Instructions for Use
Life Science Kits & Assays

blackPREP FFPE DNA Kit

Innuscreen GmbH

Order No.:

845-BP-0021010 10 reactions
845-BP-0021050 50 reactions
845-BP-0021250 250 reactions

Publication No.: HB_BP-0021_e_220406

This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **blackPREP FFPE DNA Kit** has been designed as a tool for very fast and efficient isolation of genomic DNA from FFPE tissue samples/ sections. The extraction procedure is based on a new kind of chemistry, which combines an efficient lysis step with a subsequent efficient binding of genomic DNA on a Spin Filter surface followed by washing of the bound DNA and finally eluting of the DNA. The recovery of DNA and the quality are excellent. The new kind of chemistry allows the isolation of DNA from FFPE samples without the deparaffinising step using toxic and hazardous components like octane or xylene.

The **blackPREP FFPE DNA Kit** is not designed for use with other starting materials as described above and the kit performance has not been evaluated for other starting materials.

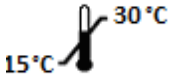
The extraction process is finished within 2.5 hours. The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications like amplification reactions.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> reactions.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Expiry date**
	**Lot number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → „Notes on the use of this manual and the kit “ p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before use to guarantee your own safety and a trouble-free operation. Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don’t eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!

Do not add bleach or acidic components to the waste after sample preparation!

NOTE

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

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

All other components of the blackPREP FFPE DNA Kit should be stored dry at room temperature (15 °C to 30 °C). If stored as recommended, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the blackPREP FFPE DNA Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com.
For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications“ p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	845-BP-0021010	845-BP-0021050	845-BP-0021250
Lysis Solution MA	5 ml	25 ml	120 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Washing Solution C	8 ml	30 ml	135 ml
Washing Solution BS (conc.)	2 ml	10 ml	18 ml
Elution Buffer	2 x 2 ml	15 ml	2 x 30 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- ddH_2O for dissolving **Proteinase K**
- 1.5 ml and 2.0 ml tubes
- 96–99.8 % ethanol, non-denatured or methylated
- RNase A (10 mg/ml); optional

7 Product specifications

1. **Starting material:**
   - FFPE (formalin fixed paraffin embedded) tissue samples
   - Approx. 8 mg (approx. 12 µl) paraffin correspond to:
     ≈ 4 sections of 10 µm thickness and each of 300 mm^2 area
     ≈ 3 sections of 10 µm thickness and each of 400 mm^2 area
     ≈ 2 sections of 10 µm thickness and each of 600 mm^2 area
     ≈ 1 sections of 10 µm thickness and each of 1,200 mm^2 area
   - Maximum amount of tissue: 50 mg

NOTE
It is possible to process more than the amount of starting material indicated above. In such case, it is the costumer's responsibility to validate the blackPREP FFPE DNA Kit for this new purpose.

2. **Time for isolation:**
   - Approximately 2.5 hours (all steps included)

3. **Typical yield:**
   - Depends on type and amount of starting material
   - The extracted genomic DNA (gDNA) can be used for a wide range of different molecular biology applications.

8 Initial steps before starting

- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-BP-0021010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-BP-0021050 845-BP-0021250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to each **bottle Washing Solution BS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-BP-0021010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
845-BP-0021050	Add 90 ml ethanol to 10 ml Washing Solution BS (conc.).
845-BP-0021250	Add 162 ml ethanol to 18 ml Washing Solution BS (conc.).

- Add 1.5 Heat thermal mixer or water bath to 65 °C, followed by 90 °C.
- Centrifugation steps should be carried out at room temperature.

9 Examples of application

9.1 Extraction of different FFPE tissue sample

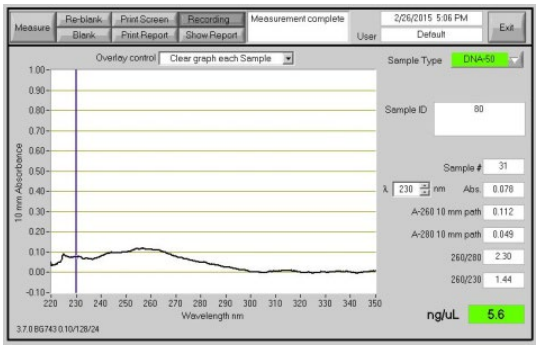
- Extraction of 12 different FFPE tissue samples
  (approx. 3 x 7 µm each sample)
- Spectrophotometric measurement of all 12 samples
- Subsequent amplification of a human specific target sequence

Results

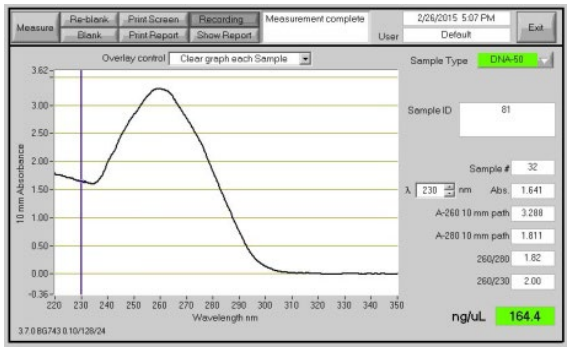
After the extraction process, the amount of isolated DNA (eluted in 100 µl Elution Buffer) was measured by spectrophotometric method and subsequent amplification of a human specific target sequence by Real-time PCR:

Sample ID	DNA [ng/µl]	Ratio A260/280	Ratio A260/230	Ct values	
1	5.78	1.95	1.50	30.88	31.22
2	159.27	1.82	1.96	24.41	23.55
3	50.85	1.70	1.59	27.40	27.51
4	60.80	1.83	2.18	26.85	26.85
5	34.09	1.85	2.23	29.48	29.47
6	14.93	2.03	2.35	27.94	27.91
7	195.22	1.81	2.18	27.43	27.27
8	122.79	1.84	2.28	25.61	25.60
9	56.45	1.80	2.19	28.49	28.72
10	109.9	1.66	1.79	30.21	30.42
11	50.18	1.78	2.22	27.74	28.99
12	15.54	1.85	2.01		

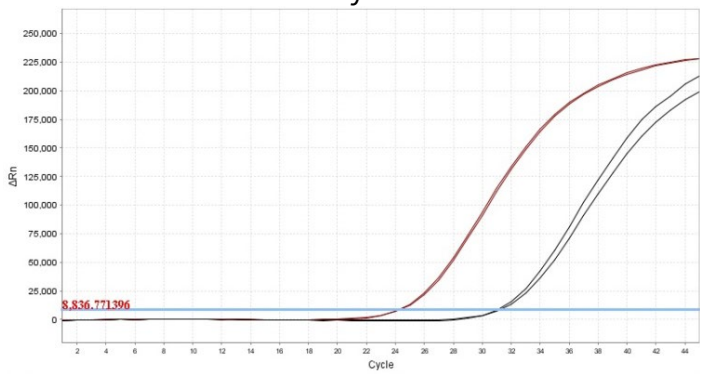
Analysis of sample 1 (adipose tissue) and sample 2 (pancreatic tissue):



Sample 1: FFPE tissue sample with low yield.

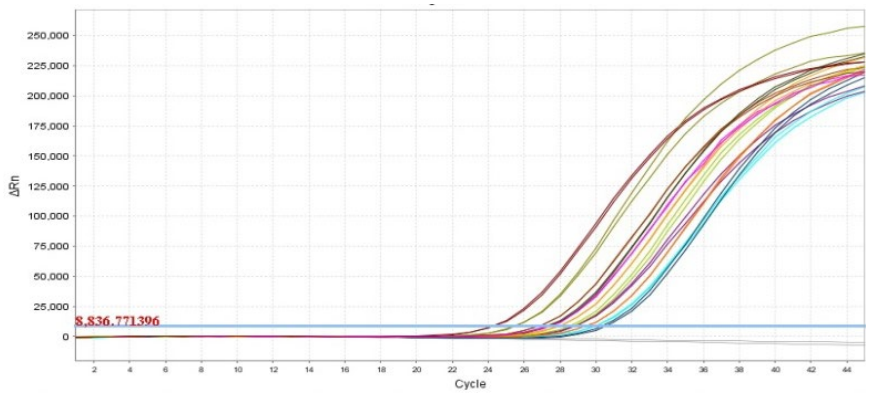


Sample 2: FFPE tissue sample with high yield.



The Ct values of sample 1 (■) and sample 2 (■) correspond to the yield as shown above.

Analysis of sample 3 up to sample 11 (pancreatic tissue) and sample 12 (duodenum tissue):



The Ct values of different FFPE tissue samples correspond to the yield as shown above. Sample 3 (■), sample 4 (■), sample 5 (■), sample 6 (■), sample 7 (■), sample 8 (■), sample 9 (■), sample 10 (■), sample 11 (■) and sample 12 (■).

Data kindly provided by Dr. L.F. Grochola, Department of Research / Centre for Surgery, University Hospital Zurich, Switzerland.

9.2 DNA Extraction trom FFPE samples with different amount of starting material

- DNA Extraction from FFPE tissue samples with different amount of starting material
- Spectrophotometric measurement of all samples
- Subsequent amplification of a human specific target sequence

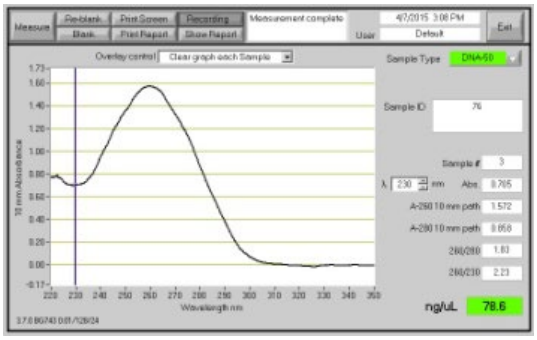
Sample ID	Number of sections	Thickness of sections	Weight of tissue	Paraffin surface
A	4	10 µm	~ 2 mg	1,200 mm^2
B	2	10 µm	~ 2 mg	600 mm^2
C	1	10 µm	~ 2 mg	300 mm^2
D	2	10 µm	~ 4 mg	600 mm^2
E	3	10 µm	~ 6 mg	900 mm^2

Results

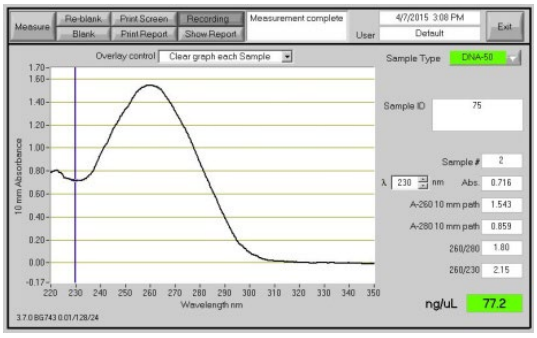
After the extraction process, the amount of isolated DNA (eluted in 100 µl Elution Buffer) was measured by spectrophotometric method and subsequent amplification of a human specific target sequence by Real-time PCR:

Sample ID	DNA [ng/µl]	Ratio A260/280	Ratio A260/230	Ct values
A	78.6	1.83	2.23	29.67 29.58
B	77.2	1.80	2.15	29.63 29.96
C	74.6	1.73	2.12	29.71 29.76
D	116.8	1.83	2.21	28.91 28.75
E	261.5	1.79	2.14	28.28 28.33

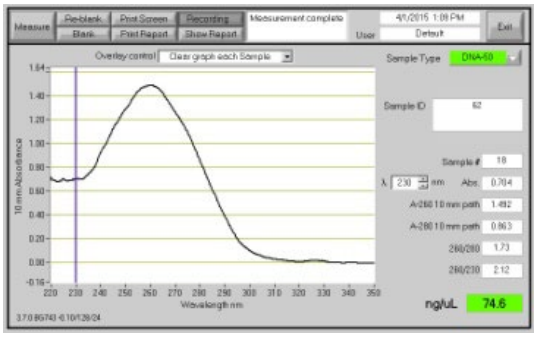
Spectrophotometric analysis of sample A–E:



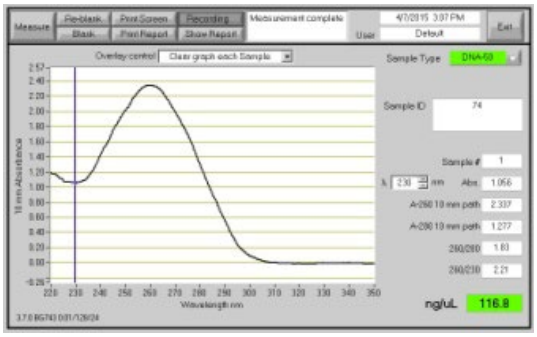
Sample A: 4 sections, 2 mg tissue, 1,200 mm^2 paraffin.



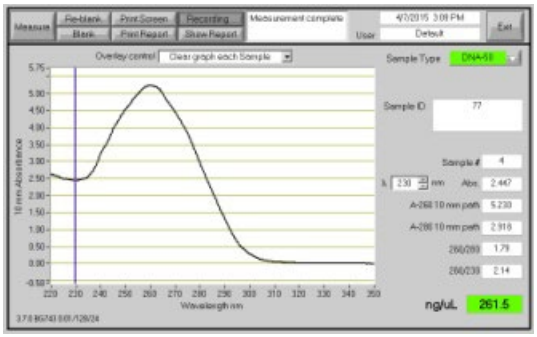
Sample B: 2 sections, 2 mg tissue, 600 mm^2 paraffin.



Sample C: 1 section, 2 mg tissue, 300 mm^2 paraffin.

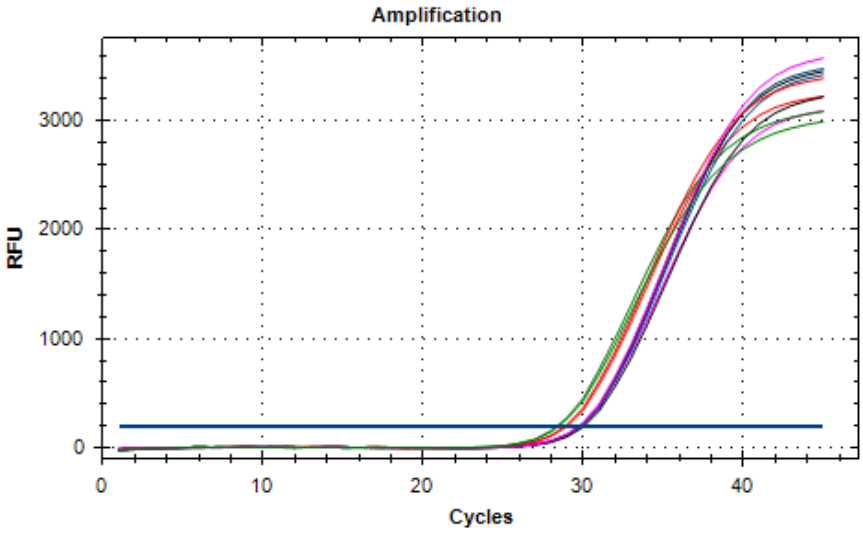


Sample D: 2 sections, 4 mg tissue, 600 mm^2 paraffin.



Sample E: 3 sections, 6 mg tissue, 900 mm^2 paraffin.

Analysis of sample A up to sample E by Real-time PCR:



The Ct values of different FFPE tissue samples correspond to the yield as shown above. Sample A (■), sample B (■), sample C (■), sample D (■) and sample E (■).

10 Protocol for DNA isolation from paraffin embedded tissue samples

1. Place the **FFPE material** into a 1.5 ml or 2.0 ml reaction tube and centrifuge the reaction tube at maximum speed for 1 minute.

NOTE

For correct sample amount see „Product specifications“ on p. 8.

2. Open the reaction tube and add **400 µl Lysis Solution MA and 40 µl Proteinase K** to the sample, mix vigorously by pulsed vortexing for 10 seconds.

IMPORTANT

The FFPE material has to be completely covered by the **Lysis Solution MA**, if necessary spin down briefly to remove drops from the lid!

3. Incubate the reaction tube at 65 °C for 1 hour in a thermal mixer under continuous shaking at 1,000 rpm.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively: vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

IMPORTANT

If the residual tissue sample is still visible after 1 hour, prolong the incubation step until the tissue is completely lysed.

4. After lysis step place the sample into a thermal mixer pre-heated to 90 ℃ and incubate the sample for 1 hour under continuous shaking at 1,000 rpm.

IMPORTANT

Do not place the sample into the thermal mixer, before the temperature of 90 ℃ is achieved! Longer incubation at 90 ℃ may lead to lower yield!

5. Incubate the sample for 5 minutes at room temperature.

NOTE

To remove RNA from the sample (if necessary) add 10 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 min at room temperature. RNA removal from the sample by RNase A could lead partial loss of DNA.

6. Centrifuge the sample at maximum speed for 2 minutes and transfer the sample as much as possible into a new 1.5 ml reaction tube under considering following notes. Try to avoid carryover of residual FFPE material!

NOTE

Depending on the amount of paraffin material in the sample, a layer forms on the top of lysed sample. Try to avoid carryover of this formed paraffin layer in a new 1.5 ml reaction tube.

Depending on the sample a pellet forms on the bottom of the reaction tube. Avoid to carryover this pellet in a new 1.5 ml reaction tube.

7. Add **400 µl of ethanol absolute (96–99 %)** to the sample, mix vigorously by pulsed vortexing for 10 seconds or pipetting up and down several times.

NOTE

It is important that the sample and the ethanol absolute are mixed vigorously to get a homogeneous solution.

8. Apply the sample onto a Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

9. Open the Spin Filter and add **500 µl Washing Solution C**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

10. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

    Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

11. Open the Spin Filter and add **650 µl ethanol absolute (96–99 %)**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

    Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

12. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

13. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

NOTE

The DNA can be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Paraffin layer on lysed sample	
Too much starting material	If the lysed sample is covered by a paraffin layer after centrifugation step (step 6). Transfer carefully all solution by piercing the paraffin layer using a 100 µl pipette. Avoid carryover of paraffin to the next tube!
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.
Insufficient mixing with ethanol absolute	Mix sample with ethanol absolute by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much Elution Buffer was used in the elution step	Elute the DNA with lower volume of Elution Buffer
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately after taking in liquid nitrogen or at -18 °C to -22° C! For long time storage continuously store at -78 °C to -82° C! Avoid thawing of the material.
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.

Problem / probable cause	Comments and suggestions
RNA contamination	
Extracted DNA is contaminated with RNA	Perform an RNase A digestion.
Insufficient quality of extracted DNA	
Carryover of paraffin or pellet	Carefully transfer all solution by piercing the paraffin layer using a 100 µl pipette after centrifugation step (step 6). Avoid carryover of paraffin to the next tube!
Sample diffuses out from the electrophoresis gel	
Ethanol was not completely removed from the Spin Filter	Centrifuge at maximum speed for 3 minuntes (step 12) with open lid of the Spin Filter. Prolong the centrifugation step at maximum speed before elution step (step 12). Incubate eluted samples in elution tube with opened lied at 37 °C for 30 minutes.

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