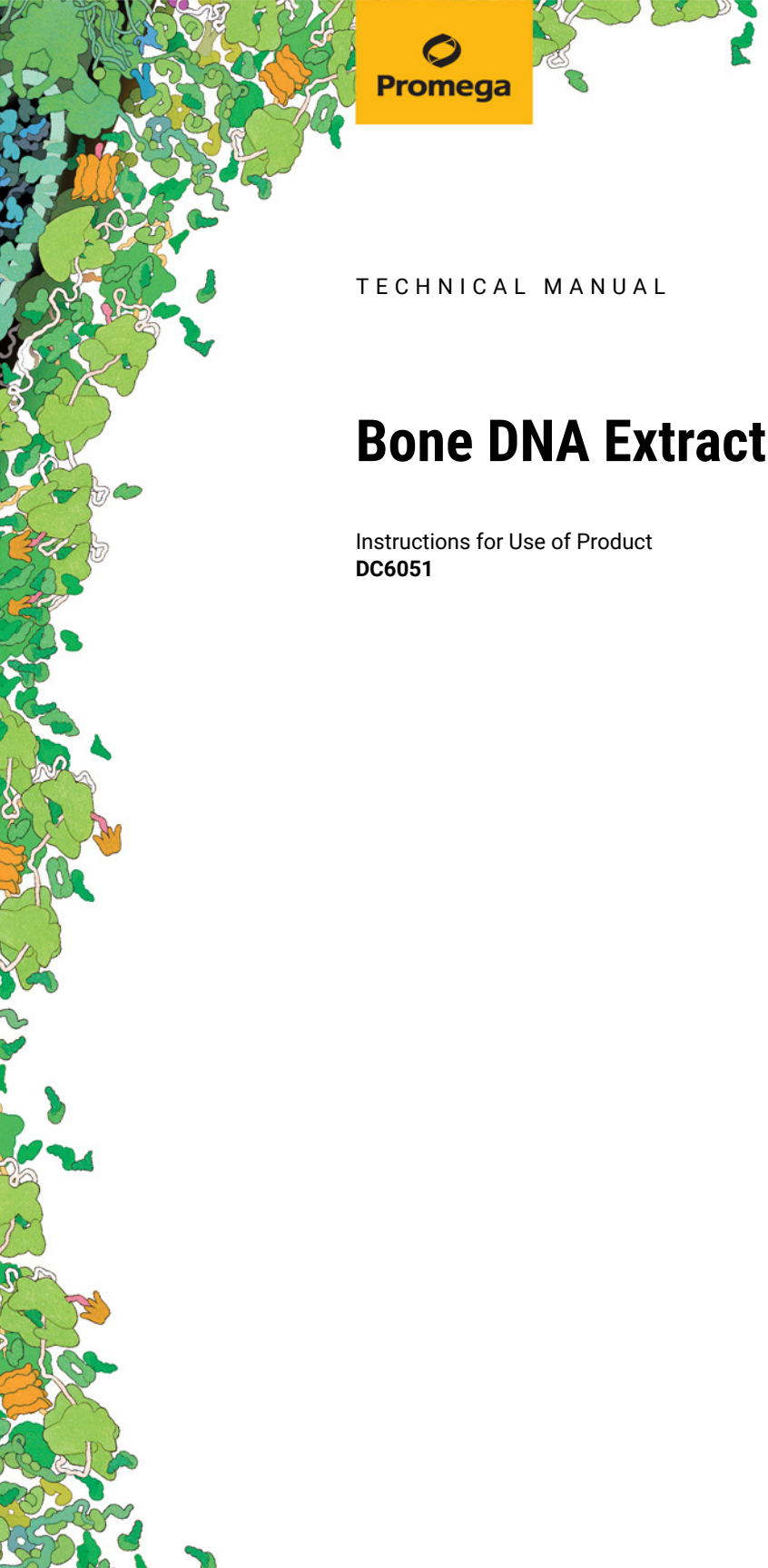

TECHNICAL MANUAL

Bone DNA Extraction Kit

Instructions for Use of Product
DC6051

Revised 8/22
TM691

Promega

Bone DNA Extraction Kit

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 Manual.
E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

1. Description

Bone and teeth are traditionally considered difficult DNA extraction sample types due to their unique composition. Typical preprocessing lysis reagents used for casework samples are not effective in efficiently extracting DNA from the calcium matrix. This Bone DNA Extraction protocol is based on a Demineralization Buffer protocol developed by the Armed Forces DNA Identification Lab, and has been used in combination with many purification protocols, including organic extraction (1).

The Bone DNA Extraction Kit contains reagents needed for preprocessing pulverized bone samples. The preprocessing step can be followed up with automated (Maxwell® Instrument) or manual methods of DNA purification using DNA IQ™ chemistry. Protocols for both methods are included in this technical manual.

Note: The formulation of Bone DNA Extraction Kit reagents is identical to the formulation for Bone DNA Extraction Kit Custom, reagents. Studies showcasing the performance of custom kit with challenging samples have been documented in the literature (2,3).

For additional details regarding manual DNA purification, refer to *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin* #TB296. For additional details regarding DNA purification using Maxwell® instruments, refer to the DNA IQ™ Casework Kit protocol in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499. Purified DNA is suitable for downstream processing applications, such as human DNA quantification using Plexor® HY and PowerQuant® Systems and STR amplification using PowerPlex® reagents.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Bone DNA Extraction Kit	**100 preps**	**DC6051**

Not For Medical Diagnostic Use. Includes:

- 50ml Demineralization Buffer
- 3 × 0.9ml 1-Thioglycerol
- 4ml Proteinase K (PK) Solution
- 150ml Lysis Buffer

Storage Conditions: Store the Bone DNA Extraction Kit at +15 to +30°C. Upon receipt, store 1-Thioglycerol at +2 to +10°C.

3. Before You Begin

The quality of an STR profile from a bone sample depends on the type, age and environmental storage condition of the bone. The success of purifying nuclear DNA from bone also depends on DNA integrity. Soil conditions and humidity have a profound effect on DNA quality.

Bone must be preprocessed to efficiently extract DNA from the calcium matrix. The success of the extraction process also depends on the degree of grinding, which can be accomplished by physical grinding or with a drill operated at low speed to reduce heat buildup. The extraction process works most efficiently with finely ground bone in which cells interspersed in the bone matrix are more accessible for lysis.

Materials to Be Supplied by the User

- DNA IQ™ System (Cat.# DC6700 and DC6701)
- PolyATract® System 1000 Magnetic Separation Stand (Cat.# Z5410) or MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5332, Z5342)
- Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550)
- Maxwell® FSC (Cat.# AS4600) or Maxwell® RSC 48 (Cat.# AS8500) Instrument
- 56°C heater/shaker
- 65°C heater/shaker
- 95–100% ethanol
- isopropyl alcohol
- microcentrifuge tubes, 1.5ml
- aerosol-resistant micropipette tips

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4. Preprocessing Using Demineralization Buffer and Purification Using DNA IQ™ Chemistry

For more information, refer to *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin* #TB296.

Note: This protocol uses more DNA IQ™ Resin than the standard amount listed in Technical Bulletin #TB296 and will provide approximately 54 purifications with the DNA IQ™ System (Cat.# DC6701) or 180 purifications with the DNA IQ™ System (Cat.# DC6700). DNA IQ™ reagents are available as stand-alone products.

4.A. Preprocessing Method

1. Add 100mg of pulverized bone powder to 1.5ml microcentrifuge tubes for processing.
2. Prepare bone lysis cocktail A as follows, preparing enough for an excess of n + 2 samples:

Reagent	Volume for n = 1 Sample
Demineralization Buffer	400µl
Proteinase K (PK) Solution	40µl
1-Thioglycerol	10µl

Note: Pipet 1-Thioglycerol slowly; it is viscous.

3. Add 400µl of bone lysis cocktail A to each 1.5ml tube containing bone powder.
4. Vortex each tube for approximately 10 seconds to mix.
5. Incubate the tubes on a heater/shaker set at 56°C for 2.5 hours, shaking at 1,000rpm.
6. Remove the tubes from the heater/shaker and vortex for approximately 10 seconds to mix.
7. Centrifuge the tubes at 13,000 × *g* for 5 minutes.
8. Carefully transfer supernatant to new 1.5ml microcentrifuge tubes, taking care not to disturb the pellet. Dispose of tubes containing bone pellets.
9. Prepare bone lysis cocktail B as follows, preparing enough for an excess of n + 2 samples:

Reagent	Volume for n = 1 Sample
Lysis Buffer	990µl
1-Thioglycerol	10µl

Note: Pipet 1-Thioglycerol slowly; it is viscous.

10. Add 800µl of bone lysis cocktail B to each 1.5ml microcentrifuge tube containing lysate from Step 8.

Note: If performing a manual protocol, do not discard the remaining volume of bone lysis cocktail B; 100µl per sample is used in Section 4.B, Step 3.

11. Vortex each tube for approximately 10 seconds to mix.

4.B. Purification using DNA IQ™ Manual Chemistry

For more information, refer to *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin* #TB296.

1. Vortex the DNA IQ™ Resin to resuspend the magnetic beads. Add 15µl of resin to each tube containing the preprocessed lysate, vortex for 3–5 seconds and incubate at room temperature for 5 minutes, vortexing every 2 minutes to mix.
2. Vortex for 3–5 seconds and place in the magnetic stand. Allow the beads to collect on the side, then gently remove the lysate and discard. Do not disrupt the beads.
3. Add 100µl of bone lysis cocktail B (prepared in Section 4.A, Step 9), vortex for 3–5 seconds and collect the beads with the magnetic stand. Gently remove and discard the bone lysis cocktail B, including any remaining droplets from the cap.
4. Dilute the 2X Wash Buffer to 1X as follows:

Reagent	Volume to Add for 1X
2X Wash Buffer	30ml
isopropyl alcohol	15ml
95–100% ethanol	15ml

5. Add 100µl of 1X Wash Buffer, vortex for 3–5 seconds and collect the beads with the magnetic stand. Remove and discard the Wash Buffer, including any remaining droplets from the cap. Repeat the washes with 1X Wash Buffer two more times for a total of three washes.
6. With the lid open, air-dry the beads for 5 minutes.
7. Add 50µl of Elution Buffer, close the lid and vortex for 3–5 seconds, and incubate at 65°C for 5 minutes.
8. Vortex for 3–5 seconds, collect the beads using the magnetic stand and transfer the eluted DNA to a sterile 1.5ml microcentrifuge tube.
9. Following the run, the eluted DNA can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

5. Preprocessing Using Demineralization Buffer and Purification Using Maxwell® FSC or Maxwell® RSC 48 Instrument

5.A. Preprocessing Method

1. Weigh out 100mg of pulverized bone powder into 1.5ml tubes for processing.
2. Prepare bone lysis cocktail A as follows, allowing for an excess of n + 2 samples:

Reagent	Volume for n = 1 Sample
Demineralization Buffer	400µl
Proteinase K (PK) Solution	40µl
1-Thioglycerol	10µl

Note: Pipet 1-Thioglycerol slowly; it is viscous.

3. Add 400µl of bone lysis cocktail A to each 1.5ml tube containing bone powder.
4. Vortex each tube for approximately 10 seconds to mix.
5. Incubate the tubes on a heater/shaker set to 56°C for 2.5 hours, shaking at 1,000rpm.
6. Remove the tubes from the heater/shaker and vortex for approximately 10 seconds to mix.
7. Centrifuge the tubes at 13,000 × *g* for 5 minutes.
8. Carefully transfer supernatant to new 1.5ml tubes, taking care not to disturb the pellet. Dispose of tubes containing bone pellets.
9. Prepare bone lysis cocktail B as follows, allowing for an excess of n + 2 samples:

Reagent	Volume for n = 1 Sample
Lysis Buffer	990µl
1-Thioglycerol	10µl

Note: Pipet 1-Thioglycerol slowly; it is viscous.

10. Add 800µl of bone lysis cocktail B to each 1.5ml tube containing lysate from Step 8.
11. Vortex each tube for approximately 10 seconds to mix.

5.B. Purification Using Maxwell® FSC or Maxwell® RSC 48 Instrument

For details, please refer to *Maxwell® FSC DNA IQ™ Casework Technical Manual* #TM499, Section 4.

1. Prepare a Maxwell® FSC Deck Tray with Maxwell® FSC Cartridges, FSC Plunger Pack (Cat.# AS7101) and Elution Tubes 0.5ml. Add 50µl of Elution Buffer supplied in the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) to the bottom of each Elution Tube.
2. Transfer the entire volume of eluate from Section 5.A, Step 11 (approximately 1.1ml) to well 1 of the Maxwell® FSC Cartridge.
3. Mix the sample in well #1 using the pipette tip to ensure that all sample has been transferred. Change pipette tips between samples. After mixing, run the DNA IQ™ Casework protocol.
4. Following the run, the eluted DNA can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

6. 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**

6.A. Bone DNA Extraction Kit Troubleshooting

Symptoms	Causes and Comments
Low DNA yield	Consider an alternate skeletal powder preparation method. Try using a mortar and pestle under liquid nitrogen for sample powder preparation.
No STR result	Use the PowerQuant® System (Cat.# PQ5002) to assess DNA quantity, quality or both.

6.B. Maxwell® FSC DNA IQ™ Casework Kit Troubleshooting

Symptoms	Causes and Comments
Low DNA yield	Insufficient sample was processed. Add more starting material for preprocessing to increase yield.
Instrument over run or under run errors	Verify that nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly.
	Perform a Self Test from the Settings menu. The instrument will rehome itself. If the error recurs, contact Promega for service.
	The cartridges were not completely seated on the platform. Ensure the cartridges are pressed firmly into place.
	Incorrect elution tube was used. To prevent a z-axis collision, use only the 0.5ml Elution Tube provided with the Maxwell® FSC DNA IQ™ Casework Kit. Other tubes may have different dimensions.

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6.B. Maxwell® FSC DNA IQ™ Casework Kit Troubleshooting (continued)

Symptoms	Causes and Comments
Resin carryover during elution	The presence of a small amount of resin particles in the Elution Tube will not affect the final DNA concentration or downstream applications. If desired, final elution tubes can be centrifuged to pellet resin and eluates can be transferred to a clean tube or an additional resin capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341).
Instrument unable to pick up plungers	Make sure you are using the plungers in the Maxwell® FSC DNA IQ™ Casework Kit; the plungers for the Maxwell® FSC reagent kits are different than those of other Maxwell® reagent kits.
Instrument calibration error	The cartridges were not completely seated in the deck tray. Ensure the cartridges are pressed firmly into place.
	Verify nothing is physically blocking the movement of the platform, plunger bar or magnet bar.
	Confirm that you are using the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) with FSC Plungers.
	Turn the machine off then on to cycle the power. The instrument will rehome itself. If the calibration error occurs again after power cycling, contact Promega for service.
	Turn the machine off then on to cycle the power. After cycling power, run the DNA IQ Casework method without a cartridge in the machine. If another calibration error occurs during the run, contact Promega for service.
	Incorrect elution tube was used. Use only the 0.5ml Elution Tube provided with the Maxwell® FSC DNA IQ™ Casework Kit. Other tubes may have different dimensions.

7. Composition of Buffers and Solutions

Demineralization Buffer

0.5M EDTA, pH 8.0
1% Lauroylsarcosine

8. References

1. Lorielle, O.M. *et al.* (2007) High efficiency DNA extraction from bone by total demineralization. *Forensic Sci. Int. Genet.* **1**, 191–5.
2. Calacal, G.C. *et al.* (2021) Improved autosomal STR typing of degraded femur samples extracted using a custom demineralization buffer and DNA IQ™. *Forensic Sci. Int. Synerg.* **3**, 100131.
3. Duijs, F.E., Sijen, T. (2020) A rapid and efficient method for DNA extraction from bone powder. *Forensic Science Int. Reports*. **2**, 100099.

9. Appendix

9.A. Maxwell® FSC Cartridge Setup

1. Change gloves before handling Maxwell® FSC Cartridges, FSC Plungers and Elution Tubes. Place the cartridges to be used in the deck tray(s). Use the Maxwell® Deck Trays appropriate to the Maxwell® Instrument. Place each cartridge in the deck tray with well #1 (the largest well) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge.

   Notes:

   - Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
   - Clean sample or reagent spills on any part of the deck tray with a detergent-water solution, followed by 70% ethanol and then water. Do not use bleach on any instrument parts.
2. Transfer each preprocessed sample to well #1 (the largest well) of each cartridge (Figure 1).
3. Mix the sample in well #1 using the pipette tip to ensure that all sample has been transferred. Change pipette tips between samples.
4. Place one plunger into well #8 of each cartridge.
5. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 50µl of Elution Buffer to the bottom of each elution tube.

9.A. Maxwell® FSC Cartridge Setup (continued)

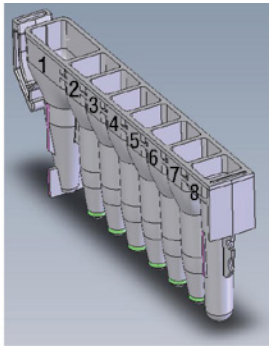

Figure 1. Maxwell® FSC Cartridge. Preprocessed sample is added to well #1, and a plunger is added to well #8.

Figure 2. Setup and configuration of the deck tray. Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

9.B. Maxwell® Instrument Setup and Run

For detailed information, refer to the technical manual specific to your Maxwell® Instrument:

Instrument	Technical Manual
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® software on the Tablet PC. The instrument will proceed through a self-check and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method. Touch the DNA IQ™ Casework method.
3. Following the run, the eluted DNA can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.
4. If applicable to your Maxwell® Instrument model, verify that the DNA IQ Casework method has been selected, and touch the **Proceed** button. If requested by the software, scan or enter kit lot and expiration information as required by the Administrator.
5. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect the positions that will be used during this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, touch the **Front** and **Back** buttons to select or deselect cartridge positions on each deck tray.
6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, that cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

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10. Related Products

Product	Size	Cat.#
DNA IQ™ System	400 reactions	DC6700
	100 reactions	DC6701
PolyATract® System 1000 Magnetic Separation Stand	1 each	Z5410
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
Maxwell® FSC DNA IQ™ Casework Kit	48 preps	AS1550
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® RSC 48 Instrument	1 each	AS8500

Accessory Products

Product	Size	Cat.#
DNA IQ™ Resin	8ml	A8258
	50ml	A8251
ClickFit Microtube, 1.5ml	100/pack	V4745

11. Summary of Changes

The following changes were made to the 8/22 revision of this document:

1. Added Note in Section 4.
2. Made minor text edits in Sections 4.A, 4.B and 9.B.
3. Added Related Products, Section 10.
4. Updated trademark information and document font.