TECHNICAL BULLETIN

DNA IQ™ System—Small Sample Casework Protocol

Instructions for Use of Products
DC6700 and DC6701

Revised 8/19
TB296

DNA IQ™ System—Small Sample Casework Protocol

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Biological material encountered in forensic casework often contains substances that interfere with downstream applications such as PCR. Isolation of DNA that is free of inhibitors can improve the chances of obtaining a usable and interpretable STR profile.

The DNA IQ™ System is a DNA isolation system designed specifically for forensic and paternity laboratories. This system employs novel paramagnetic particles to prepare clean samples for short tandem repeat (STR) analysis easily and efficiently. The DNA IQ™ System can be used to extract DNA from a variety of sample types, including buccal swabs, liquid blood, and stains on FTA® and other blood cards.

When working with larger sample volumes, such as those found in paternity and databasing, the DNA IQ™ System delivers a consistent amount of total DNA without extra quantitation steps prior to amplification. In addition, no harmful organic solvents such as phenol and chloroform are used, so use of a hood is not required and disposal of hazardous chemicals is eliminated.

For more information about implementing these methods, contact Technical Services at **genetic@promega.com**

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
DNA IQ™ System	**400 samples**	**DC6700**

This system includes:

- 3ml Resin
- 150ml Lysis Buffer
- 70ml 2X Wash Buffer
- 50ml Elution Buffer

PRODUCT	SIZE	CAT.#
DNA IQ™ System	**100 samples**	**DC6701**

This system includes:

- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer

Storage Conditions: Store all DNA IQ™ System reagents at 15–30°C.

3. Preprocessing

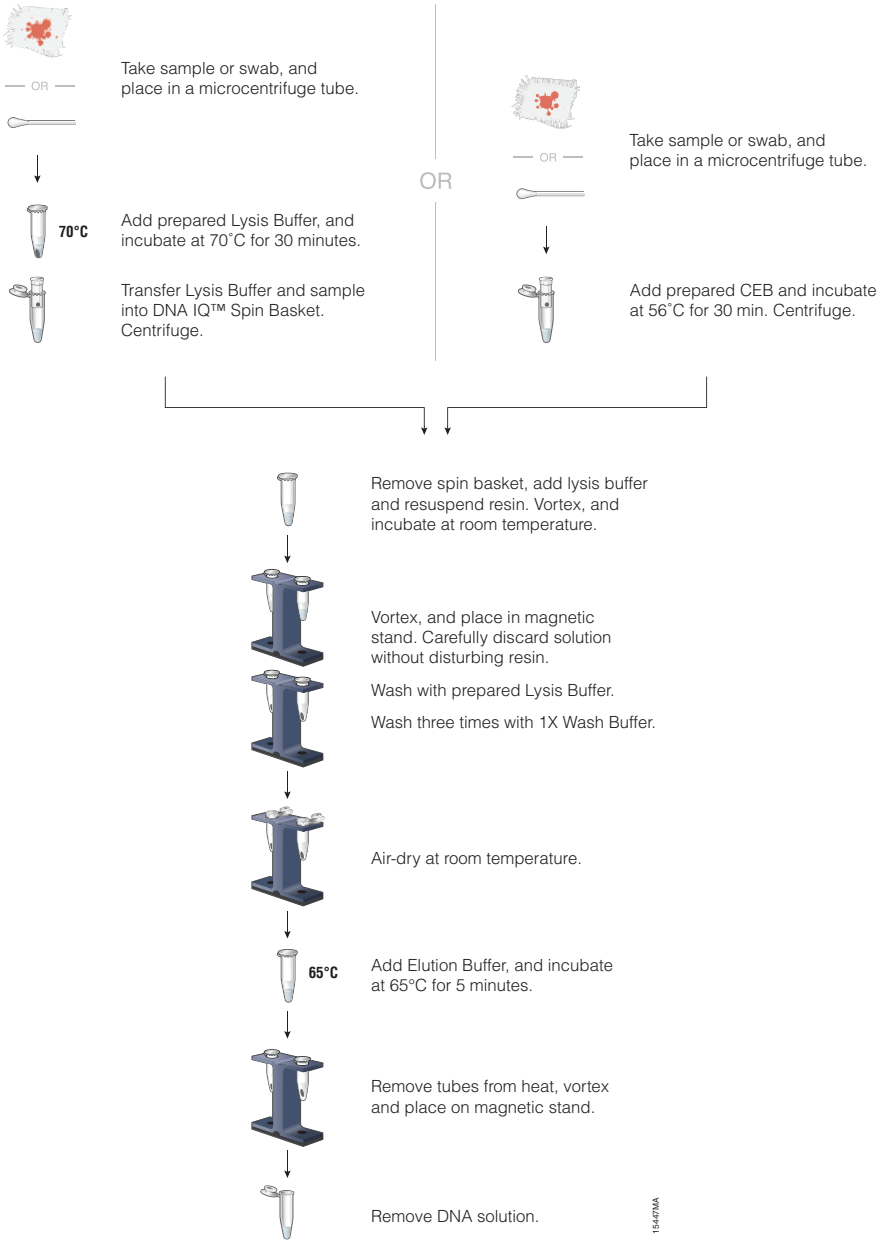

Figure 1. Schematic of DNA isolation from stains on solid material using the DNA IQ™ System. See Section 4.B. for a detailed protocol.

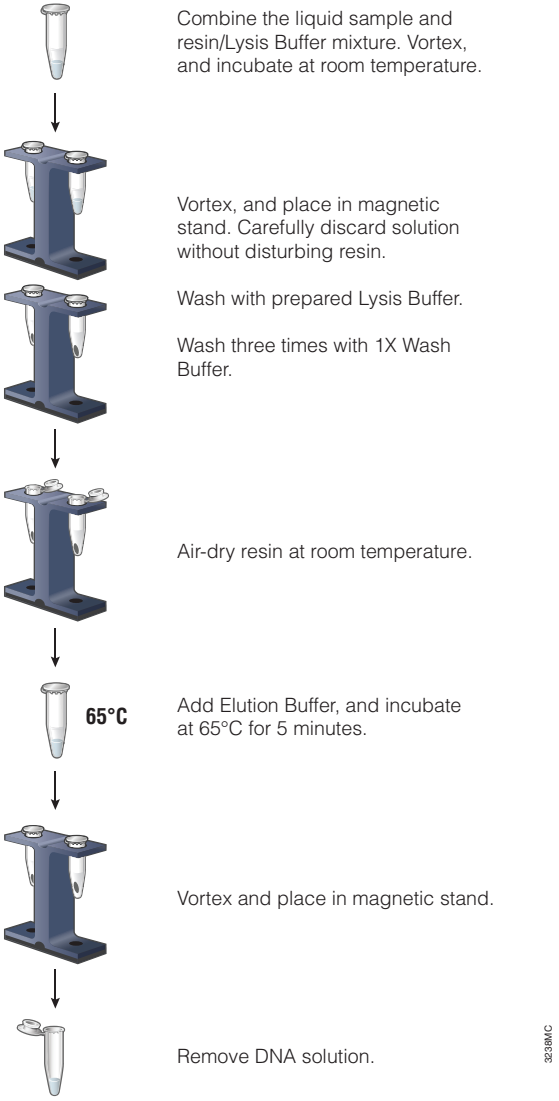

Figure 2. Schematic of DNA purification from liquid samples using the Lysis Buffer preprocessing method and the DNA IQ™ System. See Section 4.F. for a detailed protocol.

4. Protocol for the DNA IQ™ System

Samples may be preprocessed using Casework Extraction Buffer (CEB) or Lysis Buffer before purification of DNA using the DNA IQ™ Resin.

Materials to Be Supplied by the User

Casework Extraction Buffer Preprocessing followed by DNA IQ™ Purification

- Casework Extraction Kit (Cat.# DC6745)
- 95–100% ethanol
- isopropyl alcohol
- preprocessing spin baskets and tubes (use option 1 or 2 listed below):
  1. DNA IQ™ Spin Baskets (Cat.# V1225) with ClickFit Microtubes, 1.5ml (Cat.# V4745)
  2. CW Spin Baskets (Cat.# AS8101) with CW Microfuge Tubes, 1.5ml (Cat.# AS8201)
- 65°C heat block, water bath or thermal cycler (for DNA elution step)
- 56°C heat block, water bath or thermal cycler (for preprocessing step)
- vortex mixer
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5332 or Z5342)

Lysis Buffer Preprocessing followed by DNA IQ™ Purification

- 95–100% ethanol
- isopropyl alcohol
- 1M DTT
- proteinase K, to process samples listed in Table 5
- preprocessing spin baskets and tubes (use option 1 or 2 listed below):
  1. DNA IQ™ Spin Baskets (Cat.# V1225) with ClickFit Microtubes, 1.5ml (Cat.# V4745)
  2. CW Spin Baskets (Cat.# AS8101) with CW Microfuge Tubes, 1.5ml (Cat.# AS8201)
- 65°C heat block, water bath or thermal cycler (for DNA elution step)
- 70°C heat block, water bath or thermal cycler (for stain or swab extraction)
- vortex mixer
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5332 or Z5342)

We highly recommend the use of gloves and aerosol-resistant pipette tips.

4.A. Preparation of Reagents

Preparation of Stock Proteinase K Solution for Sample Preprocessing using Casework Extraction Buffer

Add 556µl of Nuclease-Free Water to one tube of lyophilized Proteinase K, and gently invert to dissolve. The final concentration of Proteinase K will be 18mg/ml. Store Proteinase K Solution at –30°C to –10°C.

Preparation of Lysis Buffer

1. Determine the total volume of prepared Lysis Buffer to be used (Table 1), and add 1µl of 1M DTT for every 100µl of Lysis Buffer.

   Note: Increasing the DTT concentration in the prepared Lysis Buffer to 60mM can improve the recovery of DNA from the sperm fraction of sperm-containing samples (Section 4.D). To prepare Lysis Buffer with a final concentration of 60mM DTT, add 6µl of 1M DTT to 100µl of Lysis Buffer.

Table 1. Volume of Prepared Lysis Buffer Required Per Sample.

Sample	Lysis Buffer[1]	Lysis Buffer[2]	Total Volume
Liquid blood (up to 40µl)	100µl	100µl	200µl
1 Cotton swab	250µl	100µl	350µl
1/4th CEP swab	250µl	100µl	350µl
1–2 4mm punches of S&S 903 paper	150µl	100µl	250µl
1–3 2mm punches of FTA® paper	150µl	100µl	250µl
Cloth, up to 25mm^2	150µl	100µl	250µl

[1]For use in Section 4.B, Step 2, or Section 4.C, Step 1.
[2]For use in Section 4.B, Step 9, or Section 4.C, Step 8.

2. Mix by inverting several times.
3. Mark and date label to record the addition of DTT. This solution can be stored at room temperature for up to a month if sealed.

   Note: If prepared Lysis Buffer forms a precipitate, warm solution to 37–60°C.

Preparation of 1X Wash Buffer

1. For DC6701 (100 samples) add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.

   For DC6700 (400 samples) add 35ml of 95–100% ethanol and 35ml of isopropyl alcohol to the 2X Wash Buffer.
2. Mix by inverting several times.
3. Mark label to record the addition of alcohols. Label bottle as 1X Wash Buffer. Solution can be stored at room temperature. Be sure bottle is closed tightly to prevent evaporation.

4.B. Preprocessing Solid Samples

Preprocessing Using Casework Extraction Buffer

Option 1: Extraction of Samples on a Solid Support with DNA IQ™ Spin Basket

1. Place the solid substrate (e.g., fabric or swab head) at the bottom of a labeled ClickFit Microtube, 1.5ml.
2. Prepare the Extraction Mix by adding the final volume of each reagent listed in Table 2 to a clean tube.

Table 2. Extraction Mix for Solid Support Samples with DNA IQ™ Spin Basket.

Extraction Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Extraction Buffer	386µl	×		=	
Proteinase K (18mg/ml)	10µl	×		=	
1-Thioglycerol	4µl	×		=	
Total Reaction Volume	400µl	×		=	

Note: 1-Thioglycerol is viscous. Pipet slowly.

3. Briefly vortex the Extraction Mix, and dispense 400µl to each ClickFit Microtube containing solid substrate.
4. Close the tube, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
5. Place a DNA IQ™ Spin Basket into a clean labeled ClickFit Microtube, 1.5ml. Transfer the sample to the DNA IQ™ Spin Basket with forceps, being sure to orient the swab or fabric toward the bottom of the spin basket. Transfer the lysate from the incubation tube to the spin basket, and close the tube.
6. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Carefully remove the DNA IQ™ Spin Basket.
7. Add 400µl of Lysis Buffer to the tube containing extract.
8. Close the lid of the tube, and vortex the sample for 5–10 seconds.

4.B. Preprocessing Solid Samples (continued)

Preprocessing Using Casework Extraction Buffer (continued)

Option 2: Extraction of Samples on a Solid Support with a CW Spin Basket

1. Place a CW Spin Basket into a labeled CW Microfuge Tube, 1.5ml.
2. Place the solid substrate (e.g., fabric or swab head) at the bottom of a CW Spin Basket.
3. Prepare the Extraction Mix by adding the final volume of each reagent listed in Table 3 to a clean tube.

Table 3. Extraction Mix for Solid Support Samples with CW Spin Basket.

Extraction Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Extraction Buffer	286µl	×		=	
Proteinase K (18mg/ml)	10µl	×		=	
1-Thioglycerol	4µl	×		=	
Total Reaction Volume	300µl	×		=	

Note: 1-Thioglycerol is viscous. Pipet slowly.

4. Briefly vortex the Extraction Mix, and dispense 300µl to each CW Spin Basket containing the solid substrate.
5. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
6. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Carefully remove the CW Spin Basket.
7. Add 400µl of Lysis Buffer to the tube containing the extract.
8. Close the lid of the tube, and vortex the sample for 5–10 seconds.

Preprocessing Using Lysis Buffer

1. Place the sample in a 1.5ml microcentrifuge tube (e.g., ClickFit Microtube, 1.5ml, Cat.# V4745).

   Note: The use of the ClickFit Microtube is specifically recommended to avoid any problem with tube caps opening during incubation.

2. Add the appropriate volume of prepared Lysis Buffer. Different samples require different volumes of prepared Lysis Buffer; see Column 2 of Table 1 for the appropriate volume to add at this point. Additional prepared Lysis Buffer may be used to cover the entire sample. Close the lid, and incubate tube at 70°C for 30 minutes.

   Exceptions:

   - Heat-sensitive fabrics (e.g., polyester and nylon): Extract without heating.
   - Leather: Lysis Buffer extraction with or without heat may not work on some leathers. Extract in a small volume of aqueous buffer (100–200µl), then add 2 volumes of Lysis Buffer after removing matrix.

Note: For small stains, an alternative approach is to place the stained material in a DNA IQ™ Spin Basket (Cat.# V1225) seated in a ClickFit Microtube (Cat.# V4745). Add 100–150µl of prepared Lysis Buffer to the basket. Carefully close the lid, and incubate at 70°C for 30 minutes. Most of the buffer should remain in the basket if the indicated tubes and spin baskets are used. Proceed to Step 4.

3. Remove the tube from the heat source, and transfer the prepared Lysis Buffer and sample to a DNA IQ™ Spin Basket seated in a ClickFit Microtube.

4. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Remove the spin basket.

   Note: Centrifuge the prepared Lysis Buffer and stained matrix to obtain maximum recovery.

4.C. Preprocessing Bone Samples

Please refer to the *Purification of DNA from Bone Samples Using Bone DNA Extraction Kit, Custom and DNA IQ™ Chemistry Application Note* #AN343 for a detailed protocol for using Demineralization Buffer, Proteinase K and 1-thioglycerol for preprocessing bone and teeth samples. The extracted DNA can be purified using DNA IQ™ protocol listed in Section 4.E. of this technical manual.

4.D. Preprocessing Sperm-Containing Mixture Samples

Please refer to the *Differex™ System—For Use With the Differex™ Magnet Technical Manual* #TM331 or the *Differex™ System Technical Bulletin* #TBD020 for preprocessing mixture samples containing sperm. The extracted DNA can be purified using the DNA IQ™ protocol listed in Section 4.E. of this technical manual.

4.E. DNA Purification Using DNA IQ™ Resin

The maximum DNA yield will depend on the sample type, even samples that contain DNA amounts in excess of the DNA-binding capacity of the resin. As expected for samples that do not contain DNA amounts exceeding the DNA-binding capacity, the yield will vary with the sample type and amount. Samples containing small amounts of DNA will have high efficiencies of recovery; as the DNA content approaches the maximum DNA-binding capacity, efficiency decreases (see Figure 1 of the *DNA IQ™ System—Database Protocol*, #TB297).

DNA can be purified from solid samples preprocessed using Casework Extraction Buffer, prepared Lysis Buffer, Bone DNA Extraction Kit, Custom and Differex System using the protocol below. For liquid samples, see Section 4.F.

1. Vortex the stock resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Add 7µl of DNA IQ™ Resin to the preprocessed sample. Keep the resin resuspended while dispensing to obtain uniform results.

2. Vortex sample/Lysis Buffer/resin mixture for 3 seconds at high speed. Incubate at room temperature for 5 minutes. Vortex mixture for 3 seconds once every minute during this 5-minute incubation.

3. Vortex the tube for 2 seconds at high speed. Place the tube in the magnetic stand. Separation will occur instantly.

   Note: If the resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place it back in the stand.

4. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.

   Note: If some resin is drawn up in the tip, gently expel the resin back into the tube to allow reseparation.

4.E. DNA Purification Using DNA IQ™ Resin (continued)

5. Add 100µl of prepared Lysis Buffer. Remove the tube from the magnetic stand, and vortex for 2 seconds at high speed.
6. Return the tube to the magnetic stand, then remove and discard all Lysis Buffer.
7. Add 100µl of prepared 1X Wash Buffer. Remove the tube from the magnetic stand, and vortex for 2 seconds at high speed.
8. Return the tube to the magnetic stand, then remove and discard all Wash Buffer.
9. Repeat Steps 7 and 8 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.
10. With the tube in the magnetic stand and the lid open, air-dry the resin for 5 minutes.

! Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

11. Add 25–100µl of Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.
12. Close the lid, and vortex the tube for 2 seconds at high speed. Incubate the tube at 65°C for 5 minutes.
13. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place the tube on the magnetic stand.

! Tubes must remain hot until placed in the magnetic stand or yield will decrease.

14. Carefully transfer the DNA-containing solution to a container of choice.

    Note: DNA can be stored at 4°C for short-term storage or at –20 or –70°C for long-term storage.

4.F. DNA Isolation from Liquid Samples

This protocol is recommended for liquid samples, excluding liquid blood. A protocol for DNA purification from liquid blood can be found in the *DNA IQ™ System—Database Protocol* #TB297.

1. Prepare a stock solution of resin and Lysis Buffer by using the ratio of 7µl of resin to 93µl of prepared Lysis Buffer per sample (prepare extra to allow for losses during pipetting). The following equation will help determine the exact volumes to be made. Vortex the resin container for 10 seconds at high speed or until the resin is thoroughly mixed.

   (Number of samples + 1) × 7µl = µl of resin

   (Number of samples + 1) × 93µl = µl of prepared Lysis Buffer

2. Mix the liquid sample gently, and place an aliquot of up to 40µl into a ClickFit Microtube (Cat.# V4745).
3. Vortex the resin/Lysis Buffer mixture for 3 seconds at high speed to ensure suspension of the resin, and add 100µl of the mixture to the tube containing the liquid sample. The resin/Lysis Buffer mixture should be mixed again if the resin begins to settle while dispensing aliquots.
4. Vortex the sample/Lysis Buffer/resin mixture for 3 seconds at high speed. Incubate 5 minutes at room temperature. Vortex the mixture for 3 seconds once every minute during this 5-minute incubation.

5. Vortex for 2 seconds at high speed. Place the tube in the magnetic stand. Separation will occur instantly.

   Note: If the resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place it back in the stand.

6. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.
7. Add 100µl of prepared Lysis Buffer. Remove the tube from the magnetic stand, and vortex for 2 seconds at high speed.
8. Return the tube to the magnetic stand, and remove and discard all Lysis Buffer.
9. Add 100µl of prepared 1X Wash Buffer. Remove the tube from the magnetic stand, and vortex for 2 seconds at high speed.
10. Return the tube to the magnetic stand. Dispose of all Wash Buffer.
11. Repeat Steps 9 and 10 two more times for a total of three washes. Be sure all of the solution has been removed after the last wash.
12. With the tube in the magnetic stand and the lid open, air-dry the resin for 5 minutes.

! Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

13. Add 25–100µl of Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.
14. Close the lid, and vortex the tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.
15. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place it on the magnetic stand.

! Tubes must remain hot until placed in the magnetic stand, or yield will decrease.

16. Carefully transfer the DNA-containing solution to a container of choice.

    Note: DNA can be stored at 4°C for short-term storage or at –20 or –70°C for long-term storage.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: www.promega.com. E-mail: genetic@promega.com

Symptoms	Causes and Comments
Poor yield	Too much sample was used. Excessive amounts of sample can reduce the efficiency of DNA binding to the resin. Use less sample or more resin.
	Poor extraction. After heating stain in prepared Lysis Buffer, centrifuge the buffer with matrix. Be sure enough liquid is present to wash out the DNA.
	Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.
	For sperm-containing samples, increasing the DTT concentration in the prepared Lysis Buffer to 60mM may improve yield (see Section 4.D).
Poor resin "pellet" formed	The resin settled before the tube was placed in the magnetic stand. Samples should be placed in the magnetic stand immediately after mixing. Repeat mixing, and place the tube in the magnetic stand.
	Excessive input material was used relative to the recommended volumes of reagents. Use less initial sample. Consult protocols for recommended quantities of initial sample. Alternatively, use more resin per isolation. A proportional increase in resin will allow DNA capture from more initial sample. The increase in yield will be roughly proportional to the increase in resin.
Coloration in final wash or eluted solution (may affect results of downstream assays)	Insufficient washing. Remove all fluid during washes. Be sure that the resin is completely resuspended during each wash step. Be sure a distinct resin pellet is formed during all washes.
	Use less initial sample.
	Perform additional washes with 1X Wash Buffer.

Symptoms	Causes and Comments
Resin present in final eluted solution (may affect results of downstream assays)	Resin is occasionally transferred by rapid pipetting or is caught in the meniscus of the final eluate. Vortex or mix the solution, place it in the magnetic stand and transfer the eluate to a new tube.
Inconsistent yield (may affect results from downstream assays)	Inconsistent amounts of resin. Vortex the resin stock just before making aliquots. Be sure to vortex the resin/Lysis Buffer mixture while dispensing aliquots.
	Excessive drying of resin. Do not dry samples for more than 20 minutes, as overdrying the resin inhibits DNA elution.
	Contaminating nonhuman DNA in the initial sample can decrease yield of human DNA. The DNA IQ™ System captures total DNA, including single- and double-stranded DNA.

6. References

1. Greenspoon, S. and Ban, J. (2002) Robotic extraction of mock sexual assault samples using the Biomek® 2000 and the DNA IQ™ System. *Profiles in DNA* **5**, 3–5.
2. Gill, P. *et al.* (1985) Forensic application of DNA 'fingerprints'. *Nature* **318**, 577–9.

7. Appendix

Sample Types Examined

DNA from the following sample types have been successfully purified at Promega or by external forensic laboratories. Due to the nature of casework samples (i.e., the samples may have been exposed to environmental factors for long periods of time and the amount of biological material may be limiting), DNA yields may vary, and DNA may not be obtained from all samples. Please see the most updated list at: **www.promega.com/dnaiqsamples/**. Tissue masses, including hair, bone, and sperm, require a proteinase K digestion to obtain reliable amounts of DNA. Contact Promega Technical Services (**genetic@promega.com**) for the latest information on available protocols.

Table 4. Types of Samples From Which DNA Has Been Successfully Isolated Using the DNA IQ™ System.

Sample Type	Promega	External	Comments
Fresh blood	Yes	Yes	Works with the following anti-clotting reagents: EDTA, citrate, heparin, ACD.
Frozen blood	Yes	Yes	Old blood may produce lower yields.
Bloodstains			
S&S 903	Yes	Yes	
FTA® paper	Yes	Yes	
Cotton	Yes	Yes	
Blue denim	Yes	Yes	
Black denim	Yes	Yes	
Soil	Yes		
Leather	Yes	Yes	
Surface to swab		Yes	
Buccal swabs			
Cotton	Yes	Yes	
Rayon	Yes		
CEP paper	Yes		
Swab to FTA® paper		Yes	
Foam swab to paper		Yes	
Cigarette butt	Yes	Yes	Use paper wrapping; filter may form gel if heated with prepared Lysis Buffer.
Toothbrush		Yes	Soak bristles in prepared Lysis Buffer at 60°C for 30 minutes.
Envelope		Yes	Soak in 0.5% SDS before adding 2 volumes of prepared Lysis Buffer.
Urine		Yes	Sample from bladder cancer patient.

Table 5. Samples Requiring a Proteinase K Digestion Prior to Addition of Twice the Recommended Volume of Lysis Buffer.

Sample Type	Promega	External	Comments
Tissue			
Fresh	Yes	Yes	See the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* TB307.
Formalin-fixed	Yes	Yes	See the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* TB307.
Hair	Yes	Yes	See the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* TB307.
Bone*		Yes	From pulverized bone samples.
Antler*		Yes	From drill shavings.
Differential extractions		Yes	See Section 4.D.

*Requires the Bone Incubation Buffer containing 1mg/ml proteinase K for DNA purification. Bone Incubation Buffer composition and DNA isolation protocol are available in Section 8 of this technical bulletin.

8. Composition of Buffers and Solutions

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA

Bone Incubation Buffer

10mM Tris (pH 8.0)
100mM NaCl
50mM EDTA
0.5% SDS

9. Related Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
DNA IQ™ Spin Baskets*	50/pack	V1225
ClickFit Microtube, 1.5ml	100/pack	V4745
CW Spin Baskets	50/pack	AS8101
CW Microfuge Tubes	50/pack	AS8201
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740
DTT, Molecular Grade (Dry Powder)	5g	V3151
	25g	V3155
Bone DNA Extraction Kit, Custom	100 preps	AX6780
Differex™ System	50 samples	DC6801
	200 samples	DC6800

*Not For Medical Diagnostic Use.

10. Summary of Changes

The following changes were made to the 6/19 revision of this document:

1. Updated error in Section 4.E regarding which steps of the protocol are repeated in Step 9.

[a]European Pat. No. 1 204 741 and Japanese Pat. No. 4425513.