January 2020

GeneRead™ Size Selection Handbook

For fast and reliable removal of DNA fragments <150 bp for library preparation in next-generation sequencing (NGS) applications

Sample to Insight

QIAGEN

Contents

Kit Contents

GeneRead Size Selection Kit **Catalog no.** **Number of reactions**	**(50)** **180514** **50**
MinElute® Spin Columns	50
Buffer SB1*	60 ml
Buffer TE	30 ml
Buffer EB	15 ml
Collection Tubes (1.5 ml)	100
Collection Tubes (2 ml)	50
Quick-Start Protocol	1

* Buffer SB1 contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. See "Safety Information" on page 4 for further information.

Shipping and Storage

Upon arrival, open the kit and store MinElute spin columns at 2–8°C. The remaining kit components can be stored at room temperature (15–25°C). Under these conditions, the GeneRead Size Selection Kit can be stored for up to 12 months without showing any reduction in performance and quality, if not otherwise stated on the label. Check buffers for precipitate before use and redissolve at 37°C if necessary. The kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Intended Use

The GeneRead Size Selection Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

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

Buffer SB1 contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach.

In case liquid containing this buffer 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the GeneRead Size Selection Kit tested against predetermined specifications to ensure consistent product quality.

Product Specifications

Feature	Specification
Compatibility	DNA in common elution buffers, such as Buffers TE, EB and AE, and DNA libraries created using the GeneRead DNA Library Prep I (QIAGEN) and the TruSeq DNA LT Sample Prep Kit (Illumina®)
Maximum binding capacity	6 µg
Full recovery of DNA	300 bp and longer
Cut-off limit	<150 bp
Maximum sample input volume	140 µl
Elution volume	17 µl
Volume of eluate	15 µl
Capacity of column reservoir	800 µl

Introduction

The GeneRead Size Selection Kit enables precise size selection and purification of DNA fragments for next-generation sequencing (NGS) applications. The kit contains MinElute columns and an optimized binding buffer for the selection of DNA fragments larger than 150 bp.

The GeneRead Size Selection Kit is suitable for use with DNA libraries prepared using:

- GeneRead DNA Library Prep I Kit (QIAGEN) (for instruments from Illumina)
- TruSeq DNA LT Sample Prep Kit (Illumina)

Principle and procedure

The GeneRead Size Selection Kit combines the speed and convenience of spin-column technology with the selective properties of a uniquely designed binding buffer. MinElute spin columns are designed to give high concentrations of purified DNA for subsequent reactions. The special buffer provided with the kit is optimized for efficient removal of small DNA fragments, such as adapter monomers and adapter dimers (Figure 1). Larger DNA fragments adsorb to the silica membrane in the presence of optimized concentrations of salt, while contaminants and smaller fragments pass through the column. This leads to a cut-off limit (defined as the length of the shortest fragment that can be visualized by electropherogram) of 150 bp. Impurities, such as enzymes from previous reactions, are efficiently washed away, and pure, size-selected DNA is eluted. For optimal adapter monomer and dimer removal, together with high DNA recovery, two subsequent purification steps are performed for size selection during library preparations for next-generation sequencing.

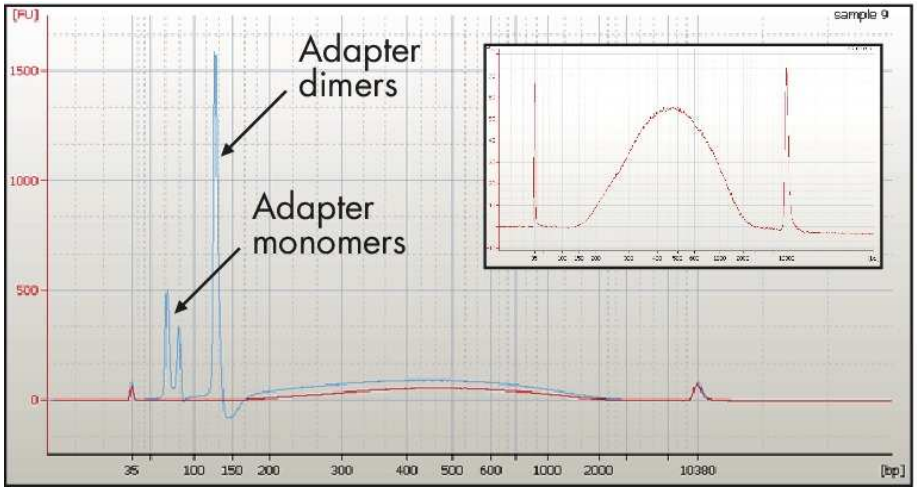


Figure 1. Precise size selection. The GeneRead Size Selection Kit effectively removes adapter dimers and adapter monomers following library preparation. A scaled-up image of the above data showing the correct size distribution of Illumina-compatible library fragments following size selection is shown in inset. **FU**: Fluorescence units.

Optimized binding buffer for size selection and cleanup

The GeneRead Size Selection Kit includes MinElute spin columns and an optimized binding buffer for the selection of DNA fragments >150 bp.

This size selection is compatible with DNA in common elution buffers as TE, EB and AE, as well as the buffers used in the respective steps of the following kits: QIAGEN's GeneRead DNA Library Prep I Kit and TruSeq DNA LT Sample Prep Kit (Illumina).

Washing

During the DNA adsorption step, unwanted DNA fragments, such as adapter monomers and adapter dimers, as well as salts, enzymes and other impurities, do not bind to the silica membrane, but flow through the column. Salts are quantitatively washed away by ethanol. Any residual ethanol, which may interfere with subsequent enzymatic reactions, is removed by an additional centrifugation step.

Elution in low-salt solutions

Elution efficiency is strongly dependent on the salt concentration and pH of the elution buffer. Buffers EB, AE and TE are suitable. However, final elution with Buffers TE and AE is not recommended for all applications because the presence of EDTA in the buffers may inhibit subsequent enzymatic reactions.

For removal of adapters and adapter dimers in NGS library preparations, the DNA is cleaned up and eluted twice. The first elution uses Buffer TE. Partially size-selected DNA is then rebound to the column and washed again. The second elution is facilitated with 17 µl Buffer EB. The volume of the final eluate is 15 µl. Final elution with Buffer TE is not recommended because the EDTA present in the buffer may inhibit subsequent enzymatic reactions.

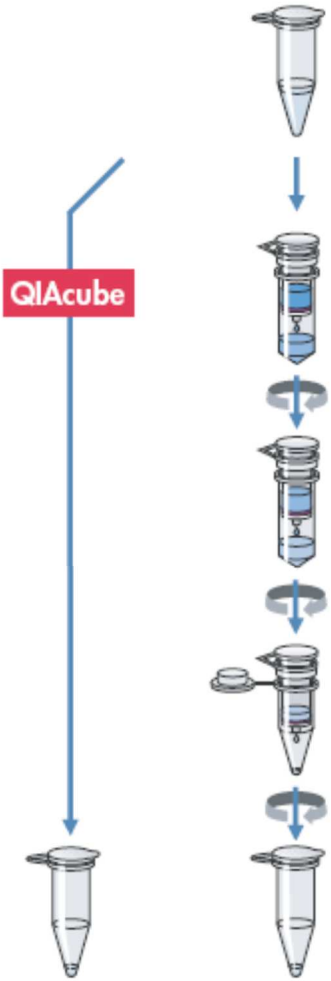
Automated purification of DNA on QIAcube Instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the GeneRead Size Selection Handbook for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.

GeneRead Size Selection Principle



Mix the sheared DNA sample or the DNA library with Buffer SB1

Bind DNA >150 bp

Wash with 80% ethanol

Elute

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.

- 80% ethanol (prepared from 96–100% ethanol)*
- Microcentrifuge

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

Protocol: GeneRead Size Selection of Sheared DNA in Common Elution Buffers

This protocol is optimized for the purification of double-stranded DNA fragments larger than 150 bp in high concentrations. The DNA is size selected and purified using MinElute spin columns in a microcentrifuge, while contaminants, such as salts, enzymes and small fragments, of DNA are effectively removed.

Important points before starting

- DNA should be sheared in common elution buffers, such as Buffers EB, TE or AE.
- All centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge at room temperature (15–25°C).

Things to do before starting

- Wash steps should be performed using 80% ethanol prepared from 96–100% ethanol.

Procedure

1. Add 4 volumes of Buffer SB1 to 1 volume of the sheared DNA sample, and mix.
   Usually 400 µl Buffer SB1 is added to 100 µl DNA.
2. To bind DNA, apply the sample to the MinElute spin column and centrifuge for 1 min.
   For maximum recovery, transfer all traces of the sample to the column.
3. Discard the flow-through and place the MinElute spin column back into the same tube.
4. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
5. Discard the flow-through and place the MinElute spin column back into the same tube.
6. Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
7. Discard the flow-through and place the MinElute spin column back into the same tube.

8. Centrifuge the MinElute spin column for an additional 1 min.

   IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.

9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).

10. For elution, add 17 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.

    Note: A second elution step can be beneficial for higher yield. To avoid dilution of the eluate, use a different microcentrifuge tube.

Protocol: GeneRead Size Selection of DNA Libraries Prepared with the GeneRead DNA Library Prep I Kit

This protocol is optimized for the size selection and cleanup of DNA libraries prepared using the GeneRead DNA Library Prep I Kit (for use with instruments from Illumina). The convenient, spin-column-based procedure ensures precise size selection of the DNA library, while effectively removing adapter dimers or monomers, and contaminants, such as salts and enzymes, using MinElute spin columns in a microcentrifuge.

Important point before starting

- All centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge at room temperature (15–25°C).
- Please refer to step 10 of the *GeneRead DNA Library Prep I Kit Quick-Start Protocol*.

Things to do before starting

- Wash steps should be performed using 80% ethanol prepared from 96–100% ethanol.

Procedure

1. Add 4 volumes of Buffer SB1 to 1 volume of the DNA library sample prepared at step 10 of the GeneRead DNA Library Prep I Kit procedure, and mix.

   Usually 360 µl Buffer SB1 is added to 90 µl sample.
2. To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 min.

   For maximum recovery, transfer all traces of the sample to the column.
3. Discard the flow-through and place the MinElute spin column back into the same tube.
4. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.

5. Discard the flow-through and place the MinElute spin column back into the same tube.
6. Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
7. Discard flow-through and place the MinElute spin column back into the same tube.
8. Centrifuge the MinElute spin column for an additional 1 min.

   IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).
10. Add 90 µl Buffer TE to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane. Keep the spin column and the flow-through.
11. Place the MinElute spin column into a new 2 ml collection tube (provided). Add 4 volumes of Buffer SB1 to 1 volume of the flow-through, and mix.

    Usually ~360 µl Buffer SB1 is added to ~90 µl DNA.
12. Re-apply the sample to the MinElute spin column and centrifuge for 1 min.

    For maximum recovery, transfer all traces of the sample to the column.
13. Discard the flow-through and place the MinElute spin column back into the same tube.
14. To wash, add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.
15. Discard the flow-through and place the MinElute spin column back into the same tube.
16. Add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.
17. Discard the flow-through and place the MinElute spin column back into the same tube.
18. Centrifuge the MinElute spin column for an additional 1 min.

    IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
19. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).

20. For elution, add 17 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.

Protocol: GeneRead Size Selection of DNA Libraries Prepared with the TruSeq DNA LT Sample Prep Kit (Illumina)

This protocol is optimized for the cleanup of DNA libraries prepared using the TruSeq DNA LT Sample Prep Kit from Illumina. The procedure effectively removes adapter dimers and monomers, as well as contaminants, such as salts and enzymes, using MinElute spin columns in a microcentrifuge.

Important point before starting

- All centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge at room temperature (15–25°C).

Things to do before starting

- Wash steps should be performed using 80% ethanol prepared from 96–100% ethanol.

Cleanup of end-repair reactions

1. After the end-repair reaction has been incubated for 30 min, transfer the DNA reaction mixture to a microcentrifuge tube and add 4 volumes of Buffer SB1 to 1 volume of sample, and mix.

   Usually 400 µl Buffer SB1 is added to 100 µl sample.
2. To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 min.

   For maximum recovery, transfer all traces of sample to the column.
3. Discard the flow-through and place the MinElute spin column back into the same tube.
4. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
5. Discard the flow-through and place the MinElute spin column back into the same tube.

6. Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
7. Discard the flow-through and place the MinElute spin column back into the same tube.
8. Centrifuge the MinElute spin column for an additional 1 min.

   IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).
10. For elution, add 17.5 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.
11. Use 15 µl eluate for 3′ adenylation.

Cleanup of ligation reactions

1. After the ligation reaction is stopped by the addition of the stop ligation buffer, transfer the DNA reaction mixture to a microcentrifuge tube and add 4 volumes of Buffer SB1 to 1 volume of sample, and mix.

   Usually 170 µl Buffer SB1 is added to 42.5 µl sample.
2. To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 min.

   For maximum recovery, transfer all traces of the sample to the column.
3. Discard the flow-through and place the MinElute spin column back into the same tube.
4. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
5. Discard the flow-through and place the MinElute spin column back into the same tube.
6. Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
7. Discard the flow-through and place the MinElute spin column back into the same tube.

8. Centrifuge the MinElute spin column for an additional 1 min.

   IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.

9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).
10. Add 90 µl Buffer TE to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane. Keep the spin column and the flow-through.

11. Place the MinElute spin column into a new 2 ml collection tube (provided). Add 4 volumes of Buffer SB1 to 1 volume of flow-through, and mix.

    Usually ~360 µl Buffer SB1 is added to ~90 µl DNA.

12. Re-apply the sample to the MinElute spin column and centrifuge for 1 min.

    For maximum recovery, transfer all traces of sample to the column.

13. Discard the flow-through and place the MinElute spin column back into the same tube.
14. To wash, add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.
15. Discard the flow-through and place the MinElute spin column back into the same tube.
16. Add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.
17. Discard the flow-through and place the MinElute spin column back into the same tube.
18. Centrifuge the MinElute spin column for an additional 1 min.

    IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.

19. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).
20. For elution, add 22.5 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.

21. Use 20 µl of eluate for DNA fragment enrichment.

Cleanup of PCR

1. After amplification, transfer the DNA sample to a microcentrifuge tube, add 4 volumes of Buffer SB1 to 1 volume of sample, and mix.

   Usually 200 µl Buffer SB1 is added to 50 µl sample.

2. To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 min.

   For maximum recovery, transfer all traces of the sample to the column.

3. Discard the flow-through and place the MinElute spin column back into the same tube.
4. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
5. Discard the flow-through and place the MinElute spin column back into the same tube.
6. Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.
7. Discard the flow-through and place the MinElute spin column back into the same tube.
8. Centrifuge the MinElute spin column for an additional 1 min.

   IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.

9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).
10. For elution, add 32.5 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

    IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.

11. Use 30 µl eluate for library validation or enrichment.

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 or no recovery	
a) Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of a low-salt buffer.
b) Elution buffer incorrectly dispensed	Add elution buffer to the center of the MinElute membrane to ensure that the buffer completely covers the membrane.
Incomplete adapter removal	
Ethanol in buffer	Be sure not to add ethanol or other alcohol to the Buffer SB1 and sample mix as it will disturb the size selection properties.
DNA does not perform well in downstream applications	
a) Salt concentration in the eluate too high	Modify the wash step by incubating the MinElute spin column for 5 min at room temperature (15–25°C) after adding 700 µl of 80% ethanol, and then centrifuge.
b) Eluate contains residual ethanol	Ensure that the wash flow-through is drained from the collection tube and that the MinElute spin column is then centrifuged at ≥10,000 x *g* for 1 min.

Ordering Information

Product	Contents	Cat. no.
GeneRead Size Selection Kit (50)	For 50 reactions: Spin columns and buffers	180514
Related products		
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
GeneRead Kits — for next-generation sequencing applications		
GeneRead Library Quant Kit	PCR arrays with accessory components for sample library quantification prior to next-generation sequencing	180612
GeneRead DNA Library I Core Kit (12)	For 12 reactions: Buffers and reagents for end-repair, A-Addition and ligation, for use with Illumina instruments	180432
GeneRead DNA I Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Illumina instruments	180455
QIAamp® Kits — for genomic DNA purification		
QIAamp DNA Mini Kit	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA FFPE Tissue Kit	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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.

Document Revision History

Date	Changes
January 2020	Updated text, ordering information and intended use for QIAcube Connect.

Limited License Agreement for GeneRead Size Selection Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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