at room temperature for prolonged periods should be avoided. Reconstituted QIAGEN Protease can be stored at –20°C for up to 6 months, but repeated freezing and thawing should be avoided. We recommend dividing the reconstituted QIAGEN Protease into aliquots before storing at –20°C.

Intended Use

The BioSprint 15 DNA Blood Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). 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 SDS for each QIAGEN kit and kit component.

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

Buffers AL and AW1 contain 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.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of BioSprint 15 DNA Blood Kit is tested against predetermined specifications to ensure consistent product quality.

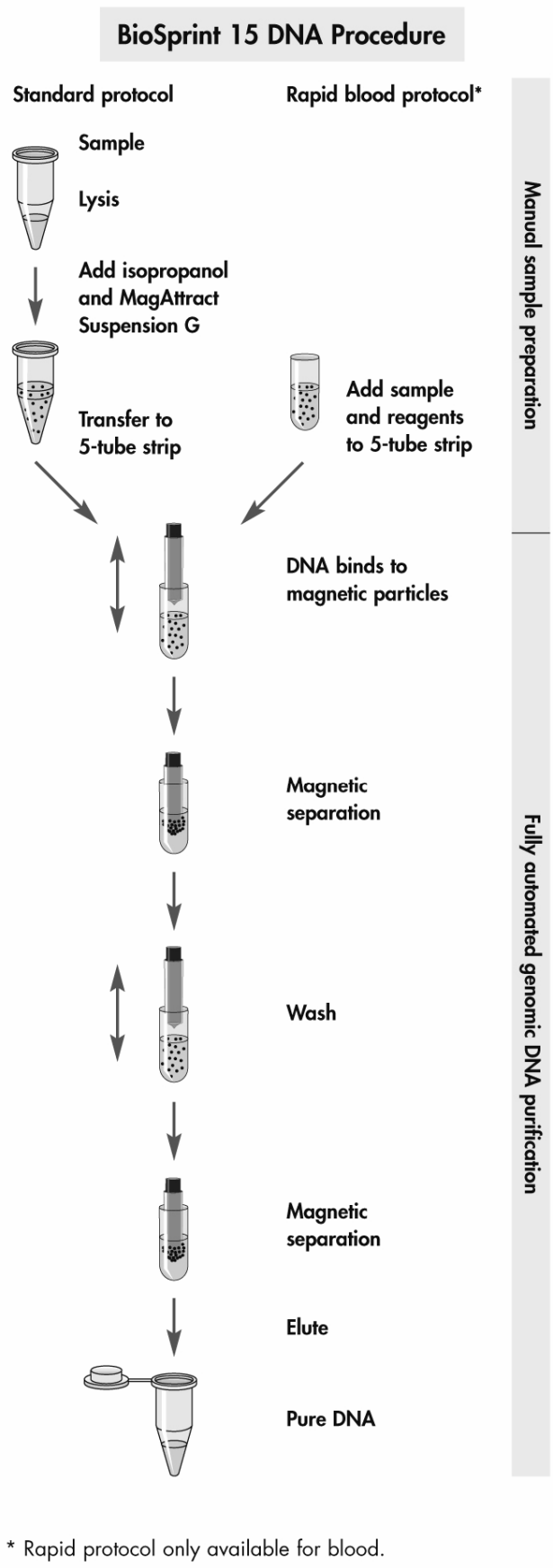
Introduction

The BioSprint 15 DNA Blood Kit is designed for purification of total DNA (i.e., genomic and mitochondrial DNA) from whole blood, buffy coat, cultured cells, tissues, rodent tails, buccal swabs, dried blood spots, and other samples using the BioSprint 15 workstation. The BioSprint 15 DNA Blood Kit provides high-quality DNA that is free of protein, nucleases, and other contaminants or inhibitors. The DNA is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions.

Principle and procedure

The BioSprint 15 DNA Blood Kit uses MagAttract magnetic-particle technology for DNA purification. MagAttract technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, next page). DNA binds to the silica surface of MagAttract magnetic particles in the presence of a chaotropic salt. DNA bound to the magnetic particles is then efficiently washed. Two different wash buffers are used, followed by a rapid rinse with distilled water or an air drying step, which considerably improves the purity of the DNA. High-quality DNA is eluted in Buffer AE. DNA yields depend on sample type, sample storage, and, if purifying from whole blood, white blood cell content.

Supplementary protocols for processing other sample types or for purification of different target molecules using the BioSprint 15 workstation are available at www.qiagen.com/literature/protocols or from QIAGEN Technical Services. BioSprint Software protocols for automated sample processing are available from QIAGEN Technical Services.



* Rapid protocol only available for blood.

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 safety data sheets (SDSs), available from the product supplier.

- BioSprint 15 workstation (cat. no. 9000850)
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Multidispenser (e.g., Finnpipette® Stepper from Thermo Electron see www.thermo.com)*
- Water bath or a shaker–incubator (not required for rapid blood protocol) (e.g., Eppendorf® Thermomixer Comfort, cat. no. 5355 000.011)*
- Ethanol (96–100%)†
- Isopropanol (100%)
- Phosphate-buffered saline (PBS) (may be required for diluting samples)
- Buffer AE, cat. no. 19077 (may be required for diluting samples)
- Disposable gloves
- Vortexer
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the BioSprint worktable
- Buffer ATL, cat. no. 19076, if processing tissues, rodent tails, swabs, or dried blood spots
- QIAGEN Proteinase K (2 ml), cat. no. 19131, or QIAGEN Proteinase K (10 ml), cat. no. 19133, if processing tissues, rodent tails, swabs, or dried blood spots
- DNase-free RNase A (required if purified DNA needs to be RNA-free) (not required if processing swabs or dried blood spots)
- Microcentrifuge tubes (1.5 ml) required for lysis steps when processing blood (not needed for rapid blood protocol), cells, tissues, or rodent tails
- Microcentrifuge tubes (2 ml) required for lysis steps when processing buffy coat, swabs, or dried blood spots
- Microcentrifuge (not needed for rapid blood protocol)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Swabs, such as sterile Omni Swabs (available from Whatman, www.whatman.com), or Puritan® applicators with plastic shafts and cotton or Dacron® tips (available from Hardwood Products, www.hwppuritan.com) if processing buccal swabs*
- Filter paper (e.g., 903® Specimen Collection Paper, Blood Stain Card, or FTA® Card [Whatman, www.whatman.com], or comparable blood cards) if processing dried blood spots. We recommend using 903 Specimen Collection Paper with the BioSprint 15 workstation*
- Manual paper punch, 3–6 mm (1/8–1/4 inch) if processing dried blood spots

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Starting material

The amounts of starting material for use in BioSprint 15 DNA procedures are shown in Table 1.

Table 1. Sample volumes used in BioSprint 15 DNA procedures

Sample	Amount of sample
Whole blood*	100–300 µl
Buffy coat	100–200 µl
Cultured cells	Up to 5 x 10^6 diploid cells
Tissues	Up to 25 mg
Rodent tails	1.2 cm (approximately 25 mg)
Dried blood spots	1–3 punches (3–6 mm [1/8–1/4 inch] diameter)
Buccal swabs	1 swab

* We recommend using 100–200 µl animal blood containing non-nucleated erythrocytes. If necessary, the volume of animal blood used can be reduced and the sample volume adjusted to 200 µl with Buffer AE. For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 20 µl blood and adjust the sample volume to 200 µl with Buffer AE.

Storing blood samples

Whole blood samples treated with EDTA, ACD, or heparin can be used, and may be either fresh or frozen. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results.

For short-term storage of up to 10 days, collect blood in tubes containing EDTA as an anticoagulant, and store at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –70°C.

Preparing buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 900–1100 x *g* for 10 minutes at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should be harvested from 10 ml centrifuged whole blood, which gives 10x enrichment. To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10x enrichment. If buffy coat samples are of >10x enrichment, use less starting material in the DNA purification procedure.

Yield and quality of purified DNA

DNA yields depend on the sample type, the number of nucleated cells in the sample, and the protocol used for DNA purification. Typical DNA yields obtained from a variety of sample types are shown in Table 2, page 13. Elution in smaller volumes increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

The BioSprint 15 DNA procedure yields pure DNA, with A_{260}/A_{280} ratios greater than 1.7. The purified DNA is up to 50 kb in size (Figures 1 and 2, pages 14 and 15), and is suitable for all downstream applications, including Southern blotting, PCR, and real-time PCR (Figure 3, page 15).

Table 2. Typical DNA yields from a range of sample types

Sample type	Amount of sample	Typical DNA yield (µg)*
Bovine tissue		
Muscle	25 mg	16.2 ± 2.5
Heart	25 mg	5.9 ± 2.6
Spleen	25 mg	69.1 ± 23.6
Lung	25 mg	13.8 ± 7.2
Liver	25 mg	77.8 ± 29.4
Kidney	25 mg	26.2 ± 18.8
Sheep tissue		
Ear	30 mg	20.3 ± 1.8
Mouse tissue		
Tail	1.2 cm (~25 mg)	32.7 ± 4.6
Cultured cells		
HL-60	2 x 10^6 cells	10.1 ± 4.7
Blood		
Human (5–7 x 10^6 cells/ml)	200 µl	4.5–9.0
Horse	200 µl	5.5–6.7
Bovine	200 µl	6.2–8.0
Sheep	200 µl	5.6–11.2
Pig	200 µl	4.5–9.0
Dog	200 µl	6.7–12.3
Cat	100 µl	4.4–8.3
Mouse	100 µl	2.0–8.0
Rat	100 µl	1.0–4.0
Bird†	10 µl	15.0 ± 6.3
Fish†	5 µl	7.1–10.0

* Genomic DNA was purified from the indicated samples. † Sample volume adjusted to 200 µl with Buffer AE.

Table 2 continued on next page.

Table 2 continued from previous page.

Sample type	Amount of sample	Typical DNA yield (µg)*
Dried blood spots		
903 Specimen Collection Paper	1 punch (6 mm [1/4 inch] diameter)	0.30
FTA Card	1 punch (6 mm [1/4 inch] diameter)	0.12
Swabs		
Buccal swabs	1 swab	0.8–2.0

* Genomic DNA was purified from the indicated samples.

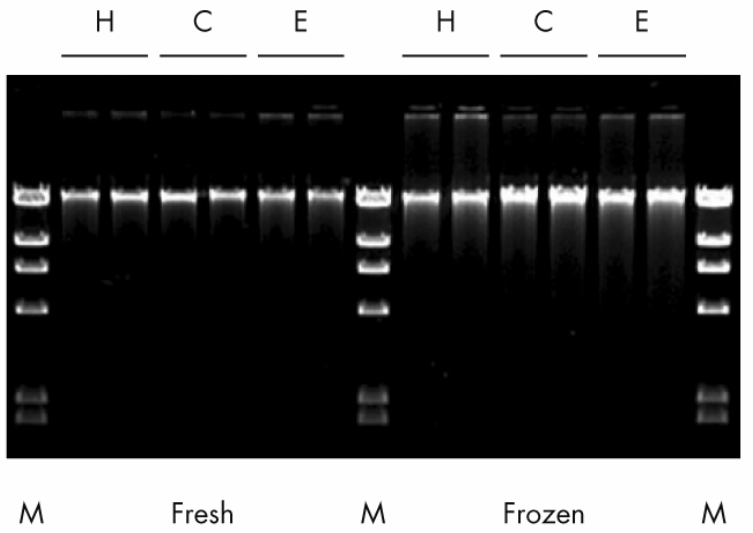


Figure 1. Purification of high-quality DNA from fresh and frozen blood. Human blood was collected and treated with one of 3 anticoagulants: heparin (**H**), citrate (**C**), or EDTA (**E**). DNA was purified from 200 µl blood immediately after blood collection (**Fresh**) and after one cycle of freezing and thawing (**Frozen**) using the BioSprint 15 DNA Blood Kit. DNA was eluted in 200 µl elution buffer. Eluates (15 µl) were run on a 0.8% agarose gel in 1x TBE. **M**: markers (Lambda *Hin*dIII).

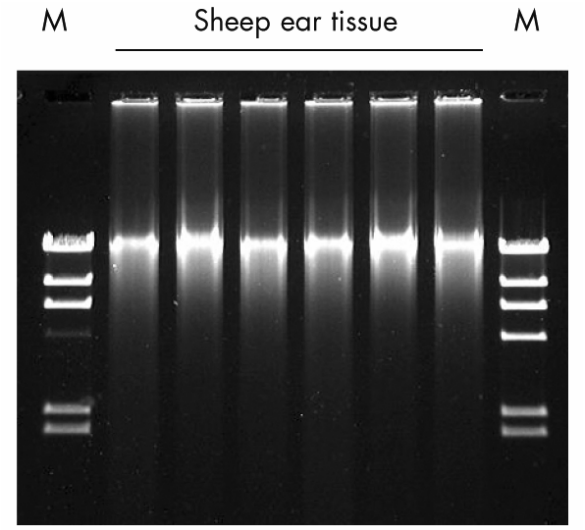


Figure 2. Reproducible purification of genomic DNA from sheep ear tissue. Sheep ear tissue samples were treated with 180 µl Buffer ATL and 20 µl QIAGEN Proteinase K at 56°C overnight. Genomic DNA was purified from the lysed tissue samples using the BioSprint 15 DNA Blood Kit with the BioSprint 15 DNA Tissue protocol. DNA was eluted in 200 µl elution buffer. Eluates (5 µl) were run on a 0.8% agarose gel in 1x TBE. **M**: markers (Lambda *Hin*dIII).

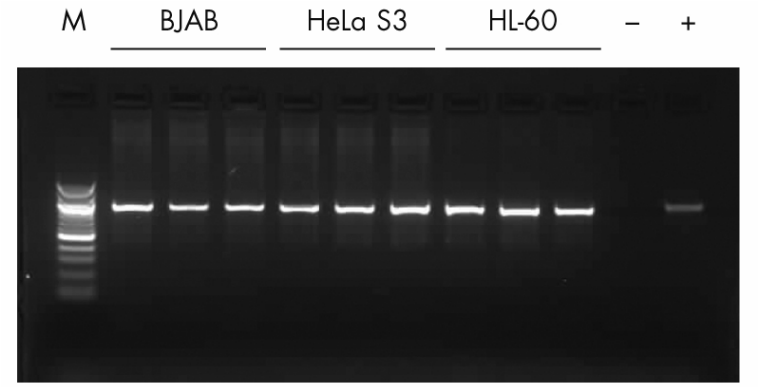


Figure 3. Efficient amplification of the single-copy gene MECL-1. The single-copy gene MECL-1 was amplified using 5 µl purified DNA from the indicated cultured-cell samples in a final reaction volume of 50 µl. Amplification reactions were performed using the QIAGEN *Taq* PCR Core Kit. A 5 µl aliquot of each PCR was run on a 1.5% agarose gel. **–**: negative control; **+**: positive control; **M**: 100 bp ladder.

Preparing reagents

QIAGEN Protease

Pipet Protease Solvent (which is nuclease-free water containing 0.04% (w/v) sodium azide) into the vial containing lyophilized QIAGEN Protease, as described on the vial label.

Reconstituted QIAGEN Protease is stable for up to 2 months when stored at 2–8°C. Storing reconstituted QIAGEN Protease at room temperature (15–25°C) for prolonged periods should be avoided. Reconstituted QIAGEN Protease can be stored at –20°C for up to 6 months, but repeated freezing and thawing should be avoided. We recommend dividing the reconstituted QIAGEN Protease into aliquots before storing at –20°C.

Buffer AL

Mix Buffer AL thoroughly by shaking before use. Buffer AL is stable for up to 1 year when stored at room temperature (15–25°C).

Note: Do not add QIAGEN Protease directly to Buffer AL.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as described on the bottle label; see also Table 3.

Table 3. Preparing Buffer AW1

Volume of AW1 concentrate (ml)	Volume of ethanol to add (ml)	Final volume (ml)
19	25	44
98	130	228

Tick the check box on the bottle to indicate that ethanol has been added. Store reconstituted Buffer AW1 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW1 before use by shaking the bottle 5 times.

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as described on the bottle label; see also Table 4.

Table 4. Preparing Buffer AW2

Volume of AW2 concentrate (ml)	Volume of ethanol to add (ml)	Final volume (ml)
17	40	57
81	190	271

Tick the check box on the bottle to indicate that ethanol has been added. Store reconstituted Buffer AW2 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW2 before use by shaking the bottle 5 times.

MagAttract Suspension G

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.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA, but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See the appendix, page 49, for more information.

Loading 5-tube strips and 5-rod covers into the BioSprint 15

Up to fifteen 5-tube strips can be loaded 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.

5-tube strips are loaded into the tube strip tray so that the tab of each 5-tube strip faces to the left. The 5-tube strips should be fully inserted into the tray and not skewed.

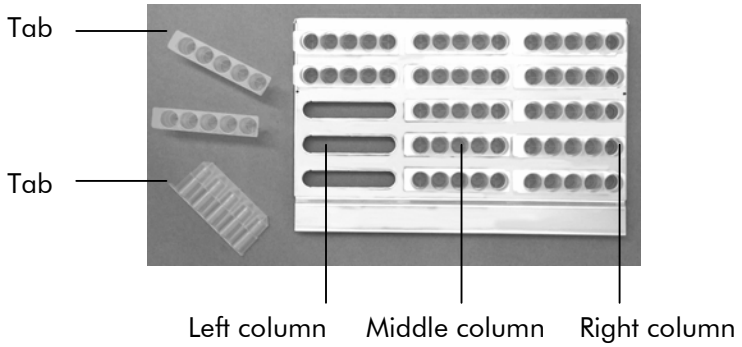


Figure 4. Correct loading of 5-tube strips in the tube strip tray.

Up to three 5-rod covers can be loaded into the rod cover slots. There must always be a 5-rod cover above a column of 5-tube strips.

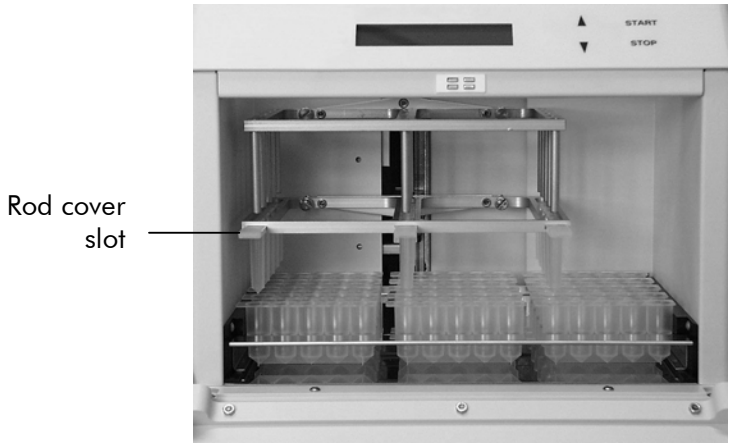


Figure 5. Rod cover slot.

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.

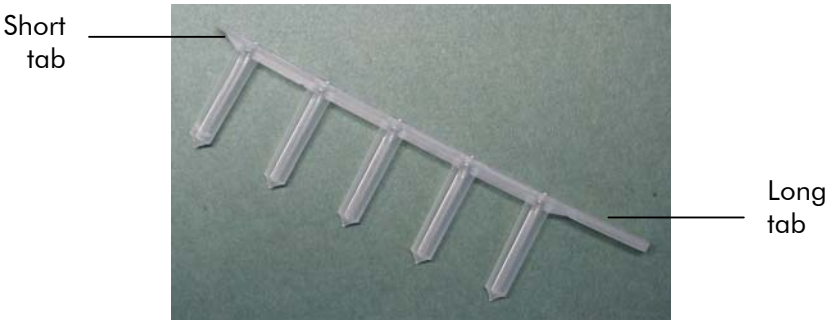


Figure 6. Tabs of the 5-rod cover

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

Protocol: Purification of DNA from Blood

This protocol is for purification of total (genomic and mitochondrial) DNA from whole blood or blood products using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit. Human blood samples can be 100 µl, 200 µl, or 300 µl. Animal blood samples containing non-nucleated erythrocytes can be 100 µl or 200 µl. Buffy coat samples can be 100 µl or 200 µl.

Important points before starting

- Check that QIAGEN Protease, Buffer AW1, and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate by shaking the bottle. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Human blood samples must be in the range of 100–300 µl. Animal blood samples must be in the range of 100–200 µl. If necessary, the volume of animal blood used can be reduced and the sample volume adjusted to 100 µl or 200 µl with Buffer AE. Since bird and fish blood contain nucleated erythrocytes, use less than 20 µl blood and adjust the sample volume to 200 µl with Buffer AE. Buffy coat samples must be 100–200 µl.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.
- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 100 µl blood samples; choose ▲ if processing 200 µl blood samples; choose ● if processing 300 µl blood samples.

Things to do before starting

- Thaw and equilibrate up to 15 whole blood samples at room temperature (15–25°C), or prepare buffy coat samples according to page 12.
- Set a water bath or shaker–incubator to 70°C for use in step 7 of the procedure.
- All samples in a single procedure must have the same volume (100 µl, 200 µl, or 300 µl). If the volume of a sample needs to be increased, add the appropriate volume of PBS (human blood samples) or Buffer AE (animal, bird, and fish blood samples).
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

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. See page 17 for more information.

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

Well	Reagent	Volume of reagent (µl) ■	▲	●
1	Lysate*	325	650	975
2	Buffer AW1	500	700	1000
3	Buffer AW2	500	500	500
4	Buffer AW2	500	500	500
5	Buffer AE	100	200	300

* Added at steps 11 and 12; includes volume of sample, QIAGEN Protease, Buffer AL, isopropanol, and MagAttract Suspension G.

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

5. **Pipet ■ 10 µl, ▲ 20 µl, or ● 30 µl QIAGEN Protease into the bottom of a microcentrifuge tube (not supplied); use a 1.5 ml tube for blood samples and a 2 ml tube for buffy coat samples. Add ■ 100 µl, ▲ 200 µl, or ● 300 µl sample to the QIAGEN Protease.**

   Note: It is possible to add QIAGEN Protease to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding QIAGEN Protease.

6. **Add ■ 100 µl, ▲ 200 µl, or ● 300 µl Buffer AL, and mix by pulse-vortexing for 15 s.**

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

7. **Incubate at 70°C for 10 min.**

   Maximum DNA yields are achieved after lysis at 70°C for 10 min. Longer incubation times should be avoided.

8. **Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.**
9. **Add ■ 100 µl, ▲ 200 µl, or ● 300 µl isopropanol, and mix by pulse-vortexing for 10 s.**
10. **Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.**
11. **Transfer the entire lysate into well 1 of the 5-tube strip.**

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

    Note: If processing more than one sample, record in which 5-tube strips you load the samples.

12. **Add ■ 15 µl, ▲ 30 µl, or ● 45 µl MagAttract Suspension G to the lysate in well 1 of the 5-tube strip.**

    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.

13. **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. See page 17 for more information.**

    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.

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

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

14. **Slide back the tube strip tray fully into the BioSprint 15.**
15. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

16. **Select the protocol ■ "BS15 DNA Blood 100", ▲ "BS15 DNA Blood 200", or ● "BS15 DNA Blood 300" using the ▲ and ▼ keys. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

17. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

18. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

19. **Switch off the BioSprint 15 at the power switch.**
20. **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 5 for safety information.

Protocol: Rapid Purification of DNA from Human Whole Blood

This protocol is for rapid purification of total (genomic and mitochondrial) DNA from human whole blood using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit. Blood samples can be 100 µl, 200 µl, or 300 µl. This shorter protocol has less manual handling steps than the standard protocol (see "Protocol: Purification of DNA from Blood Using the BioSprint 15", page 19), but yield and purity of the purified DNA may be lower.

Important points before starting

- Check that QIAGEN Protease, Buffer AW1 and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- This protocol is suitable for human whole blood. Blood samples must be in the range of 100–300 µl.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.
- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 100 µl blood samples; choose ▲ if processing 200 µl blood samples; choose ● if processing 300 µl blood samples.

Things to do before starting

- Thaw and equilibrate up to 15 whole blood samples at room temperature (15–25°C).
- All samples in a single procedure must have the same volume (100 µl, 200 µl, or 300 µl). If the volume of a sample needs to be increased, add the appropriate volume of PBS.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table on the next page for use in step 6 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, ■ 225 µl, ▲ 450 µl, or ● 650 µl master mix is required per sample (see step 6 of the protocol). The starting volume of master mix should be increased accordingly.

	Volume of reagent per sample (µl)		
Reagent	■	▲	●
Buffer AL	100	200	300
Isopropanol	100	200	300
MagAttract Suspension G	15	30	45

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. See page 17 for more information.

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

Well	Reagent	Volume of reagent (µl) ■	▲	●
1	Lysate*	325	650	975
2	Buffer AW1	500	700	1000
3	Buffer AW2	500	500	500
4	Buffer AW2	500	500	500
5	Buffer AE	100	200	300

* Added at steps 5 and 6; includes volume of sample, QIAGEN Protease, Buffer AL, isopropanol, and MagAttract Suspension G.

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

5. **Pipet ■ 10 µl, ▲ 20 µl, or ● 30 µl QIAGEN Protease into the bottom of well 1 of each 5-tube strip. Add ■ 100 µl, ▲ 200 µl, or ● 300 µl sample to the QIAGEN Protease.**

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

   Note: If processing more than one sample, record in which 5-tube strips you load the samples.

6. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see "Things to do before starting"). Add ■ 215 µl, ▲ 430 µl, or ● 645 µl master mix to each sample in well 1 of each 5-tube strip.**

   Note: If using a multidispenser, add ■ 225 µl, ▲ 450 µl, or ● 650 µl master mix to each sample.

7. **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. See page 17 for more information.**

   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.

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

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

8. **Slide back the tube strip tray fully into the BioSprint 15.**

9. **Close the front door of the BioSprint 15.**

   Closing the front and top doors protects the samples from contamination.

10. **Select the protocol ■ "BS15 DNA Blood 100", ▲ "BS15 DNA Blood 200", or ● "BS15 DNA Blood 300" using the ▲ and ▼ keys. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

11. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

12. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

13. **Switch off the BioSprint 15 at the power switch.**

14. **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 5 for safety information.

Protocol: Purification of DNA from Cultured Cells

This protocol is for purification of total (genomic and mitochondrial) DNA from up to 5 x 10^6 diploid cells per sample using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit.

Important points before starting

- Check that QIAGEN Protease, Buffer AW1, and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Set a water bath or shaker–incubator to 70°C for use in step 8 of the procedure.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the lysate in step 5 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table below for use in step 11 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Reagent	Volume of reagent per sample (µl)
Isopropanol	200
MagAttract Suspension G	30

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. See page 17 for more information.

   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.

4. **Add reagents into each 5-tube strip according to the table below.**

Well	Reagent	Volume of reagent (µl)
1	Lysate*	650
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	200

* Added at steps 10 and 11; includes volume of sample, QIAGEN Protease, Buffer AL, isopropanol, and MagAttract Suspension G.

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

5. **Centrifuge the appropriate number of cells (up to 5 x 10^6) for 5 min at 300 x *g*. Discard the supernatant and resuspend the cell pellet in 200 µl PBS (not supplied).**

   When using a frozen cell pellet, allow cells to thaw until the pellet can be dislodged by gently flicking the tube before adding PBS.

   Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells.

   Optional: RNase treatment of the sample. Add 4 µl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).

6. **Add 20 µl QIAGEN Protease to the sample.**

7. **Add 200 µl Buffer AL and mix by pulse-vortexing for 15 s.**

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogenous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

8. **Incubate at 70°C for 10 min.**

   Maximum DNA yields are achieved after lysis at 70°C for 10 min. Longer incubation times should be avoided.

9. **Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.**

10. **Transfer the entire lysate to well 1 of the 5-tube strip.**

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

    Note: If processing more than one sample, record in which 5-tube strips you load the samples.

11. **Vortex the master mix containing isopropanol and MagAttract Suspension G for 1 min (see "Things to do before starting"). Add 230 µl master mix to each sample in well 1 of each 5-tube strip.**

    Note: If using a multidispenser, add 225 µl master mix to each sample.

12. **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. See page 17 for more information.**

    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.

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

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

13. **Slide back the tube strip tray fully into the BioSprint 15.**

14. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

15. **Select the protocol "BS15 DNA Blood 200" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

16. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

17. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

18. **Switch off the BioSprint 15 at the power switch.**
19. **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 5 for safety information.

Protocol: Purification of DNA from Tissues

This protocol is for purification of total (genomic and mitochondrial) DNA from up to 25 mg of tissue per sample using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit.

Important points before starting

- Buffer ATL and QIAGEN Proteinase K are required for this protocol. See "Equipment and Reagents to Be Supplied by User", page 9.
- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Set a shaker–incubator to 56°C for use in step 3 of the procedure.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the lysate in step 3 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table on the next page for use in step 10 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, 450 µl master mix is required per sample (see step 10 of the procedure). The starting volume of master mix should be increased accordingly.

Reagent	Volume of reagent per sample (µl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	30

Procedure

1. **Cut ≤25 mg of each tissue sample into small pieces. Place a tissue sample into a 1.5 ml microcentrifuge tube (not supplied), and add 180 µl Buffer ATL (not supplied).**
2. **Add 20 µl QIAGEN Proteinase K (not supplied), and close the 1.5 ml microcentrifuge tube.**
3. **Place the 1.5 ml microcentrifuge tube in a shaker–incubator, and incubate at 56°C with shaking until the tissue is completely lysed.**

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

   Optional: Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. If RNA-free genomic DNA is required, add 4 µl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).
4. **Towards the end of proteinase K digestion, switch on the BioSprint 15 at the power switch.**
5. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
6. **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. See page 17 for more information.

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

Well	Reagent	Volume of reagent (µl)
1	Lysate*	630
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	200

* Added at steps 9 and 10; includes volume of sample, Buffer ATL, QIAGEN Proteinase K, Buffer AL, isopropanol, and MagAttract Suspension G.

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

8. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**
9. **Transfer the entire lysate to well 1 of the 5-tube strip.**

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

   Note: If processing more than one sample, record in which 5-tube strips you load the samples.
10. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see "Things to do before starting"). Add 430 µl of master mix to each sample in well 1 of each 5-tube strip.**

    Note: If using a multidispenser, add 450 µl master mix to each sample.
11. **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. See page 17 for more information.**

    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.

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

    Note: Do not push 5-rod covers further after they click into place, otherwise an instrument crash will occur.
12. **Slide back the tube strip tray fully into the BioSprint 15.**
13. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

14. **Select the protocol "BS15 DNA Tissue" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

15. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

16. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

17. **Switch off the BioSprint 15 at the power switch.**
18. **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 5 for safety information.

Protocol: Purification of DNA from Rodent Tails

This protocol is for purification of total (genomic and mitochondrial) DNA from up to 1.2 cm (approximately 25 mg) of rodent tail per sample using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit.

Important points before starting

- Buffer ATL and QIAGEN Proteinase K are required for this protocol. See "Equipment and Reagents to Be Supplied by User", page 9.
- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Set a shaker–incubator to 56°C for use in step 3 of the procedure.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in step 3 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table on the next page for use in step 10 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, 450 µl master mix is required per sample (see step 10 of the procedure). The starting volume of master mix should be increased accordingly.

Reagent	Volume of reagent per sample (µl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	30

Procedure

1. **Cut ≤1.2 cm of each rodent tail sample into small pieces. Place the tissue sample into a 1.5 ml microcentrifuge tube (not supplied), and add 180 µl Buffer ATL (not supplied).**
2. **Add 20 µl QIAGEN Proteinase K (not supplied), and close the 1.5 ml microcentrifuge tube.**
3. **Place the 1.5 ml microcentrifuge tube in a shaker–incubator, and incubate at 56°C with shaking until the tissue is completely lysed.**

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

   Optional: If RNA-free genomic DNA is required, add 4 µl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).
4. **Towards the end of proteinase K digestion, switch on the BioSprint 15 at the power switch.**
5. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
6. **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. See page 17 for more information.

   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.

7. **Add reagents into each 5-tube strip according to the table below.**

Well	Reagent	Volume of reagent (µl)
1	Lysate*	630
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	200

* Added at steps 9 and 10; includes volume of sample, Buffer ATL, QIAGEN Proteinase K, Buffer AL, isopropanol, and MagAttract Suspension G.

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

8. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**
9. **Transfer the entire lysate to well 1 of the 5-tube strip.**

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

   Note: If processing more than one sample, record in which 5-tube strips you load the samples.
10. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see "Things to do before starting"). Add 430 µl of master mix to each sample in well 1 of each 5-tube strip.**

    Note: If using a multidispenser, add 450 µl master mix to each sample.
11. **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. See page 17 for more information.**

    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.

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

    Note: Do not push 5-rod covers further after they click into place, otherwise an instrument crash will occur.
12. **Slide back the tube strip tray fully into the BioSprint 15.**
13. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

14. **Select the protocol "BS15 DNA Tissue" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

15. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

16. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

17. **Switch off the BioSprint 15 at the power switch.**

18. **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 5 for safety information.

Protocol: Purification of DNA from Buccal Swabs

This protocol is for purification of total (genomic and mitochondrial) DNA from buccal swabs using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit. The procedure is optimized for air-dried buccal swabs with cotton or Dacron tips, and brushes or swabs with an ejectable head (e.g., Whatman® Omni Swab). Other swab types may also be used.

Important points before starting

- Buffer ATL and QIAGEN Proteinase K are required for this protocol. See "Equipment and Reagents to Be Supplied by User", page 9.
- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Set a shaker–incubator with an adapter for 2 ml microcentrifuge tubes to 56°C for use in step 4 of the procedure.
- Prepare a master mix according to the table below for use in step 12 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Reagent	Volume of reagent per sample (µl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	20

Procedure

1. **Place the swab in a 2 ml microcentrifuge tube (not supplied).**

   If using an Omni Swab, eject the swab head by pressing the end of the inner shaft towards the swab head.

   If using a cotton or Dacron swab, separate the swab head from its shaft by hand or by using scissors.

2. **If using an Omni Swab, add 500 µl Buffer ATL (not supplied) to the 2 ml microcentrifuge tube. If using a cotton or Dacron swab, add 400 µl Buffer ATL to the 2 ml microcentrifuge tube.**
3. **Add 20 µl QIAGEN Proteinase K (not supplied), close the 2 ml microcentrifuge tube, and mix thoroughly by pulse vortexing for 10 s.**
4. **Place the 2 ml microcentrifuge tube in a shaker–incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.**

   If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

5. **Towards the end of proteinase K digestion, switch on the BioSprint 15 at the power switch.**
6. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
7. **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. See page 17 for more information.

   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.

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

Well	Reagent	Volume of reagent (µl)
1	Lysate*	620
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	200

* Added at steps 11 and 12; includes volume of Buffer ATL, QIAGEN Proteinase K, Buffer AL, isopropanol, and MagAttract Suspension G.

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

9. **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. See page 17 for more information.**

   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.

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

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

10. **Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from the inside of the lid.**
11. **Carefully transfer 200 µl of the lysate to well 1 of the 5-tube strip, leaving the swab in the 2 ml microcentrifuge tube.**

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

    Note: If processing more than one sample, record in which 5-tube strips you load the samples.

12. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see "Things to do before starting"). Add 420 µl master mix to each sample in well 1 of each 5-tube strip.**

    Note: If using a multidispenser, add 400 µl master mix to each sample.

13. **Slide back the tube strip tray fully into the BioSprint 15.**
14. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

15. **Select the protocol "BS15 DNA Swab" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.

16. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).

17. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

18. **Switch off the BioSprint 15 at the power switch.**
19. **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 5 for safety information.

Protocol: Purification of DNA from Dried Blood Spots

This protocol is for purification of total (genomic and mitochondrial) DNA from blood card punches using the BioSprint 15 workstation and the BioSprint 15 DNA Blood Kit. This protocol is suitable for untreated blood or blood treated with anticoagulants, such as EDTA, citrate, or heparin. The blood must be spotted and dried on filter paper according to the manufacturer's instructions. We recommend using 903 Specimen Collection Paper with the BioSprint 15.

Important points before starting

- Buffer ATL and QIAGEN Proteinase K are required for this protocol. See "Equipment and Reagents to Be Supplied by User", page 9.
- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Set a shaker–incubator with an adapter for 2 ml microcentrifuge tubes to 56°C for use in steps 4 and 12 of the procedure.

Procedure

1. **Cut 3 mm (1/8 inch) or 6 mm (1/4 inch) diameter punches from a dried blood spot with a single-hole paper punch. Place up to 3 blood card punches into a 2 ml microcentrifuge tube (not supplied).**
2. **Add 200 µl Buffer ATL (not supplied).**
3. **Add 20 µl QIAGEN Proteinase K (not supplied), close the 2 ml microcentrifuge tube, and mix thoroughly by pulse vortexing for 10 s.**

   Note: Make sure that the punches are fully covered with buffer. If necessary, briefly centrifuge the 2 ml microcentrifuge tube.
4. **Place the 2 ml microcentrifuge tube in a shaker–incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.**
5. **Towards the end of proteinase K digestion, switch on the BioSprint 15 at the power switch.**
6. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**

7. **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. See page 17 for more information.

   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.

8. **Add reagents into each 5-tube strip according to the table below.**

Well	Reagent	Volume of reagent (µl)
1	Lysate*	640
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	125

* Added at steps 16 and 17; includes the volume of Buffer ATL, QIAGEN Proteinase K, Buffer AL, isopropanol, and MagAttract Suspension G.

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

9. **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. See page 17 for more information.**

   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.

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

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

10. **Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from the inside of the lid.**
11. **Add 200 µl Buffer AL, close the 2 ml microcentrifuge tube, and mix by pulse-vortexing for 10 s.**

    Note: Make sure that the punches are fully covered with buffer. If necessary, briefly centrifuge the 2 ml microcentrifuge tube.

12. **Place the 2 ml microcentrifuge tube in a shaker–incubator, and incubate at 56°C with shaking at 900 rpm for 10 min.**
13. **Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from inside the lid.**
14. **Add 200 µl isopropanol, close the lid of the 2 ml microcentrifuge tube, and mix by pulse-vortexing for 10 s.**
15. **Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from inside the lid.**
16. **Carefully transfer the entire lysate from step 15 into well 1 of the 5-tube strip.**

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

    Note: If processing more than one sample, record in which 5-tube strips you load the samples.
17. **Add 20 µl MagAttract Suspension G to the lysate in well 1 of the 5-tube strip.**

    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.
18. **Slide back the tube strip tray fully into the BioSprint 15.**
19. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.
20. **Select the protocol "BS15 DNA Dried Blood" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    See the *BioSprint 15 User Manual* for safety information.
21. **After the protocol run ends, press "STOP", slide out the tube strip tray, and 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.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix, page 49).
22. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See page 5 for safety information.

23. **Switch off the BioSprint 15 at the power switch.**
24. **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 5 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Low DNA yield	
a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.
b) Inefficient cell lysis due to decreased protease activity	Repeat the DNA purification procedure with a new sample and with freshly reconstituted QIAGEN Protease. Be sure to store QIAGEN Protease at 2–8°C immediately after use. Ensure that QIAGEN Protease is not added directly to Buffer AL.
c) No isopropanol added to the lysate before adding MagAttract Suspension G	Repeat the DNA purification procedure with a new sample.
d) 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.
e) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of ethanol (96–100%) (see pages 16–17). Repeat the DNA purification procedure with a new sample.
f) Frozen blood samples were not mixed properly after thawing	Thaw frozen blood samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.

DNA does not perform well in downstream applications

a) Insufficient DNA used in downstream application	Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 49).
b) Excess DNA used in downstream application	Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 49).

A_{260}/A_{280} ratio for purified DNA is low

a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.
b) Inefficient cell lysis due to decreased protease activity	Repeat the DNA purification procedure with a new sample and with freshly reconstituted QIAGEN Protease. Be sure to store QIAGEN Protease at 2–8°C immediately after use. Ensure that QIAGEN Protease is not added directly to Buffer AL.
c) No isopropanol added to the lysate before adding MagAttract Suspension G	Repeat the DNA purification procedure with a new sample.
d) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of ethanol (96–100%) (see pages 16–17). Repeat the DNA purification procedure with a new sample.
e) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see the appendix, page 49).

Appendix: Handling, Quantification, and Determination of Purity of DNA

Storage of DNA

Purified DNA may be stored at 2–8°C for 24 hours or at –20°C for longer periods.

Minimizing magnetic particle carryover in the DNA

If the purified DNA is to be analyzed by real-time PCR, 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) 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.

Quantification and determination of purity of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per ml ($A_{260} = 1 \rightarrow 50$ µg/ml). Use a low-salt buffer of neutral pH (e.g., 10 mM Tris·HCl,* pH 7) to dilute DNA samples and to calibrate the spectrophotometer.

The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity. For accurate results, use a slightly alkaline buffer (e.g., 10 mM Tris·HCl, pH 7.5) to dilute DNA samples and to calibrate the spectrophotometer. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

Carryover of magnetic particles in the eluates may affect the A_{260} and A_{280} readings, but should not affect the performance of the DNA in downstream applications. Measure the absorbance at 320 nm, 280 nm, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Concentration of DNA sample $= 50$ µg/ml x $(A_{260} - A_{320})$ x dilution factor

Total amount of DNA isolated = concentration x volume of sample in ml

Purity of DNA sample $= (A_{260} - A_{320})/(A_{280} - A_{320})$

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

Ordering Information

Product	Contents	Cat. no.
BioSprint 15 DNA Blood Kit (45)	For 45 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	940014
BioSprint 15 DNA Blood Kit (360)	For 360 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	940017
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Buffer AE (240 ml)	240 ml Elution Buffer	19077
Accessories		
BioSprint 15 Plasticware (130)	For 130 preps: 26 x 5-Rod Covers and 130 x 5-Tube Strips for use with the BioSprint 15	1030058
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
BioSprint 96 DNA Blood Kit — for rapid purification of DNA from cells, tissue, blood, buffy coat, buccal swabs, and dried blood spots using the BioSprint 96 workstation		
BioSprint 96 DNA Blood Kit (48)*	For 48 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	940054

* Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
BioSprint DNA Plant Kits — for rapid purification of total DNA from plant tissue using BioSprint workstations		
BioSprint 15 DNA Plant Kit (360)	For 360 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	941517
BioSprint 96 DNA Plant Kit (576)*	For 576 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941557

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Other kit sizes are available; see www.qiagen.com.

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Limited License Agreement for BioSprint 15 DNA Kit

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