MGIEasy

Exome Capture V5 Probe Set User Manual

Cat No.: 940-000187-00 (16 RXN)

Kit Version: V1.0

Manual Version: 1.0

Revision History

Manual Version	Kit Version	Date	Description
1.0	V1.0	Dec. 2021	• Update Cat. No.

Note: Please download the latest version of the manual and use it with the corresponding kit.

Search manual by Cat. No. or product name from website:

https://en.mgi-tech.com/download/files.html

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Chapter 1 Product Description

1.1 Introduction

The MGIEasy Exome Capture V5 Probe Set consists of a MGIEasy Exome Capture V5 Probe Kit and a MGIEasy Exome Capture Hybridization and Wash Kit (Box1 and Box2). As a probe set, it can be used for exome library construction and hybridization combined with MGIEasy Exome FS Library Prep Set or MGIEasy Exome Universal Library Prep Set on MGI sequencing platform. It can also be used with DNA library prep kit on other sequencing platforms. The detailed combination strategies are listed below (see table 1).

⚠ **Note: If both the library construction and hybridization are based on MGIEasy series, you could choose MGIEasy Exome V5 FS Library Prep Set or MGIEasy Exome V5 Universal Library Prep Set directly. If based on other high-throughput sequencing platform, remember to set the main insert size around 280bp. It recommended to use the double size selection before adapter ligation for selecting the size band.**

Table 1 The Combination Strategies of MGIEasy Exome Capture V5 Probe Set

No.	Probe and Wash Kit	DNA Library Prep Kit
1	MGIEasy Exome Capture V5 Probe Set (MGI, Cat. No.: 940-000187-00)	MGIEasy Exome FS Library Prep Set (MGI, Cat. No.1000009658)
2		MGIEasy Exome Universal Library Prep Set（MGI, Cat. No.1000009657）
3		DNA Library Prep Kit on other sequencing platforms

1.2 Application

This set is used for samples derived from Human and achieves the capture for whole exome collocated with Probes.

1.3 Platform Compatibility

Constructed libraries are compatible with

MGISEQ-2000RS (PE100/PE150); DNBSEQ-G400RS (PE100/PE150)

BGISEQ-500RS (PE100);

Or other high-throughput sequencing platforms.

1.4 Contents

Each set consists of a MGIEasy Exome Capture V5 Probe Kit and a MGIEasy Exome Capture Hybridization and Wash Kit (Box1 and Box2). Further information on Cat. No., Components and Specifications are listed below.

Table 2 MGIEasy Exome Capture V5 Probe Set (16 RXN) (Cat No.: 940-000187-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Exome Capture V5 Probe Kit Cat. No.: 1000007741	MGI Exome V5 Probe	Black	80 μL/tube × 1 tube
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) Cat. No.: 940-000168-00	Block 1	Yellow	40 μL/tube × 1 tube
	Block 2	Yellow	40 μL/tube × 1 tube
	Block 5	Yellow	8 μL/tube × 1 tube
	Hyb Buffer 3	Green	64 μL/tube × 1 tube
MGIEasy Exome Capture Hybridization and Wash Kit (Box 2) Cat. No.: 940-000169-00	Hyb Buffer 1	Green	160 μL/tube × 1 tube
	Hyb Buffer 2	Green	7 μL/tube × 1 tube
	Hyb Buffer 4	Green	90 μL/tube × 1 tube
	Binding Buffer	White	12800 μL/bottle × 1 bottle
	Wash Buffer I	White	8000 μL/bottle × 1 bottle
	Wash Buffer II	White	24000 μL/bottle × 1 bottle

1.5 Storage Conditions and Shelf Life

MGIEasy Exome Capture V5 Probe Kit

- Storage Temperature: -80°C
- Transport Conditions: transported on dry ice

MGIEasy Exome Capture Hybridization and Wash Kit (Box 1)

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice.

MGIEasy Exome Capture Hybridization and Wash Kit (Box 2)

- Storage Temperature: room temperature
- Transport Conditions: transported at room temperature

*Production Date and Expiration Date: refer to the label

* Please ensure that an abundance of dry ice remains after transportation.

* Performance of products is guaranteed until the expiration date, under appropriate transport, storage, and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 3 Equipment and Materials Required but not Provided

Equipment	Pipets Vortex Mixer Desktop Centrifuge Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96) or equivalent Thermocycler Thermomixer or water bath equipment Nutator or other nutating mixer/shaker Eppendorf Concentrator (Eppendorf, Cat. No. 5305000398)
Reagents	Dynabeads™ M-280 Streptavidin (Invitrogen, Cat. No. 112.06D) Or Dynabeads MyOne Streptavidin T1 (Invitrogen, Cat. No. 65601) Nuclease free water (NF water) (Ambion, Cat. No. AM9937)
Consumables	0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or equivalent 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C) or equivalent 1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C) 2.0 mL centrifuge tubes (Axygen, Cat. No. MCT-200-C) or equivalent 8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips (Axygen, Cat. No. PCR-02CP-C) or equivalent Filter Tips (Axygen, Cat. No. TF-100) or equivalent Clear Adhesive Film (ABI, Cat. No. 4306311) Blade or knife
DNA Library Prep Kit	MGIEasy Exome FS Library Prep Set (MGI, Cat. No.1000009658) or MGIEasy Exome Universal Library Prep Set (MGI, Cat. No.1000009657) or DNA library Prep Kit designed for other high-throughput sequencing platforms MGIEasy Exome Capture Accessory Kit (MGI, Cat. No.: 1000007743)

1.7 Precautions and Warning

- This product is intended for research use only. Clinical diagnostic application is strictly prohibited. Please read this manual carefully before use.
- Instructions provided in this manual are intended for general use only, and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other equipment.
- This probe set can reach the optimal performance with MGIEasy DNA Library Prep Kit. It's compatible for using other DNA Prep Kit. But the insert main size should be 280bp, and it's better to take the small batch preliminary test for the success library construction.
- For the MGI Exome V5 Probe, centrifuge briefly and place on ice for further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- Avoid contact of skins and eyes with all samples and reagents. Do not swallow. Seek medical attention immediately if these contacts occur during the process.
- Dispose of all samples and waste in accordance with local regulations.
- If you have other questions, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation

2.1 Sample Requirement

The PCR products for hybridization can be constructed from MGIEasy FS Library Prep Set or MGIEasy Universal Library Prep Set or any library Prep Kit on other high-throughput sequencing platform. If product based on other high-throughput sequencing platforms, remember to set the main insert size around 280bp.

2.2 Reagent Requirement

- MGIEasy Exome Capture V5 Probe Kit should be put in -80°C immediately once you receive the product. The probe should be stored in -80°C. Remove the probe from storage beforehand, and thaw it on ice, centrifuge briefly and put on ice for further use.
- MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) should be put in -20°C immediately once you receive the product. Remove the reagents from storage beforehand, and thaw it on ice or room temperature, centrifuge briefly and put on ice for further use.
- MGIEasy Exome Capture Hybridization and Wash Kit (Box 2) should be put at room temperature immediately once you receive the product. Mix thoroughly the reagents before usage, and put at room temperature for further use. The buffers may have some precipitation which will not affect the function. If a precipitate forms, warm the buffer at 65°C for 5 minutes until the precipitation disappear, then mix thoroughly for use.

Chapter 3 Library Construction Protocol

3.1 Pre-Hybridization Preparation

⚠ **Note: A 96-well PCR plate is recommended for the hybridization reaction. Other steps can use strip tubes depending on the number of samples.**

Note: Remember to confirm the main insert size of PCR products, excluding the adapter length, for hybridization around 280bp.

3.1.1 If only one sample per hybridization reaction, transfer around 1000 ng of PCR products according to the concentrations. If multi-plex in hybridization is needed, ≥250 ng of each sample is required, and the total amount of PCR products should be between 1000ng and 4000 ng. An 8-plex is the maximum pooling possible with this kit. Please refer to Appendix A The combination barcode Adapters strategies for the pooling strategy of samples.

3.1.2 Prepare the Block mixture on ice (see Table 4).

Table 4 Block Mixture

Components	Volume
Block 1	2.5 μL
Block 2	2.5 μL
Block 3	1 μL
Block 4	10 μL
Total	16 μL

⚠ **Note: Block 3 and Block 4 are reagents from the MGIEasy Exome Capture Accessory Kit, which are designed exclusively for the MGISEQ/DNBSEQ platform. When preparing samples for a different platform, use reagents applicable for that platform's adaptor sequences.**

3.1.3 Transfer 16 μL of Block mixture to each of the PCR products from step 3.1.1 for preparation of the Pre-hybridization Mixture. Uncap and place the tubes containing the mixture on the concentrator, set the temperature at 65°C for spin vacuum around 30 min until the final concentrated volume is 9 μL. If the final volume is less than 9 μL, add NF water to reach a final volume of 9 μL.

3.1.4 Place the 9 μL of Pre-hybridization Mixture from step 3.1.3 into a thermocycler and run the program in Table 5.

Table 5 Pre-hybridization Reaction Conditions

Temperature	Time
Heated lid	on
95°C	5 min
65°C	Hold

3.2 Hybridization and Capture

3.2.1 Prepare the Hybridization Mixture in a new 0.2 mL PCR tube (see Table 6).

Table 6 Hybridization Mixture

Components	Volume
Hyb Buffer 1	10 µL
Hyb Buffer 2	0.4 µL
Hyb Buffer 3	4 µL
Hyb Buffer 4	5.6 µL
Total	20 µL

3.2.2 Incubate the Hybridization Mixture from step 3.2.1 in a thermocycler at 65°C for at least 5 min. Before use, ensure there is no precipitation in the mixture.

3.2.3 Prepare the Probe mixture on ice (see Table 7) in a new 96-well PCR plate (recommended).

Table 7 Probe Mixture

Components	Volume
NF water	1.5 µL
Block 5	0.5 µL
MGI Exome V5 Probe	5 µL
Total	7 µL

⚠ **Note: The MGI Exome V5 Probe must be thawed on ice and added last to the mixture.**

3.2.4 Tightly close the Strip Domed Caps on the 96-well plate from step 3.2.3, then place the Probe Mixture into the thermocycler and run the program in Table 8

Table 8 Probe Mixture Incubation

Temperature	Time
Heated lid	on
65°C	2 min
65°C	Hold

3.2.5 Keeping all the mixtures above (pre-hybridization mixture, hybridization mixture, and probe mixture) at 65°C, quickly transfer 13 μL of the Hybridization Mixture from step 3.2.2 into 9 μL Pre-hybridization Mixture from step 3.1.4 at 65°C. Pipette up and down to mix thoroughly.

⚠ **Note: 100 μL Filter Tips are recommended for this step.**

3.2.6 Transfer 22 μL of the combined pre-hybridization and hybridization mixtures from step 3.2.5 into the 96-well PCR plate with 7 μL of the Probe Mixture from step 3.2.4. Pipette up and down to mix thoroughly.

⚠ **Note: 100 μL Filter Tips are recommended for this step.**

3.2.7 Cover the PCR plate with Clear Adhesive Film, press the film firmly against the plate to make sure all wells are completely sealed. Repeat this step to make sure film is completely sealed.

3.2.8 Keep the 96-well plate at 65°C (set the heated lid of thermocycler to 105°C) for hybridization for 24 hours according to the program in Table 9.

Table 9 Hybridization Reaction Conditions

Temperature	Time
Heated lid (105°C)	On
65°C	Hold

3.3 Pre-Elution Preparation

⚠ **Note: Please make sure no precipitation in buffers before use. If a precipitate forms, warm the buffer at 65°C for 5 minutes until the precipitation disappear, then mix thoroughly for use.**

3.3.1 Turn on a Thermomixer and set at 65°C at least 30 min before beginning the elution. For each hybridization reaction, add 1.8 mL of Wash Buffer II in a new 2.0 mL Microcentrifuge Tube and place in the Thermomixer for preheating.

3.3.2 Vigorously vortex Dynabeads until evenly mixed. For each hybridization reaction, transfer 50 μL of Dynabeads to a new 2.0 mL Microcentrifuge Tube.

3.3.3 Add 200 μL of Binding Buffer to each tube containing Dynabeads and vigorously vortex for 5 s to re-suspend the magnet beads.

3.3.4 Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette

3.3.5 Repeat step 3.3.3 to 3.3.4 twice.

3.3.6 Add 200 μL of Binding Buffer to each tube containing Dynabeads to re-suspend the beads.

3.4 Elution

3.4.1 Keep the mixtures from step 3.2.8 on the thermocycler following the 24 hours incubation. Slice the adhesive film in-between each row of wells and carefully peel off the film row by row. Quickly use a pipette to estimate the remaining hybridization solutions one by one and transfer each mixture into a separate tube from step 3.3.6 containing prepared Dynabeads.

⚠ **Note: If the volume of remaining hybridization solution is less than 19 μL, the yield may be low.**

Note: While handling large number of samples, in order to reduce the vaporization of the hybridization solution, it is recommended to handle the samples in sets of six to eight reactions. In-between sets, the user should re-adhere the film and close the heated lid of the PCR thermal cycler cover for ten seconds before continuing with the next set of reactions.

3.4.2 Fix the tubes from step 3.4.1 on a Nutator or other similar mixer for mixing by 360° rotation and incubate at room temperature for 30 min.

3.4.3 Take the tubes off the mixer.

3.4.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.5 Add 500 μL of Wash Buffer I, turn tubes upside down several times to re-suspend the bead mixture, then incubate at room temperature for 15 min.

3.4.6 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.7 Add 500 μL of pre-heated Wash Buffer II from step 3.3.1. Put the tubes in the Thermomixer and set speed to 1000 rpm. Press 'short' for 10 s to make sure all the beads are re-suspended, then set speed to 0 rpm. Incubate at 65°C in the Thermomixer for 10 min.

3.4.8 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 30s until the liquid

becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.9 Repeat step 3.4.7 to 3.4.8 twice.

3.4.10 Add 100 μL NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 1.5 mL Microcentrifuge Tube and centrifuge briefly.

3.4.11 Place the centrifuge tube from step 3.4.10 onto a Magnetic Separation Rack for 2 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.12 Add 44 μL of NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 0.2 mL PCR tube.

3.5 Post-Capture PCR

3.5.1 Prepare the Post-capture PCR mixture on ice (see Table 10).

Table 10 Post- capture PCR Mixture

Components	Volume
Post-PCR Enzyme Mix	50 μL
PCR Primer Mix	6 μL
Total	56 μL

⚠ **Note: Post-PCR Enzyme Mix and PCR Primer Mix are included in the 'MGIEasy Exome Capture Accessory Kit'. When preparing samples for a different platform, use proper primers applicable for that platform's adaptor sequences.**

3.5.2 Transfer 56 μL of the Post-capture PCR mixture into each of the PCR tube(s) (including beads) from step 3.4.12. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.5.3 Place the PCR tube(s) from step 3.5.2 into the thermocycler and run the program described in Table 11.

Table 11 Post-capture PCR Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	13 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

Note: If only one sample per hybridization reaction, 13 cycles are suggested. If multi-plex in hybridization reaction and the input for hybridization > 1000 ng, it is suggested to reduce the cycles to 12.

3.5.4 Centrifuge briefly to collect the solution at the bottom of the tube.

3.5.5 Place the tube(s) onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Transfer 100 µL supernatant from each tube to a new 1.5 mL Microcentrifuge tube.

3.6 Cleanup of Post-Capture PCR Product and Quantification

3.6.1 Take out DNA Clean Beads from the refrigerator and allow 30 min to bring the beads to room temperature. Vortex and mix thoroughly before use.

Note: DNA Clean Beads are included in 'MGIEasy DNA Clean Beads' (MGI, Cat. No. 1000005278). Or AMPure® XP (Beckman Coulter, Cat. No. A63882) is an alternative.

3.6.2 Transfer 100 µL DNA Clean Beads to each centrifuge tube from step 3.5.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and beads are fully dispensed from the pipette tip into the centrifuge tube before proceeding.

3.6.3 Incubate at room temperature for 5 min.

3.6.4 Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.6.5 Keep the tube(s) on the Magnetic Separation Rack and add 200 µL of freshly prepared 80% ethanol to each tube to wash the beads and the walls of the tube. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.6.6 Repeat step 3.6.5 once, remove all liquid from the tube without disrupting the beads. You may

centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, and remove remaining liquid using a small volume pipette.

3.6.7 Keep the centrifuge tube(s) on the Magnetic Separation Rack with the lid open, and air dry the beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.6.8 Remove the centrifuge tube(s) from the Magnetic Separation Rack and add **32 μL** TE Buffer to each tube to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.6.9 Incubate at room temperature for 5 min.

3.6.10 Centrifuge briefly, then place the centrifuge tube(s) back onto the Magnetic Separation Rack for 2-5 min until the liquid becomes clear. Transfer **30 μL** supernatant from each tube to a different new 1.5 mL centrifuge tube.

3.6.11 Quantify the purified post-capture PCR products with dsDNA Fluorescence Assay Kits such as the Qubit® dsDNA HS Assay Kit or the Quant-iT PicoGreen® dsDNA Assay Kit. If one single post-capture PCR product for one sequencing reaction, the desired yield for PCR products is ≥ 1 pmol. Please refer to Formula 1 to calculate the yield. For example, the desired yield for the fragmented DNA with a peak fragment size of 280 bp (Post-hybridization PCR products with a peak fragment size of 364 bp) should be ≥ 240 ng. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. Detailed information shows how to plan your sample pooling (see Appendix B). Quantify your Adapter-ligated samples before pooling. The total yield after pooling should be 1 pmol, with a total volume ≤ 48 μL.

Formula 1 Conversion between 1 pmol of dsDNA sample and Mass in ng

$$\text{Mass (ng) corresponding to 1 pmol PCR Products} = \frac{\text{DNA Fragment Size (bp)}}{1000\ \text{bp}} \times 660\ \text{ng}$$

✔ **Stopping Point: After cleanup, purified PCR Products can be stored at -20°C.**

⚠ **Note: If the library will be sequenced on MGI platform, please refer to the step '3.13 Denaturation' from 'MGIEasy Exome Universal Library Prep Set', or the step '3.15 Denaturation' from 'MGIEasy Exome FS Library Prep Set' to finish the library construction. If the library will be sequenced on other platforms, please refer to the requirement according to the platform.**

Appendix

Appendix A The Combination Barcode Adapters Strategies

- This set includes a MGIEasy DNA Adapters-16 (Tube) Kit. This kit was developed to meet requirements for batch processing of library construction and multiplex sequencing. We selected the best adapter combination based on the principle of balanced base composition. However, the number of Barcode Adapters are not always continuous. For optimal performance, please carefully read instructions in Appendix A-1.
- Our Adapters are double stranded. Please do not incubate above room temperature to avoid structural changes such as denaturation, which might affect performance.
- Before use, please centrifuge to collect liquid at the bottom of tubes. Gently remove the cap to prevent spills and cross-contamination. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use.
- Adapters from other MGI Library Prep Kits (number 501-596) are designed differently and are incompatible for mixed use. Mixed use will cause errors in barcode demultiplexing in data analysis procedures.
- Adapter quality as well as quantity directly affects the efficiency and quality of the library construction. We recommend the dilution ratio to be between 2 – 5. An excessive input of Adapters may cause Adapter dimers; whereas insufficient input may cause lower library yield and lower efficiency of library construction.

Table 12 Recommended Adapter Input According to the Amount of Sample DNA (280 bp)

DNA Sample (ng)	MGI Adapter Dilution Ratio	MGI Adapter Input after Dilution (μL)
50	No dilution	5
25	2	5
10	5	5

- Increasing Adapter input may increase the library yield to a certain extent, especially when the DNA sample ≤ 25 ng. If there is a need to optimize the efficiency of library construction, you may try increasing Adapter input (within the range of 2-10 times).

A-1 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

Based on the principles of balanced base composition, adapters must be used in specific groups. Please follow the instructions below to use Adapters in proper combination:

2 sets of 4 Adapters: (01-04) and (13-16)

1 set of 8 Adapters: (97-104)

If the sequencing data output requirement is the same for all samples in one lane, please refer to Table 13 below to choose your barcode adapter combinations.

Table 13 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

Sample(s)/lane	Instructions (Example)
1	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample. Or 2. Take a set of 8 Adapters (e.g. 97-104), mix 8 Adapters with equal volumes, then add the mixture to the sample.
2	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 97-100, then add to sample 1; Mix 101-104, then add to sample 2)
3	Requires at least 2 sets of Adapters: For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-2 and for sample 3.
4	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, respectively.) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with an equal volume in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 97-98, 99-100, 101-102, 103-104, then add respectively to samples 1, 2, 3, 4.)

5	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for samples 5-6.
7	Requires all 3 Adapter sets and follow these 3 steps: 1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set). 2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set). 3) For sample 7, use the method for (1 sample/lane) above (Use 3rd Adapter set). You can add a single Adapter within the Adapter set. Or add the Adapter mix which is mixed from all Adapters within the Adapter set with an equal volume. Note that you should use different Adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	Requires at least 1 set of Adapters: 1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume. Or 2. Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter to each sample in an equal volume.

For situations in which the sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters. For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (97-104), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

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