DNA IQ™ System– Database Protocol

Promega

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INSTRUCTIONS FOR USE OF PRODUCTS DC6700 and DC6701. *PLEASE DISCARD PREVIOUS VERSIONS.*
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I. Description

The development of human DNA databases is playing an increasingly important role in the forensic field. To this end, robust commercial STR-based systems have been developed for the analysis of standardized sets of loci. These systems can analyze sub-nanogram amounts of DNA but are limited to a well-defined range of DNA. Thus, DNA must be purified and then quantitated accurately to reduce the risk of artifacts.

Two approaches have been used to purify DNA from blood for databasing purposes where sample quantities and nonhuman DNA contamination are not a concern. The first approach is to purify and then quantitate DNA using absorbance measurements or a DNA-specific dye. This approach requires an additional series of steps and is sensitive to the quality of DNA. The second approach is to deliver DNA from a set amount of blood usually attached to a solid support such as FTA® or S&S 903 paper. This approach yields variations in DNA content due to variations in white cell counts or variations on the filter due to sample wicking and differential drying. Additionally, the DNA is bound to the filters and must be removed for quantitation purposes. The high capacity of FTA® paper requires modification of the amplification protocol to prevent excessive amplification and allele imbalances.

Promega's DNA IQ™ System[a] uses a novel approach for DNA isolation (Figure 1). A paramagnetic resin is used to capture a consistent amount of DNA. The Resin has a defined DNA capacity in the presence of excess DNA and will only bind a specific amount of DNA. This property is used to isolate approximately 100ng of DNA from a range of liquid blood, stains or swabs. The DNA is eluted into 100µl of Elution Buffer to give a DNA concentration of approximately 1ng/µl. As a result, the researcher can bypass the quantitation step typically necessary in other purification procedures.

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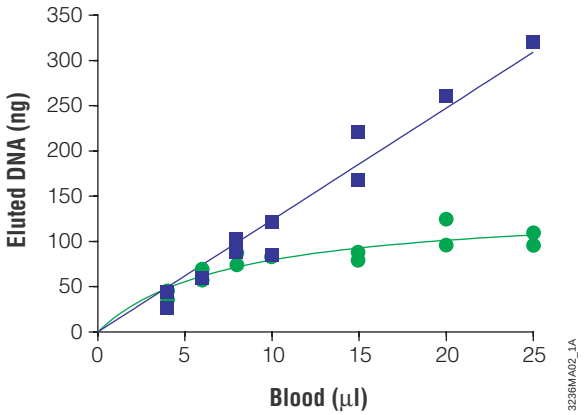

Figure 1. Effect of sample volume on the amount of DNA isolated. The high-capacity Resin shows a linear relationship between eluted DNA versus amount (µl) of blood extracted (represented by squares). In contrast, the DNA IQ™ System Resin is saturated using the blood volumes tested and thus gives approximately the same amount of DNA regardless of sample size (represented by circles).

The DNA IQ™ System avoids the use of harmful organic solvents such as phenol and eliminates multiple centrifugation steps used in some DNA purification procedures. The DNA IQ™ System procedure is performed using a few simple steps (Figures 2 and 3):

- Extraction of sample (FTA® paper, S&S 903 paper, cotton swab) and/or Lysis of sample
- DNA capture using Resin
- Washing of Resin
- Elution of Resin

II. Product Components

Product	Size	Cat.#
DNA IQ™ System	400 reactions	DC6700

For Laboratory Use. The system includes:

- 3ml Resin
- 150ml Lysis Buffer
- 70ml 2X Wash Buffer
- 50ml Elution Buffer
- 2 Protocols (Casework and Database)

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701

For Laboratory Use. This system includes:

- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer
- 2 Protocols (Casework and Database)

Storage Conditions: Store all DNA IQ™ reagents at room temperature.

III. Protocol for the DNA IQ™ System

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- 1M DTT
- 65°C heat block, water bath or thermal cycler
- 95°C heat block, water bath or thermal cycler (for stain or swab extraction)
- vortex mixer
- Microtubes, 1.5ml (Cat. # V1231)
- DNA IQ™ Spin Baskets (Cat. # V1221)
- aerosol-resistant micropipette tips
- MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342)

Use of gloves and aerosol-resistant micropipette tips is highly recommended to prevent cross-contamination.

A. Preparation of 1X Wash Buffer and Lysis Buffer

Preparation of 1X Wash Buffer

1. For DC6701 (400 samples) add 35ml of 95–100% ethanol and 35ml of isopropyl alcohol to the 2X Wash Buffer.

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

2. Replace cap and mix by inverting several times.
3. Mark label to record the addition of alcohols and label as 1X Wash Buffer. Solution can be stored at room temperature. Make sure bottle is closed tightly to prevent evaporation.

Preparation of Lysis Buffer

1. Determine the total amount of Lysis Buffer to be used (see Table 1) and add 1µl of 1M DTT for every 100µl of Lysis Solution.

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

   Table 1. Amount of Lysis Buffer required per sample.

Material	Lysis Buffer[1]	Lysis Buffer[2]	Total Buffer
Liquid blood	100µl	100µl	200µl
Cotton swab	250µl	100µl	350µl
1/4th CEP swab	250µl	100µl	350µl
15–50mm^2 S&S 903 paper	150µl	100µl	250µl
3–30mm^2 FTA® paper	150µl	100µl	250µl

[1]For use in Section III.B, Step 2, or Section III.C. Step 1
[2]For use in Section III.B, Step 9, or Section III.C, Step 7.

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.

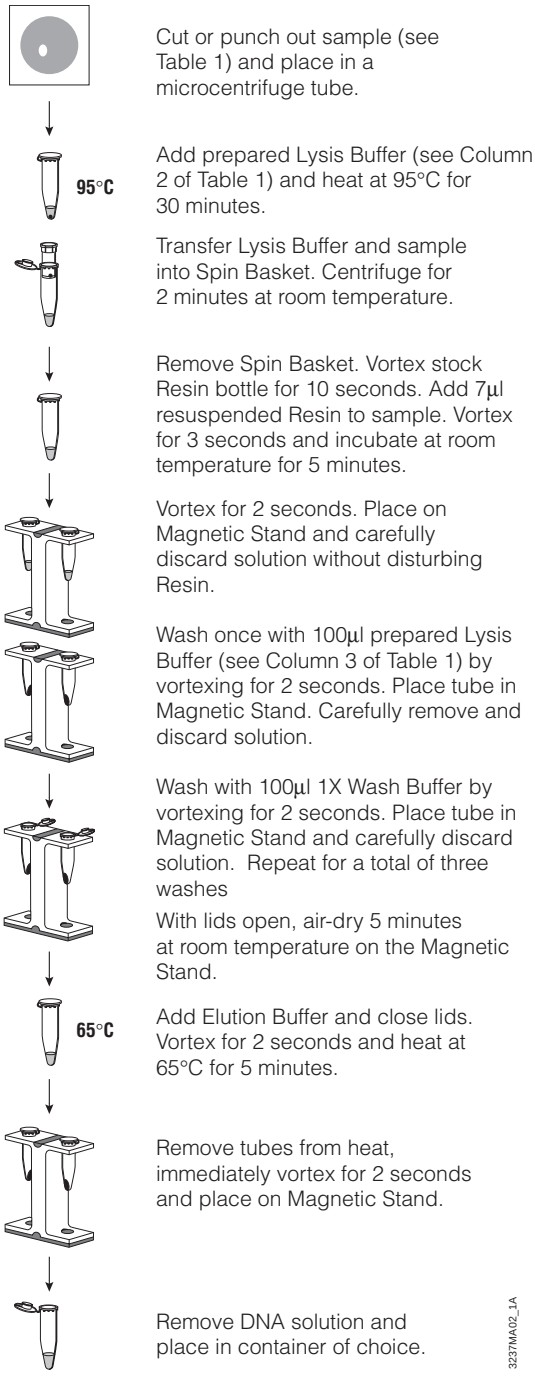

Figure 2. Schematic of DNA isolation from stains and swabs using the DNA IQ™ System. See Section III.B for a detailed protocol.

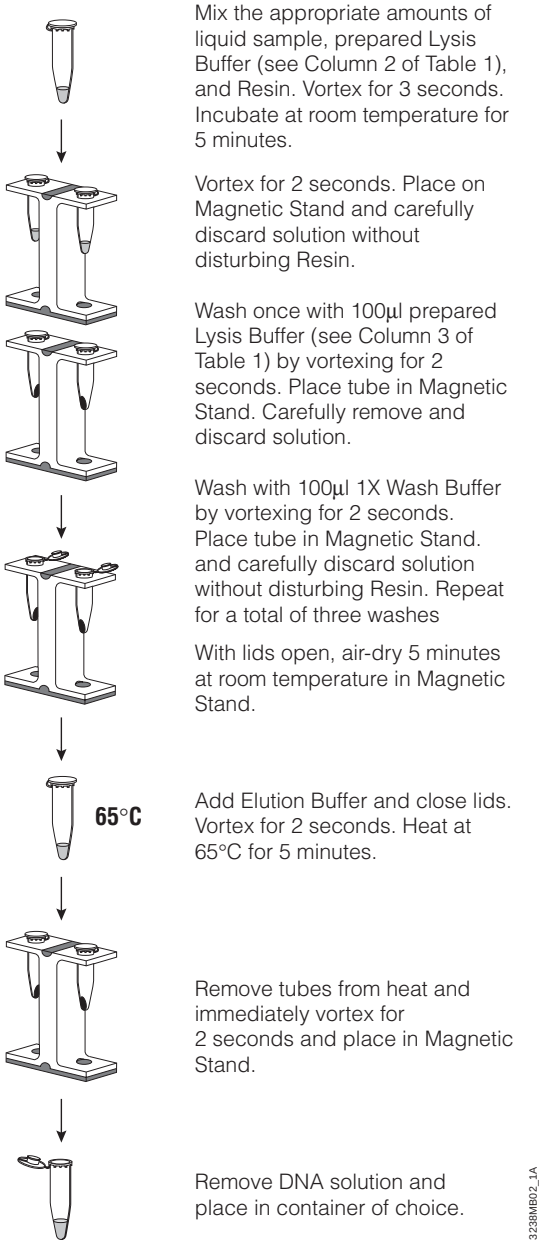

Figure 3. Schematic of DNA isolation and quantitation from liquid blood using the DNA IQ™ System. See Section III.C for a detailed protocol.

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Tip: Swab material can be scraped off the shaft after incubating a swab head in prepared Lysis Buffer for 5 minutes at room temperature. Scraped material can then be incubated in tube as described in Step 2.

B. DNA Isolation from Stains and Buccal Swabs (Figure 2)

1. Place sample (see Table 1) in a 1.5ml microcentrifuge tube. The recommended amount of Resin can capture a maximum of approximately 100ng DNA.
2. Add the appropriate amount of prepared Lysis Buffer. Different samples require different volumes of Lysis Buffer; see Column 2 of Table 1 for the appropriate volume to add at this point. Close the lid and place tube in a heat block at 95°C for 30 minutes.

   Note: For small stains, an alternative approach is to place the stained material in a DNA IQ™ Spin Basket (Cat.# V1221) seated in 1.5ml Microtubes (e.g, Promega Cat. # V1231) and add 100–150µl of prepared Lysis Buffer to the basket. Carefully close the lid and heat at 95°C for 30 minutes. Most of the Buffer should remain in the basket if the indicated Microtubes and Spin Baskets are used. Proceed to Step 4. This does not work reliably with samples requiring more than 150µl Lysis Buffer.
3. Remove the tube from the heat block and transfer the Lysis Buffer and sample to a Spin Basket placed in a standard 1.5ml conical microcentrifuge tube.

   Note: It is important to centrifuge Lysis Buffer with the stained matrix to obtain maximum recovery.
4. Centrifuge at room temperature for 2 minutes at maximum speed. Remove the Spin Basket.
5. Vortex the stock Resin bottle for 10 seconds at high speed or until thoroughly mixed. Add 7µl of Resin to the DNA solution. Keep the stock Resin resuspended while dispensing to obtain uniform results.
6. Vortex sample/Lysis Buffer/Resin mix for 3 seconds at high speed. Incubate at room temperature for 5 minutes.
7. Vortex tube for 2 seconds at high speed and place tube in the Magnetic Stand. Separation will occur instantly.

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

8. Carefully remove and discard all of the solution without disturbing the Resin on the side of the tube.
9. Add 100µl of prepared Lysis Buffer. Remove the tube from the Magnetic Stand and vortex for 2 seconds at high speed.
10. Return tube to the Magnetic Stand and discard all Lysis Buffer.
11. Add 100µl of prepared 1X Wash Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
12. Return tube to the Magnetic Stand and discard all Wash Buffer.
13. Repeat Steps 11 and 12 two more times for a total of 3 washes. Make sure that all of the solution has been removed after the last wash.
14. With lid open, air-dry the Resin in the Magnetic Stand for 5 minutes.
15. Add 100µl of Elution Buffer.
16. Close the lid, vortex the tube for 2 seconds at high speed and place tube at 65°C for 5 minutes.
17. Remove the tube from the heat block and vortex for 2 seconds at high speed. Immediately place the tube on the Magnetic Stand.

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

Tubes must remain hot until placed in the Magnetic Stand or yield will decrease.

18. Transfer the solution to a container of choice. The DNA concentration will be approximately 1ng/µl, ready for use in an amplification reaction.

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

C. DNA Isolation from Liquid Blood (Figure 3)

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 (make 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 Resin is thoroughly mixed.

   (Number of samples + 1) _____ × 7µl = _____ µl Resin

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

2. Mix blood gently and place 15µl in a 1.5ml microcentrifuge tube (10–25µl can be used routinely).
3. Vortex Resin/Lysis Buffer mixture for 2 seconds at high speed to ensure suspension of Resin and add 100µl of the solution to the tube containing blood. Resin/Lysis Buffer mixture should be mixed again if Resin begins to settle while dispensing aliquots.
4. Vortex the sample/Resin/Lysis Buffer mixture for 3 seconds at high speed. Incubate 5 minutes at room temperature.
5. Vortex tube for 2 seconds at high speed and place tube in the Magnetic Stand. Separation will occur instantly.
6. Carefully remove and dispose of all solution without disturbing the resin on the side of the tube.
7. Add 100µl of prepared Lysis Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
8. Return tube to the Magnetic Stand and remove and dispose of all Lysis Buffer.
9. Add 100µl of prepared 1X Wash Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
10. Return tube to the Magnetic Stand. Dispose of all Wash Buffer.
11. Repeat Steps 9 and 10 two more times for a total of 3 washes. Make sure that all of the solution has been removed after the last wash.
12. With lid open, air-dry the Resin in the Magnetic Stand for 5 minutes.
13. Add 100µl of Elution Buffer.
14 Close the lid; vortex tube for 2 seconds at high speed. Place at 65°C for 5 minutes.
15. Remove the tube from the heat block and vortex for 2 seconds at high speed. Immediately place on the Magnetic Stand.
16. Transfer the solution to a container of choice. The DNA concentration will be approximately 1ng/µl, ready for use in an amplification reaction.

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

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

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

Tubes must remain hot until placed in the Magnetic Stand or yield will decrease.

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

E-mail: **techserv@promega.com**

IV. Troubleshooting

Symptoms	Possible Causes	Comments
Poor Resin "pellet" formed	Resin settled before tube placed in Magnetic Stand	Samples should be placed in the Magnetic Stand immediately after vortexing/mixing. Repeat vortexing/mixing and place back in stand.
	Excessive input material relative to recommended volumes of reagents	Use less initial sample. Consult protocols for recommended quantities of initial sample.
		A proportional increase in Resin will allow DNA capture from more initial sample. **Note:** Yield increase will be roughly proportional to increase in Resin.
Coloration in final wash or eluted solution (may affect results in down-stream assays)	Insufficient washing	Remove all fluid during washes.
		Ensure a distinct Resin pellet is formed during all washes.
		Use less initial sample. Consult protocols for recommended quantities of initial sample.
Inconsistent yield (may affect results from downstream assays)	Inconsistent amounts of Resin	Vortex/Mix Resin stock just before making aliquots. Repeat vortexing/mixing Resin/Lysis Buffer while making aliquots.
		Do not remove Resin during washes.
	Excessive drying	Do not dry samples more than 20 minutes, as overdrying Resin inhibits the elution.
	Too little sample	Use more initial sample. Consult protocols for recommended quantities of initial sample.
		If concentration of DNA is consistent, volume of eluted solution used in later assays can be modified.
Resin present in final eluted solution (may affect results in down-stream assays)	Resin is occasionally transferred by rapid pipetting or is caught in the meniscus of the final eluant	Vortex/Mix eluted solution, place in Magnetic Stand and transfer eluant to new tube.

V. Composition of Buffers and Solutions

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA

VI. 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*	1,000/pk	V1221
Microtubes, 1.5ml	1,000/pk	V1231
ART® 20P, Pipet Tip, 20µl	960/pk	DY1071
ART® 200, Pipet Tip, 200µl	960/pk	DY1121
ART® 1000, Pipet Tip, 1,000µl	768/pk	DY1131
AluQuant™ Human DNA Quantitation System[a]*	80 determinations	DC1010
	400 determinations	DC1011
PowerPlex® 16 System[b,c,d]*	400 reactions	DC6530
	100 reactions	DC6531
PowerPlex® 1.1 and 2.1 Systems[b,c]*	400 reactions	DC6500
	100 reactions	DC6501
PowerPlex® 1.2 System[b,c]*	400 reactions	DC6100
	100 reactions	DC6101

*Not For Medical Diagnostic Use.

[a]Patent Pending.

[b]U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, and other patents pending.

[c]STR loci are the subject of U.S. Pat. No. 5,766,847 and German Pat. No. DE 38 34 636 C2, issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, eV, Germany. Exclusive rights have been licensed to Promega Corporation for uses in human clinical research and diagnostics applications and all forms of human genetic identity. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications.

U.S. Pat. No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA. U.S. Pat. No. 5,674,686 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO and the combination of allelic ladders for the loci CSF1PO, FESFPS and TH01. U.S. Pat. No. 5,783,406 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO. U.S. Pat. No. 6,156,512 has been issued to Promega Corporation for allelic ladders for the loci D16S539, D7S820, D13S317 and D5S818.

Use of Promega's STR Systems requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of Promega's STR Systems does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of Promega's STR Systems may, therefore, be required to obtain a patent license, depending on the country in which the systems are used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

[d] U.S. Pat. No. 6,238,863 and other patents pending.

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