April 2018

GeneRead™ Clonal Amp Q Handbook

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For clonal amplification of DNA libraries for next-generation sequencing (NGS) applications using the QIAGEN GeneRead QIAcube® instrument.

For Research Use Only. Not for use in diagnostic procedures.

REF 185001

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Sample to Insight

QIAGEN

Contents

Kit Contents

GeneRead Clonal Amp Q Kit (4)	
Catalog no.	**185001**
Number of reactions	**4**
GeneRead Clonal Amp Q Kit Box 1*	Cat. no. 1095015
GeneRead Clonal Amp Q Kit Box 2*	Cat. no. 1095016
GeneRead Clonal Amp Q Kit Box 3*	Cat. no. 1095017

* Kit Boxes 1–3 are components of the GeneRead Clonal Amp Q Kit (4).

GeneRead Clonal Amp Q Kit Box 1†	
Catalog no.	**1095015**
Master Mix	4 x 1600 µl
Primer Mix	4 x 650 µl
Enrichment Buffer	1 x 12 ml
Enrichment Buffer Additive*	1 x 120 µl

† Not for individual sale; to order reagents, see cat. no. 185001.
* For preparation of enrichment buffer, see page 29.

GeneRead Clonal Amp Q Kit Box 2‡	
Catalog no.	**1095016**
Primer Loaded PCR Beads	1 x 1000 µl
Super A Beads	4 x 600 µl
Denaturation Solution	1 x 7.5 ml

‡ Not for individual sale; to order reagents, see cat. no. 185001.

GeneRead Clonal Amp Q Kit Box 3*	
Catalog no.	**1095017**
Oil Mix	1 x 18 ml
Buffer T	1 x 12 ml
Buffer B	1 x 14 ml
Buffer E	1 x 8 ml
Buffer N	1 x 37 ml
Buffer A	1 x 90 ml
Buffer D	1 x 100 ml
Enrichment Column	4
Tube 10 ml	12
PCR Plate	4
8-strip PCR tubes	4
Sealing Film	4
Color Chart	1

* Not for individual sale; to order reagents, see cat. no. 185001.

Storage

The GeneRead Clonal Amp Q Kit is delivered in three (3) boxes.

GeneRead Clonal Amp Q Kit Box 1 is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer (not frost-free).

GeneRead Clonal Amp Q Kit Box 2 is shipped on cool packs and should be stored immediately upon receipt at 2–8°C.

GeneRead Clonal Amp Q Kit Box 3 is shipped at ambient temperature and should be stored at room temperature (15–25°C).

If stored under these conditions, the kit is stable until the date indicated on the QC label inside the kit lid.

Make sure that all reagents are at room temperature when used.

Intended Use

The GeneRead Clonal Amp Q Kit is intended for Research Use Only. Not for use in diagnostic procedures.

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.

Super A Beads and Buffer D contain sodium azide, which is known to form explosive compounds when it is combined with metal halides and many heavy metals, such as lead, copper, gold and silver. Denaturation Solution may be corrosive to metals. Keep away from oxidizing agents and acidic or alkaline products.

Use either polypropylene (PP) or fluorinated ethylene propylene (FEP) plastics for disposing of GeneRead QIAcube waste.

DO NOT USE polystyrene (PS) or polyethylene (PE) plastics for disposing of GeneRead QIAcube waste.

Quality Control

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

Introduction

Next Generation Sequencing (NGS) is a driving force for numerous applications, including cancer research, stem cell research, metagenomics, population genetics and medical research. After the completion of library preparation using the GeneRead DNA Library Q Kit, the GeneRead Clonal Amp Q Kit is used to prepare sequencing templates using clonal amplification. Figure 1 illustrates the workflow for bead preparation and enrichment.

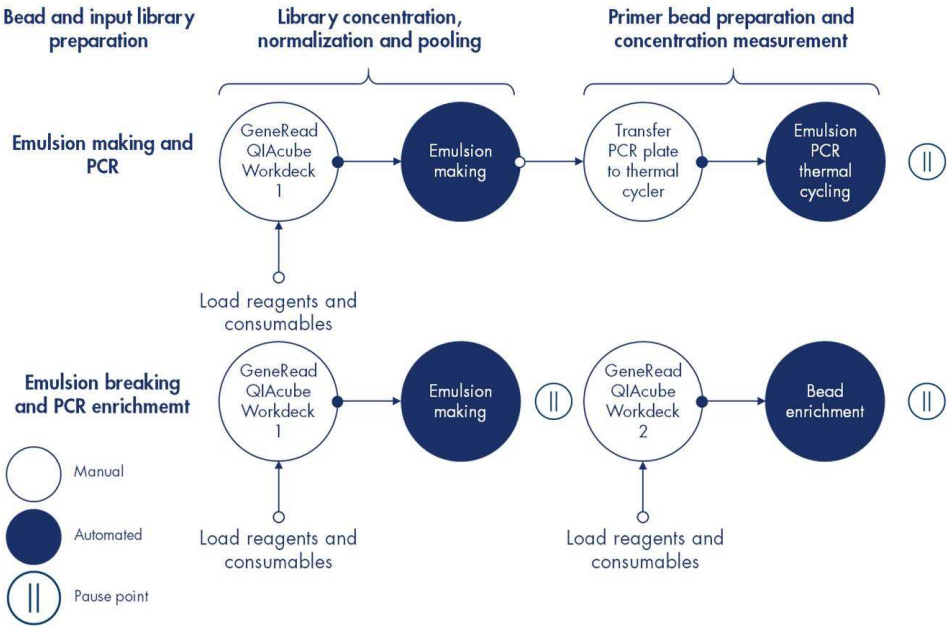

Figure 1. Workflow: bead **preparation and enrichment for NGS.**

Principle and Procedure

The GeneRead Clonal Amp Q Kit enables processing of DNA library samples and pooling of multiple DNA library samples that have been uniquely bar coded during the library preparation process. The clonal amplification workflow is comprised of emulsion making, emulsion PCR, emulsion breaking and bead enrichment. At the completion of the entire clonal amplification workflow, the DNA library samples or pools will have been clonally amplified and, after bead yield determination, are ready for sequencing.

Description of Protocols

This handbook contains the five (5) protocols for the clonal amplification of DNA library samples or pools using the GeneRead Clonal Amp Q Kit.

The 5-step workflow is outlined below:

1. Library concentration normalization and pooling

   Manual normalization of the DNA library sample concentration(s) and pooling (if multiplexing is desired) are performed.

2. Emulsion making

   The Primer Loaded PCR Beads are pre-treated to eliminate bead clumping.

   Automated emulsion making is carried out on the GeneRead QIAcube.

   Upon completion of emulsion making, the PCR plate containing the emulsions is removed from the GeneRead QIAcube Workdeck and manually loaded onto a stand-alone thermal cycler for clonal amplification.

3. Emulsion breaking

   The PCR plate containing the clonally amplified emulsions is returned to the GeneRead QIAcube for automated emulsion breaking.

4. Bead enrichment

   Super A Beads are manually washed prior to bead enrichment.

   Automated bead enrichment is conducted on the GeneRead QIAcube.

   Enriched beads are recovered and manually transferred from the GeneRead QIAcube into sample tubes.

5. Bead yield determination using either optical density or Color Chart

   Both bead determination methods are verified to evaluate yield without impact on sequencing performance.

The acceptable range of beads required for Sequencing Primer hybridization can be found in the *GeneRead Sequencing Handbook* used downstream of the GeneRead Clonal Amp Q Kit. If beads are stored longer than 48 hours and require additional processing, proceed according to the corresponding *GeneRead Sequencing Handbook*. If the amount of beads used for sequencing is outside the recommended ranges, QIAGEN is unable to guarantee optimal results. See "Troubleshooting Guide", page 35, for possible causes of abnormal bead recovery.

Optical density method

For each lot of Primer Loaded PCR Beads, a standard curve is created using Primer Loaded PCR Beads that have not been processed through the clonal amplification workflow (this standard curve may be re-used for subsequent clonal amplifications with different kits using the same Primer Loaded PCR Bead lot).

Following the clonal amplification workflow, the optical density (OD) of the enriched beads (clonally amplified and enriched library pools) is measured so that the concentration can be extrapolated using the standard curve.

Enriched beads are then aliquoted in preparation for sequencing reaction.

Color Chart method

Refer to the Color Chart supplied in Box 3 of the GeneRead Clonal Amp Q Kit to verify that the amount of the enriched beads is within the acceptable range and that the entire volume of enriched beads can be used for the sequencing reaction.

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.

- GeneRead QIAcube instrument (cat. no. 9002344 (110V), 9002345 (230V))
- Starting material: NGS library. For further information, see GeneRead DNA Library Q Kit (cat. no. 185444) at www.qiagen.com
- Pipets and filter tips
- Pipet aid and disposable serological pipets
- Laboratory timer
- 70% ethanol and isopropanol wipes or lint-free cloths and 70% ethanol or isopropanol

  Optional: Spectrophotometer capable of reading OD at 550 nm 600 nm (equivalent to TECAN® F200, Thermo Scientific Genesys™ 10S or GE®/Amersham® Biosciences Ultrospec™ 10)* and the corresponding lab ware (plates or cuvettes) for the spectrophotometer

  Note: This optional equipment is not necessary if bead yield is determined using the Color Chart supplied in Box 3 of the GeneRead Clonal Amp Q kit
- Vortexer*
- Microcentrifuge (minimum 15,000 x *g*)*
- Mini-centrifuge*
- Magnetic rack (equivalent to Life Technologies® 12321D)*

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

- PCR thermal cycler with block capable of cycling volumes of at least 100 µl (equivalent to the Bio-Rad® C1000™, MJ Research/Bio-Rad PTC 200, Eppendorf® Mastercycler Pro, Eppendorf Mastercycler, X50, or Applied Biosystems/Thermo Fisher Scientific GeneAmp 9700 TC/Veriti TC)*
- Sterile, capped 2 ml micro tubes, non-skirted (Sarstedt®, cat. no. 72.693.005)
- 2 ml capped LoBind tubes (Eppendorf, cat. no. 022431048)
- 1.5 ml capped LoBind tubes (Eppendorf, cat. no. 022431021)
- Filter-Tips, 1000 µl (1024) (QIAGEN, cat. no. 990352)
- Filter-Tips, 1000 µl, wide-bore (1024) (QIAGEN, cat. no. 990452)
- Filter-Tips, 200 µl (1024) (QIAGEN, cat. no. 990332)
- Rotor Adapters (10 x 24) (QIAGEN, cat. no. 990394)

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* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Important Notes

Contamination

An important prerequisite for any clonal amplification experiment is to maintain an amplicon- and template-controlled work environment via physical and workflow barriers and practices. Frequently change gloves when handling reagents and samples. After cleaning GeneRead QIAcube Workdecks and discarding waste, always change gloves. Disinfect the lab bench after any spills by following proper laboratory guidelines.

Cleaning the GeneRead QIAcube

When cleaning the GeneRead QIAcube, use lint-free cloths or alcohol wipes. Do not spray cleaning reagents directly into the GeneRead QIAcube. For instrument maintenance instructions refer to the *GeneRead QIAcube User Manual*.

Template preparation

The total required input for each clonal amplification workflow is 500 µl library pool. A library pool may include up to 12 uniquely bar-coded DNA library samples for multiplex clonal amplification See the *GeneRead QIAact Panels, Powered by QCI™, Handbook* for details about the level of multiplexing and the amount of library pool required for clonal amplification.

Disposing of GeneRead QIAcube waste

Use either PP or FEP plastics for disposing of GeneRead QIAcube waste.

DO NOT USE PS or PE plastics for disposing of GeneRead QIAcube waste.

Protocol: Library Concentration Normalization and Pooling

Important points before starting

- Pipettes should be used to measure correct volumes.
- The following instructions apply to pooling libraries that are equal in concentration and produced from the same target enrichment panel. Additional considerations are required if libraries from different panels are pooled because the number of amplicons in each panel will likely be unequal.

Things to do before starting

- If the libraries to be used are frozen, thaw at room temperature (15–25°C).

Procedure

Dilution of libraries and normalization of concentration to prepare working stock solutions

1. Calculate the amount of library and dilution buffer (Buffer D) required to achieve a working stock solution concentration of 100 pg/µl for each library.

   Note: All libraries must be at the same concentration before pooling.
2. Transfer the appropriate volumes of library and Buffer D to a 1.5 ml tube.
3. Close each tube and vortex for 5–10 seconds, and then pulse-centrifuge the tube.

4. Diluted libraries can be stored at –20°C for up to one month, used immediately in the clonal amplification process (see "Preparing libraries for emulsion making", page 16) or pooled together (see "Optional pooling of uniquely bar-coded libraries", page 16).

   Note: Avoid freeze–thaw cycles of stored library and/or verify library quality by analysis protocol(s) as described in the *GeneRead DNA Library Q Handbook* before clonal amplification.

Optional pooling of uniquely bar-coded libraries

Note: All libraries must be at the same concentration before pooling.

5. For each bar-coded library that is to be pooled (up to a maximum of 12, depending on the gene panel), transfer equal volumes of 100 pg/µl working stock (see above) to a 1.5 ml tube.
6. Close the tube and vortex for 5–10 seconds, and then pulse-centrifuge the tube.
7. Pooled libraries can be stored at –20°C for up to one month or use immediately in the clonal amplification process.

   Note: Avoid freeze–thaw cycles of stored library and/or verify library quality by analysis protocol(s) as described in the *GeneRead DNA Library Q Handbook* before clonal amplification.

Preparing libraries for emulsion making

Note: A "library pool" refers to only one sample if multiplexing is not performed.

8. If libraries are frozen, thaw at room temperature (15–25°C).
9. Transfer the appropriate volumes of 100 pg/µl library pool (according to the *GeneRead QIAact Panels, Powered by QCI, Handbook*) and Buffer D to a 2 ml non-skirted Sarstedt tube to achieve a final recommended concentration of DNA in 500 µl.
10. Close the tube, vortex for 5–10 seconds, and then pulse-centrifuge the tube.
11. Proceed with "Protocol: Emulsion Making", page 17.

Protocol: Emulsion Making

Important points before starting

- Consumables and reagents should be free of templates and amplicons.
- The Primer Mix and Master Mix are provided as ready-to-use premixes (see Table 3).
- Primer Loaded PCR Beads must be fully resuspended prior to use.
- Pipettes should be used to measure correct volumes.

Things to do before starting

- Remove the tube of Primer Loaded PCR Beads and the Denaturation Solution bottle from refrigeration.
- Gently invert the Denaturation Solution and buffer bottles to mix contents prior to use.

Procedure

Pre-treatment of Primer Loaded PCR Beads

1. Vortex Primer Loaded PCR Beads thoroughly for at least 1 minute.

   Note: Ensure that the beads are completely resuspended with no visible pellet.

2. Following Table 1, transfer the appropriate volumes of Primer Loaded PCR Beads and Buffer E to a 2 ml capped LoBind tube.

   Note: Use a single LoBind tube for any number (1, 2, 3 or 4) of library pools for one GeneRead QIAcube run. Refrigerate remaining Primer Loaded PCR Beads when finished.

Table 1. Primer Loaded PCR Bead dilution for emulsion making

	Volumes (µl) for number of library pools			
Reagent	**1**	**2**	**3**	**4**
Primer Loaded PCR Beads	210	420	630	840
Buffer E	290	580	870	1160
Total	**500**	**1000**	**1500**	**2000**

3. Pulse-vortex and then pulse-centrifuge the tube.
4. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.
5. Remove and discard the supernatant.
6. Add 1 ml Denaturation Solution.
7. Vortex the tube for at least 10 seconds.

   Note: Make sure that the pellet has been resuspended completely. Return remaining Denaturation Solution to refrigeration when finished.
8. Pulse-centrifuge the tube.
9. Incubate for 5 minutes at room temperature (15–25°C), off the magnetic rack.
10. Vortex the tube for 10 seconds and then pulse-centrifuge the tube.
11. Place the tube on the magnetic rack and wait at least 30 seconds for pellet to form.
12. Remove and discard the supernatant.
13. Add 750 µl Buffer T.
14. Vortex the tube for 10 seconds, and then pulse-centrifuge the tube.
15. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.
16. Remove and discard the supernatant.
17. Repeat steps 13–16 once.
18. Following
19. Table 2, add appropriate volumes of Buffer E and Buffer B.

Table 2. Primer Loaded PCR Bead resuspension for emulsion making

	Volumes (µl) for number of libraries			
Reagent	**1**	**2**	**3**	**4**
Buffer E	250	500	750	1000
Buffer B	250	500	750	1000
Total	**500**	**1000**	**1500**	**2000**

20. Pulse-vortex and then pulse-centrifuge the tube.
21. Transfer entire volume into a 2 ml non-skirted Sarstedt tube.

Preparing reagents

22. For each library pool, remove a single Master Mix tube and a single Primer Mix tube from the freezer and thaw.
23. Gently invert and roll the tubes to mix contents, and then pulse-centrifuge the tubes.

    Note: Do not vortex the Master Mix tube, as vortexing can cause protein denaturation.
24. Gently invert the Oil Mix container 5–10 times, or vortex the container for 5 seconds to make sure the contents are mixed.
25. Transfer 3500 µl Oil Mix to a 10 ml Sarstedt tube for each library pool.

Preparing the GeneRead QIAcube Workdeck 1

26. Wipe the GeneRead QIAcube Workdeck 1 with 70% alcohol.
27. Change gloves.
28. Following Table 3, load prepared reagents and consumables onto GeneRead QIAcube Workdeck 1 according to the number of library pools that will be run. Instructions are also provided on the touchscreen and the software will guide you through setup and loading.

Table 3. Reagents and consumables set up for emulsion making

	Number of library pools per run			
Item	**1**	**2**	**3**	**4**
Library pool tube	1 x 500 µl	2 x 500 µl	3 x 500 µl	4 x 500 µl
Oil Mix tube	1 x 3500 µl	2 x 3500 µl	3 x 3500 µl	4 x 3500 µl
Primer Loaded PCR Beads tube	1 x 500 µl	1 x 1000 µl	1 x 1500 µl	1 x 2000 µl
Master Mix tube	1 x 1600 µl	2 x 1600 µl	3 x 1600 µl	4 x 1600 µl
Primer Mix tube	1 x 650 µl	2 x 650 µl	3 x 650 µl	4 x 650 µl
2 ml tube, empty	2	4	6	8
PCR plate	1	1	2	2
1000 µl tips	3	6	9	12

Emulsion Making

29. Power ON the GeneRead QIAcube instrument with the hood closed.
30. After the GeneRead QIAcube initializes, open the hood.
31. Wipe the GeneRead QIAcube worktable with 70% alcohol.
32. Open the waste drawer, and discard used tips.
33. Empty the waste container following proper laboratory practice.
34. Change gloves.
35. Load the GeneRead QIAcube Workdeck 1 into the GeneRead QIAcube.

    IMPORTANT: Make sure all tubes are uncapped before starting the protocol.
36. Select **Make Emulsion** on the GeneRead QIAcube touchscreen.
37. Follow the instructions displayed on the touchscreen.
38. Upon completion of the script, "Run finished successfully" will be displayed.
39. Click **OK** when the script completes running.

40. Remove plate(s) from the GeneRead QIAcube and cover with the sealing film provided in the kit.

    Note: Verify that plate(s) contain uniform volumes of emulsion in each well.

41. Immediately proceed with PCR cycling of the PCR plates.

    Note: Time between the completion of the emulsion making run and the initiation of the PCR cycling should not exceed 30 minutes.

PCR cycling of PCR plates

42. Place plate(s) in the PCR cycler(s) and start the cycling program.
43. Perform PCR as described in Table 4.
44. After starting the PCR cycling, remove and dispose all tubes in the GeneRead QIAcube following proper laboratory practice.
45. Open the waste drawer, and discard used tips.
46. Empty the waste container following proper laboratory practice.
47. Clean the drawer with 70% alcohol, replace the waste container, and close drawer.
48. Power OFF the GeneRead QIAcube instrument.

    Note: PCR cycling run time is approximately 3 hours.

Table 4. Cycling conditions*

Time	Temperature	Number of cycles
6 minutes	94°C	1
15 seconds	94°C	
30 seconds	57°C	60
60 seconds	70°C	
2 minutes	72°C	1
Hold	4°C	∞
Lid	105°C	–

* Total volume used: 125 µl; program thermal cycler accordingly (125 µl or maximum volume allowed).

49. After the thermal cycling run has finished, remove plate(s) from the PCR cycler(s).

50. Proceed with "Emulsion breaking", page 22, on the GeneRead QIAcube. 0.

Note: After thermal cycling, plates can be stored at 4°C for ease of processing but the emulsion breaking procedure must be started within 72 hours.

Protocol: Emulsion Breaking

Important points before starting

- Start or re-start the GeneRead QIAcube before initiating emulsion breaking.
- Do not power OFF the GeneRead QIAcube after emulsion breaking is complete.
- Pipettes should be used to measure correct volumes.

Things to do before starting

- Gently invert buffer bottles to mix contents prior to use.

Procedure

Preparing reagents

1. For each library pool, transfer 10 ml Buffer A to two 10 ml tubes.
2. For each library pool, transfer 2 ml Buffer N to three 2 ml non-skirted Sarstedt tubes.

Preparing the GeneRead QIAcube Workdeck 1

3. Wipe the GeneRead QIAcube Workdeck 1 with 70% alcohol.
4. Change gloves.
5. Following Table 5, load prepared reagents and required consumables onto the GeneRead QIAcube Workdeck 1.

Table 5. Reagents and consumables setup for emulsion breaking

	Number of library pools per run			
Item	**1**	**2**	**3**	**4**
Buffer A tube	2 x 10 ml	4 x 10 ml	6 x 10 ml	8 x 10 ml
Buffer N tube	3 x 2 ml	6 x 2 ml	9 x 2 ml	12 x 2 ml
200 µl tip rack	2	4	6	8
Wide bore 1000 µl tips	8	16	24	32
Rotor Adapters	4	6	9	12
PCR plate, containing emulsion samples	1	1	2	2
Enrichment Columns	1	2	3	4

Emulsion Breaking

6. After the GeneRead QIAcube initializes, open the hood.
7. Wipe the GeneRead QIAcube worktable with 70% alcohol.
8. Open the waste drawer, and discard used tips.
9. Empty the waste container following proper laboratory practice.
10. Change gloves.
11. Place rotor adapters in centrifuge as indicated in Figure 2, according to the number of samples.

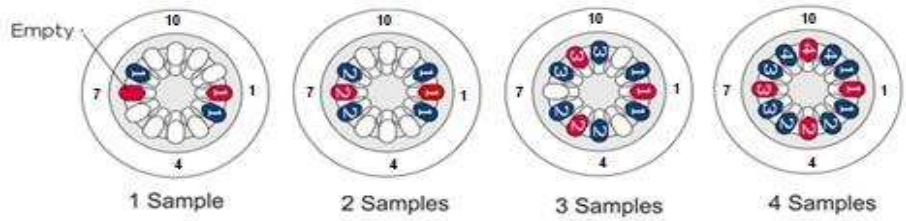

Figure 2. Rotor adapter placement in centrifuge.

12. Place enrichment column(s) in position of the rotor adapter(s), as indicated in Figure 3.
    - For 1 sample, place the enrichment column in rotor adapter 1.
    - For 2 samples, place enrichment columns in rotor adapters 1 and 7.
    - For 3 samples, place enrichment columns in rotor adapters 1, 5 and 9.
    - For 4 samples, place enrichment columns in rotor adapters 1, 4, 7 and 10.

    Note: Make sure enrichment columns are pressed down and correctly seated in the rotor adapters.

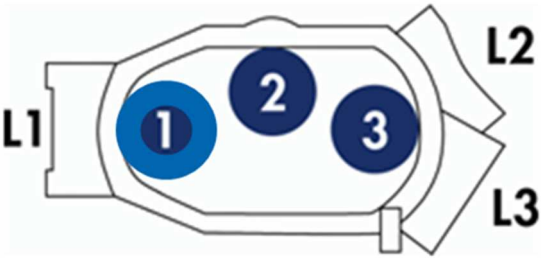

Figure 3. Rotor adapter positions.

13. Load the GeneRead QIAcube Workdeck 1 onto the GeneRead QIAcube.

    IMPORTANT: Make sure all tubes are uncapped before starting protocol.

14. Select **Breaking** on the GeneRead QIAcube touchscreen.

15. Follow the instructions displayed on the touchscreen.

16. Upon completion of the script, "Run finished successfully" will be displayed.

17. Click **OK** when the script completes running, and follow the instructions displayed on the touchscreen.

    Note: Samples can remain in the rotor adapters for ease of processing. However, bead enrichment must be started within 16 hours after completion of emulsion breaking.

    IMPORTANT: Do not remove samples or rotor adapters from the GeneRead QIAcube, do not discard rotor adapters.

18. Remove and dispose all tubes and empty PCR plate(s) from GeneRead QIAcube Workdeck 1 following proper laboratory practice.

    Note: Verify emulsion has been removed from each well.

19. Open the waste drawer, and discard used tips.

20. Empty the waste container following proper laboratory practice.

21. Replace the waste container, and close the drawer.

22. Proceed with “Bead enrichment”, page 26.

    IMPORTANT: Do not power OFF the GeneRead QIAcube after emulsion breaking is complete. It is not possible to start bead enrichment if the GeneRead QIAcube has been powered OFF after emulsion breaking.

Protocol: Bead Enrichment

Important points before starting

- Bead enrichment cannot be started independently and without performing automated emulsion breaking prior to bead enrichment.
- It is not possible to start bead enrichment if the GeneRead QIAcube has been powered OFF after emulsion breaking for any reason.
- Pipettes should be used to measure correct volumes.
- Verify that the "2" shaker adapter is installed in Workdeck 2.

Things to do before starting

- Leave all rotor adapters in place.
- Remove the GeneRead QIAcube Workdeck 1 from the GeneRead QIAcube.
- Remove Enrichment Buffer from the freezer and thaw.
- Remove Denaturation Solution bottle from refrigeration.
- Gently invert the Denaturation Solution and buffer bottles to mix contents prior to use.

Procedure

Preparation of Enrichment Buffer

1. After thawing, add 120 µl of Enrichment Buffer Additive to the Enrichment Buffer tube. After addition of the Enrichment Buffer Additive, the buffer can be stored at –20°C for up to three months.

Super A Bead pretreatment

2. For each library pool, remove a single tube of Super A Beads from refrigeration.

3. Equilibrate Super A Beads to room temperature (15–25°C) before use.
4. Centrifuge Super A Beads at maximum speed (at least 15,000 x *g*) for 1 minute.
5. Without disturbing the white Super A Bead pellet(s), remove and discard 550 µl supernatant from each tube.
6. Gently invert the Enrichment Buffer bottle to mix contents.
7. Add 550 µl Enrichment Buffer to the Super A Beads in each tube.
8. Pulse-vortex then centrifuge the tube at maximum speed for 1 minute.
9. Without disturbing the white Super A Bead pellet(s), remove and discard 400 µl supernatant from each tube, leaving 200 µl Super A Bead suspension in each tube.

Preparing reagents

10. For each library pool, transfer 950 µl Enrichment Buffer to a 2 ml non-skirted Sarstedt tube. Return remaining Enrichment Buffer to freezer when finished.
11. For each library pool, transfer 900 µl Buffer B to a 2 ml non-skirted Sarstedt tube.
12. For each library pool, transfer 900 µl Buffer N to a 2 ml non-skirted Sarstedt tube.
13. For each library pool, transfer 425 µl Buffer T to a 2 ml non-skirted Sarstedt tube.
14. For each library pool, transfer 550 µl Denaturation Solution to a 2 ml non-skirted Sarstedt tube. Return remaining Denaturation Solution to refrigeration when finished.

Preparing the GeneRead QIAcube Workdeck 2

15. Wipe the GeneRead QIAcube Workdeck 2 with 70% alcohol.
16. Change gloves.
17. Load the prepared reagents and required consumables onto the GeneRead QIAcube Workdeck 2, as described in
18. Table 6.

Table 6. Reagents and consumables setup for bead enrichment

	Number of library pools per run			
Item	**1**	**2**	**3**	**4**
Enrichment Buffer tube	1 x 950 µl	2 x 950 µl	3 x 950 µl	4 x 950 µl
Denaturation Solution tube	1 x 550 µl	2 x 550 µl	3 x 550 µl	4 x 550 µl
Buffer T tube	1 x 425 µl	2 x 425 µl	3 x 425 µl	4 x 425 µl
Buffer N tube	1 x 900 µl	2 x 900 µl	3 x 900 µl	4 x 900 µl
Buffer B tube	1 x 900 µl	2 x 900 µl	3 x 900 µl	4 x 900 µl
Super A Bead tube	1 x 200 µl	2 x 200 µl	3 x 200 µl	4 x 200 µl
8-strip PCR tubes	1	1	1	1
Wide bore 1000 µl tips	13	14	22	30
200 µl tips	3	5	8	11

Bead Enrichment

19. Load the GeneRead QIAcube Workdeck 2 onto the GeneRead QIAcube.

    IMPORTANT: Make sure all tubes are uncapped before starting protocol.

20. Follow the instructions displayed on the touchscreen.

21. Place the magnetic station cover on the magnetic station.

    Note: Upon completion of the script, "Run finished successfully" will be displayed.

22. Click **OK** on the GeneRead QIAcube touchscreen when the script is complete.

23. Remove the magnetic station cover, and carefully remove the 8-strip PCR tubes.

    Note: Verify that the 8-strip PCR tubes contain uniform pellets in each well.

24. Resuspend each pellet by carefully mixing it up and down with a 200 µl pipet.

25. Transfer each resuspended pellet into a labeled 2 ml LoBind Eppendorf tube.

26. Rinse each well of the 8-strip PCR tube with 200 µl Buffer T using a clean pipet tip for each tube. Transfer the volume into the appropriate 2 ml LoBind Eppendorf tube from the previous step.
27. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.
28. Remove and discard the supernatant.
29. Add 500 µl Buffer T.

    Note: Clonally amplified and enriched samples can be stored for 72 hours at 4°C.
30. Follow the "Determination of Bead Concentration using OD" on page 31 to aliquot sufficient sample volume for sequencing or verify the enriched bead concentration is within the acceptable range using the Color Chart. Use the entire volume of enriched beads for sequencing reaction.

    IMPORTANT: The enriched bead concentration range that may be described in the Color Chart corresponds to sequencing runs performed using the GeneRead UMI Advanced Sequencing Q Kit. However, the GeneRead Sequencing kit family is continually expanding. Therefore, if instructions for enriched bead concentration in the *GeneRead Sequencing Kit Handbook* used downstream of this GeneRead Clonal Amp Q Kit differ to those displayed in the Color Chart, please use the concentration described in the corresponding GeneRead Sequencing handbook.
31. Proceed with "Primer Hybridization" protocol according to the appropriate GeneRead Sequencing handbook.
32. Remove and dispose all consumables following proper laboratory practice.
33. Open the waste drawer, and discard used tips.
34. Empty the waste container following proper laboratory practice.
35. Clean the drawer with 70% alcohol, replace the waste container, and close the drawer.

Protocol: Bead Yield Determination

Important points before starting

- Following the clonal amplification workflow protocol for bead enrichment, clonally amplified and enriched sample yield can be evaluated by either following the "Determination of Bead Concentration using OD" below or by using the Color Chart provided in the Box 3 of the GeneRead Clonal Amp Q kit.
- Pipettes should be used to measure correct volumes.
- The OD protocol is for spectrophotometers requiring 200 µl volumes; it may be adjusted for lab ware requiring different volumes.
- The standard curve may be re-used for subsequent clonal amplifications with different kits using the same Primer Loaded PCR Bead lot.
- Primer Loaded PCR Beads must be fully resuspended prior to use.

Things to do before starting determination of bead concentration using OD

- Remove the Primer Loaded PCR Beads tube and Denaturation Solution bottle from refrigeration. Gently invert the Denaturation Solution and buffer bottles to mix contents prior to use.

Determination of Bead Concentration using OD

Bead preparation

1. Vortex Primer Loaded PCR Beads thoroughly for at least 1 minute.

   Note: Ensure that the beads are completely resuspended with no visible pellet left.
2. Add 20 µl Primer Loaded PCR Beads into a LoBind tube. Return remaining Primer Loaded PCR Beads to refrigeration when finished.
3. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.

4. Remove and discard the supernatant.
5. Add 100 µl Denaturation Solution. Return remaining Denaturation Solution to refrigeration when finished.
6. Pulse-vortex and then pulse-centrifuge the tube.
7. Incubate the tube at room temperature (15–25°C) for 5 minutes.
8. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.
9. Remove and discard the supernatant.
10. Add 100 µl Buffer E.
11. Pulse-vortex the tube.
12. Place the tube on the magnetic rack, and wait at least 30 seconds for pellet to form.
13. Remove and discard the supernatant.
14. Add 20 µl Buffer E.
15. Pulse-vortex the tube.

Serial dilution of Primer Loaded PCR Beads

16. Prepare 4 dilutions (A, B, C and D) as described in
17. Table 7.

Table 7. Serial dilution of treated Primer Loaded PCR Beads

Dilution	Volume of beads	Volume of Buffer E	Final concentration (beads/µl)
A	20 µl	80 µl	2.00×10^6
B	50 µl dilution A	50 µl	1.00×10^6
C	50 µl dilution B	50 µl	5.00×10^5
D	50 µl dilution C	50 µl	2.50×10^5

Creating a 4-point calibration curve with beads using OD_{550} or OD_{600}

18. Transfer 190 µl Buffer E to LoBind tubes.
19. Add 10 µl of each of bead dilution into a single tube, rinse pipet tip.

20. Pulse-vortex the 4 tubes.
21. Immediately transfer the entire 200 µl of each prepared dilution into appropriate lab ware for the spectrophotometer.

    Note: Volume can be adjusted according to the type of spectrophotometer.
22. Transfer 200 µl Buffer E into empty labware to use as a blank.
23. Measure blank and dilution OD at 550 nm or 600 nm.
24. Subtract the blank OD value from each dilution OD value.

Plotting a linear graph in Excel®

25. Enter the 4 bead concentrations (A, B, C and D) and their corresponding OD values into an Excel worksheet.
26. Highlight values and insert a scatterplot with the bead concentration on the y-axis and OD values on the x-axis.
27. Using the layout tab, add a trendline to the graph from the layout tab. For example:

    Layout→ Trend line→ More trendline options→ Check trendline options: linear, Display equation on chart and Display R-squared value on chart.

Measuring the OD value(s) of clonally amplified and enriched sample(s)

28. Transfer 190 µl Buffer E to a clean LoBind tube for each sample.
29. Pulse-vortex and then pulse-centrifuge the enriched bead sample tube(s).
30. Transfer 10 µl of each enriched bead sample to a single tube, and rinse pipet tip.
31. Pulse-vortex and then pulse-centrifuge the tube(s).
32. Immediately transfer the entire 200 µl of each prepared sample to appropriate lab ware for the spectrophotometer.

    Note: Volume can be adjusted according to the type of spectrophotometer.
33. Transfer 200 µl Buffer E to empty labware to use as a blank.
34. Measure blank and sample OD at 550 nm or 600 nm.
35. Subtract the blank OD value from each sample OD value.

36. Using the linear equation determined above, extrapolate the bead concentration(s) of clonally amplified samples.

    Note: The OD value is the x-value and the corresponding y-value can be calculated. By taking the dilution factor and the volume into consideration, the total bead concentration can also be calculated.

37. Using the concentration of your clonally amplified sample(s), aliquot the number of beads indicated in the corresponding GeneRead Sequencing handbook for sequencing.

    Note: The remaining clonally amplified sample(s) may be stored at 4°C. The sample(s) must be used within the recommended storage time. Follow the recommended protocol(s) for retreatment of beads before sequencing.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Emulsion making	
Primer Loaded PCR Beads are not evenly distributed in the emulsion (some rows of the plate are lighter than others)	Insufficient volume of Primer Loaded PCR Bead suspension was used. Verify that Primer Loaded PCR Beads were prepared according to Table 2 (page 19) and that the beads were mixed sufficiently.
Emulsion breaking	
Emulsions are not pooled or substantial or variable amounts of emulsion are left in plate wells	See the *GeneRead QIAcube User Manual* and check for damaged O-ring in tip adapter. Replace if necessary. Mechanical issues with GeneRead QIAcube could cause incorrect pipetting volumes or mixing speed. Check the GeneRead QIAcube. If problem persists contact QIAGEN Technical Services.
Bead enrichment	
a) Smaller than usual bead pellets are observed in the 8-strip PCR tubes after bead enrichment	An insufficient amount of library (e.g., <100 pg) was used for emulsion making. Check library concentration. Denaturation of beads was incomplete due to use of incorrect denaturation solution, insufficient mixing or inadequate incubation.
b) Larger than usual bead pellets in the 8-strip PCR tubes after bead enrichment	An excessive amount of library (e.g., >1000 pg) was used for emulsion making. Check library concentration.

		Comments and suggestions
Determination of bead concentration using OD or Color Chart		
a)	Low bead recovery	Insufficient amount of library (e.g., <100 pg) was used for emulsion making. Check library concentration. Denaturation of beads was incomplete due to use of incorrect denaturation solution, insufficient mixing or inadequate incubation. Inadequate Super A Bead pretreatment.
b)	High bead recovery	An excessive amount of library (e.g., >1000 pg) was used for emulsion making. Check library concentration.

Symbols

Symbol	Symbol definition
	Contains reagents sufficient for <N> tests
	Catalog number
	Manufacturer

Ordering Information

Product	Contents	Cat. no.
GeneRead Clonal Amp Q Kit (4)	For four (4) reactions: Buffers and reagents for clonal amplification, for use with the GeneRead QIAcube instrument	185001
Filter-Tips, 1000 µl (1024)	Disposable Filter-Tips, racked; (8 x 128); for use with the QIAcube	990352
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128); for use with the QIAcube	990332
Filter-Tips, 1000 µl, wide-bore (1024)	Disposable Filter-Tips, wide-bore, racked; (8 x 128) (not required for all protocols); for use with the QIAcube	990452
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the GeneRead QIAcube	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the GeneRead QIAcube	990392

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.

Revision History

Document revision history	
R5 04/2018	Updated recommendations for PCR thermal cyclers. Added revision history.

Notes

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Notes

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