CELEMICS

Kit User Manual

Whole Exome Sequencing
NGS Library Preparation

Enzymatic Preparation Kit (EP-Kit)

Illumina Platform

Dual Index

Version 1.0.3

Research Use Only

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Table of Contents

Materials Provided

Table 1. Library Preparation #1 **Storage condition: -20°C**

Box No.	Product Name	1 rxn (µL)	Cat No. LI1116 (16 rxns)	Cat No. LI1148 (48 rxns)	Cat No. LI1196 (96 rxns)
LI11	EP-ER/A Buffer	3	LI110716	LI110748	LI110796
	EP-ER/A Enzyme	6	LI110816	LI110848	LI110896
	Ligase Buffer	12	LI110916	LI110948	LI110996
	Ligation Enzyme	4	LI111016	LI111048	LI111096

Table 2. Library Preparation #2 (Dual Index) **Storage condition: -20°C**

Box No.	Product Name	1 rxn (µL)	Cat No. LI2016 (16 rxns)	Cat No. LI2048 (48 rxns)	Cat No. LI2096 (96 rxns)
LI20	Dual Index 501-516	2.5	LI202816 - LI204316	LI202848 - LI204348	LI202896 - LI204396
	Dual Index 701-724	2.5	LI204416 - LI206716	LI204448 - LI206748	LI204496 - LI206796
	Adapter (S)	10	LI202516	LI202548	LI202596

Additionally Provided Materials

Table 3. Additionally Provided Materials List

Product Name	Cat No.	Storage Condition (°C)	Comment
Celemics DNA purification bead (CeleMag Clean-up Bead)	CMCB50 (50 mL) CMCB500 (500 mL)	4°C	Included in the All-in-one kit
Celemics New Magnetic bead (CeleNM Bead)	CNMB0.3 (0.3 mL, 16 rxn) CNMB1.5 (1.5 mL, 96 rxn)	4°C	
CeleNM Bead binding buffer	CBBB1.8 (1.8 mL, 16 rxn) CBBB09 (9 mL, 96 rxn)	RT	
CLM Polymerase Amplification Kit PCR Ready Mix only	CMPFX.XX (X.XX: amount, mL)	-20°C	

Materials Required (Not Provided)

Table 4. Not Provided Materials List

Reagents & Consumables		
Product Name	**Suggested Supplier**	**p/n**
Nuclease-free water (not DEPC-treated)	General lab supplier	-
Ethanol (200 proof)	General lab supplier	-
Isopropanol	General lab supplier	-
Conical centrifuge tubes (15 mL or 50 mL)	General lab supplier	-
P10, P20, P200, and P1000 pipettes	General lab supplier	-
Multichannel pipette	General lab supplier	-
Sterile, nuclease-free aerosol barrier pipette tips	General lab supplier	-
0.2 mL PCR Tube Strips, Low Profile and Cap Strips	General lab supplier	-
Powder-free gloves	General lab supplier	-
DNA LoBind Tubes, 1.5 mL PCR clean	Eppendorf	022431021 or equivalent
Microtubes 1.5ml Clear, Sterile	AXYGEN	MCT-150-C-S or equivalent
Eppendorf™ twin.tec™ 96-well LoBind PCR Plates, Skirted	Eppendorf	0030129512 or equivalent
Microseal 'B' adhesive seals	Bio-Rad	MSB-1001 or equivalent
Equipment		
Product Name	**Suggested Supplier**	**p/n**
TapeStation Instrument	Agilent Technologies, Inc.	G2991BA or equivalent
Magnetic separator	Invitrogen	12321D or equivalent
Thermal Cycler	General lab supplier	-
Micro-centrifuge	General lab supplier	-
Vortex mixer	General lab supplier	-

Before you begin

It is recommended to read the manual carefully to understand the experimental procedure. In particular, read and follow the text **highlighted in bold**.

Notice

The results of this experiment can be affected by the quality of the DNA. We recommend using high-quality DNA samples. However, low-quality samples (e.g., FFPE tissue extracted gDNA) show variable efficiency due to chemical damage and fragmentation, and its small amounts. Therefore, we recommend additional experimental optimization when you prepare the NGS library with these samples.

Directions for the use of this product

1. This product should be used for research use only.
2. Handle specimens cautiously to avoid potential infections.
    1) Handle reagents cautiously and avoid contact with any area of the user's skin, eyes, and mucous membrane. In the event of reagent contact, rinse with water immediately to remove. If the reagent spills from the cartridge, dilute with water and follow the duly accredited treatment.
    2) All blood serum materials from human sources should be handled carefully and treated as potential infectious sources.
3. Genomic DNA extracted from bio-specimens such as blood, saliva, FFPE, and fresh tissue should be used as sample specimens.
4. Reagents should be used by the suggested Usage and Volume within the expiry date.
5. Reagent storage should be carried out at their suggested temperature and condition. Temperature conditions are listed below:

    Room temperature (RT, 15-25°C), Freezer(-20°C), Deep freezer (-80°C)
6. For disposal of used or expired reagents, please contact an authorized waste disposal company in accordance with relevant laws and regulations.
7. Be cautious with specific allergenic reagents.

Please maintain a nuclease-free environment during the experiment. Wear a lab coat, disposable gloves, and protective goggles. If you have any problems, questions, or related concerns, please email us at support@celemics.com

Protocol Overview

	Workflow	Time
NGS Library Preparation	Enzymatic preparation	**1 hr**
	Adapter ligation	**20 mins**
	Purification of sample using CeleMag Clean-up Bead	**40 mins**
	Amplification of the Adapter-Ligated Library	**20 mins**
	Purification using CeleNM Bead	**40 mins**
	Pre-capture pooling of samples	**40 mins**

NGS Library Preparation

Step 1. Enzymatic Preparation

[Note]

Follow the protocol in this section for human genomic DNA inputs ≥ 50 ng to construct libraries with an average insert size of ~300 bp, for targeted enrichment and direct sequencing, respectively. Fragmentation times provided are for high-quality samples. Optimization of fragmentation time may be required for samples of compromised quality (e.g., FFPE).

[Preparation]

Library Preparation Box #1	**10x EP-ER/A buffer** **5x EP-ER/A Enzyme**	Keep cool on ice (or cooling block)

[Procedure]

1. Set the program into a thermal cycler (see the details in Table 8 and 9).
2. Adjust the DNA sample volume to 21 µL as below in Table 5, if necessary. The input amount should be ≥ 50 ng of DNA.

Table 5. Adjusting the Volume per Sample

Reagent	Volume per sample (µL)
Nuclease-free water	21 - x
DNA sample	x
Total Volume	**21**

3. **Transfer the entire DNA sample** into a new 8-strip PCR tube (or 96-well plate).
4. Prepare **Enzymatic Prep Master Mix** in the 8-strip PCR tube as described in Table 6 (for multiple samples, prepare the Enzymatic Prep Master Mix of EP-ER/A Buffer and EP-ER/A Enzyme in a new 1.5 mL LoBind tube including at least 5% of additional volume).
   * Important – Because enzymes are active at room temperature and may fragment DNA to undesired sizes. Ensure that the Enzymatic Prep Master Mix is mixed thoroughly and is kept on ice until placed in the thermal cycler.

Table 6. Composition of Enzymatic Prep Master Mix

Reagent	1 Reaction (µL)	Note
EP-ER/A Buffer	3	**Enzymatic Prep Master Mix**
EP-ER/A Enzyme	6	
Total Volume	**30**	

5. **Add 9 µl of Enzymatic Prep Master Mix** to each 8-strip PCR tube (containing the DNA sample), mix well using a vortex mixer, and spin down the tube.
   * Important – Combine it on ice and mix well.
   * Important – The final reaction volume is **30 µl**.

Table 7. Composition of Enzymatic Prep Mix

Reagent	1 Reaction (µL)
Enzymatic Prep Master mix	9
Sample	21
Total Volume	**30**

6. Put the tubes on thermal cycler and run the following program in Table 8.

Table 8. Thermal Cycler Program (lid temperature: 70°C)

Step	Temperature	Time
1	4°C	1 minute
2	32°C	Desired time (Table 9)
3	65°C	30 minutes
4	4°C	Hold

* Important – Desired time for fragmentation depends on the condition of the initial input DNA amount. See Table 9 below.

Table 9. Desired Time for Fragmentation

Average insert size	Desired fragmentation time (min.) for DNA
~300 bp	18 ± 5

* Important – The optimal fragmentation times differ between enzyme production lots. Please find the fragmentation time-size table in your kit located inside the lid of Library Preparation box #1. The fragmentation times are based on the fragmentation of 200 ng of DNA with DIN≥9.

* Important – This kit can be applied to damaged samples such as FFPE. However, it should be optimized to an appropriate fragmentation time. We recommend that FFPE samples have a shorter fragmentation time depending on the level of degradation and starting size distribution.

7. After running the thermal cycler program, prepare the Ligation Master Mix immediately for the Adapter Ligation step.
* Important – Fragmented samples can be kept at 4 °C for **no more than one hour**.

IMPORTANT - Proceed with the next step right away.

Step 2. Adapter Ligation

[Preparation]

Library Preparation Box #1	**Ligation buffer** **Ligation Enzyme**	Keep cool on ice (or cooling block)
Library Preparation Box #2	**Adapter (S)**	Thawing on ice

[Procedure]

1. **Add 10 µL of Adapter (S)** directly into the PCR tube (DNA sample) where Enzymatic Preparation was performed. Mix the tube well on the vortex mixer and pulse-spin down.
2. Prepare Ligation Master Mix (ligation, Buffer, ligation Enzyme and nuclease-free water) in a new 1.5 mL LoBind tube as described in Table 10.
3. **Add 20 µL of Ligation Master Mix** into the PCR tube (DNA sample & Adapter (S), 40 µL). Mix the sample tube well using a vortex mixer and pulse-spin down.
   * Important – Combine it on ice and mix well.
   * Important – The final reaction volume is **60 µl**.

Table 10. Composition of Ligation Mix

Reagent	1 Reaction (µL)	Note
ER/A-tailed Sample	30	Mix Sample and Adapter first
Adapter (S)	10	
Nuclease-free water	4	**Prepare Ligation Master Mix** before mixing with Sample-adapter mixture
Ligation Buffer	12	
Ligation Enzyme	4	
Total Volume	**60**	

4. Incubate the Ligation mixture at 20°C for 20 minutes in thermal cycler (lid temperature: 40°C).

IMPORTANT - Proceed with the next step right away.

Step 3. Purification of sample Using CeleMag Clean-up Bead

[Preparation]

CeleMag Clean-up Bead	Keep the bead at room temperature for at least 30 minutes before use. Store the bead at 4°C after use
80% ethanol solution	Using freshly made 80% ethanol solution is preferable

[Procedures]

1. Vortex CeleMag Clean-up Bead to achieve a homogeneous state.
2. **Add 48 µL CeleMag Clean-up Bead** to a new 1.5 ml LoBind Tube (0.2mL PCR tube or 96-well plate are also available) and **transfer the A-tailed DNA (60 µL**) to the tube. Mix well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for **5 minutes**
   * Important – Avoid spinning down the bead too strongly to prevent precipitation.

   Table 11. Volumes of CeleMag Clean-up Bead (0.8x)

Reagent	Volume Ratio (Bead : Sample)
CeleMag Clean-up Bead	**0.8 : 1** (e.g., 48 µL : 60 µL)

3. Put the tube in the magnetic separator for **3-5 minutes** until the solution is clear.
4. Keep the tube in the magnetic separator and discard the supernatant
   * Important – During Steps 4 to 6, be careful not to touch the bead while you remove the supernatant.
5. Keeping the tube in the magnetic separator, **add 500 µL (180 µL for PCR tube or 96-well plate) of fresh 80% ethanol** to each tube.
6. **Incubate for 30 seconds** at room temperature, then discard the ethanol.
7. Repeat wash steps 5-6 once.
8. Spin down, put the tube in the magnetic separator and discard residual ethanol.
9. **Dry the sample tube** at room temperature for **2 minutes** or **until residual ethanol has completely evaporated.**
   * Important – Excessive drying of the bead pellets until cracks occur causes a decrease in elution efficiency. Proceed with step 10 before the crack appears.
10. **Add 22 µL of nuclease-free water** to the sample and thoroughly mix on a vortex mixer. Pulse-spin down and incubate the sample at room temperature for **2 minutes**.
11. Put the tube in the magnetic separator for **2 minutes** until the solution is clear.
12. **Transfer the supernatant (20 µL)** to a new 1.5 mL LoBind Tube and keep the supernatant for the next step.

Stop Point - If not immediately continuing to the next step, samples can be stored at -20°C.

Step 4. Amplification of the Adapter-Ligated Library

[Note]

Determine the appropriate index primers for each sample (See Table 15. Index Sequences).
***Important -** Use different index primer pairs if the samples are sequenced in the same sequencing run.

[Preparation]

CLM Polymerase	**CLM Polymerase**	Keep cool on ice (or cooling block)
Library Preparation Box #2	**Dual index 5xx primer***	Thawing on ice Keep cool on ice (or cooling block)
	Dual index 7xx primer*	

* The name of each primer differs between kits. Please find the index primers in your kit located in Library Preparation box #2.

[Procedure]

1. Set the program into a thermal cycler (see the details in Table 13).
2. Prepare new 0.2 mL PCR tubes (or 96-well PCR plates) and PCR mix as described in Table 12 (add 5% of the additional volume of reagents for multiple libraries).
3. **Add 25 µL of the CLM Polymerase** to each tube.
   * Important – Combine all reagents on ice.
4. **Add 2.5 µL of each dual index PCR Primer** to the individual tube.
   * Important – Check and record the appropriate dual index combination per sample.
5. **Add 20 µL of Adapter-ligated DNA library** to each PCR tube, mix well and spin down.
6. Put the tubes on a thermal cycler and run the PCR program shown in Table 13 (see the number of PCR cycles in Table 14).

Table 12. Composition of PCR mix

Reagent	Volume for 1 Library (µL)
CLM Polymerase	25
Dual Index 5xx Primer	2.5
Dual Index 7xx Primer	2.5
Adapter-ligated DNA Library	20
Total Volume	**50**

Table 13. PCR Program

Step	Temperature	Time
Step 1	98°C	45 seconds
Step 2	98°C	15 seconds
Step 3	65°C	30 seconds
Step 4	72°C	1 minute
Step 5	-	Repeat step 2 to step 4 (Total PCR cycles, See in Table 14)
Step 6	72°C	10 minutes
Step 7	4°C	Hold

Table 14. The Number of PCR Cycles

Genomic DNA amount (ng), from page 8 - Step 1	Total PCR Cycles
500 ng ≤ gDNA amount	3-4
100 ng ≤ gDNA amount < 500 ng	5-6
50 ng ≤ gDNA amount < 100 ng	7-8
gDNA amount < 50 ng	9-10

* The quality of gDNA can affect PCR efficiency.

Table 15. Index Sequences

Index	Sequence	Index	Sequence
Dual index 501 primer	TATAGCCT	Dual index 701 primer	ATTACTCG
Dual index 502 primer	ATAGAGGC	Dual index 702 primer	TCCGGAGA
Dual index 503 primer	CCTATCCT	Dual index 703 primer	CGCTCATT
Dual index 504 primer	GGCTCTGA	Dual index 704 primer	GAGATTCC
Dual index 505 primer	AGGCGAAG	Dual index 705 primer	ATTCAGAA
Dual index 506 primer	TAATCTTA	Dual index 706 primer	GAATTCGT
Dual index 507 primer	CAGGACGT	Dual index 707 primer	CTGAAGCT
Dual index 508 primer	GTACTGAC	Dual index 708 primer	TAATGCGC
Dual index 509 primer	GACCTGTA	Dual index 709 primer	CGGCTATG
Dual index 510 primer	CGGTGGTA	Dual index 710 primer	TCCGCGAA
Dual index 511 primer	GTTGGACT	Dual index 711 primer	TCTCGCGC
Dual index 512 primer	CTAAGATC	Dual index 712 primer	AGCGATAG
Dual index 513 primer	AATGGTTC	Dual index 713 primer	GCATAGTG
Dual index 514 primer	CCTACGAA	Dual index 714 primer	CATTCCAG
Dual index 515 primer	AGCAGATC	Dual index 715 primer	GGCAGGAT
Dual index 516 primer	GCGGAGCG	Dual index 716 primer	CAACAACA
		Dual index 717 primer	GAATAATC
		Dual index 718 primer	ATGCGGCT
		Dual index 719 primer	TTAATCAG
		Dual index 720 primer	ACTGCTTA
		Dual index 721 primer	CGTAGCTC
		Dual index 722 primer	GCCTCTCT
		Dual index 723 primer	GCCGTAGG
		Dual index 724 primer	GCGATTAA

IMPORTANT - Proceed with the next step right away.

Step 5. Purification Using CeleNM Bead

[Note]
The CeleNM bead have a distinct application use that is different from the CeleMag Clean-up Bead. Please be sure to use each separately and not to mix up the two.

[Preparation]

CeleNM Bead	Keep the bead at room temperature for at least 30 minutes before use. Store the bead at 4°C after use
Binding buffer	Store the buffer at Room temperature
100% isopropanol	Store the buffer at Room temperature
80% ethanol solution	Using freshly made 80% ethanol solution is preferable

[Procedures]

1. Thoroughly vortex CeleNM Bead to achieve a homogeneous state.
2. **Add 75 µL of binding buffer, 45 µL of 100% isopropanol, and 22.5 µL of CeleNM Bead** to a new 1.5 mL LoBind tube and **add 50 µL of amplified sample** to the tube. Combine well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes
   * Important – Avoid spinning down the CeleNM bead too strongly to prevent precipitation.

   Table 16. Volumes of CeleNM Bead

Reagent	Volume
Binding Buffer	75 µL
100% isopropanol	45 µL
CeleNM Bead	22.5 µL
Amplified sample	50 µL
Total Volume	**192.5 µL**

3. Keep the tube in the magnetic separator for **5 minutes**, then discard the supernatant.
   * Important – During Steps 3 to 6, be careful not to touch the bead while you remove the supernatant.
4. Keeping the tube in the magnetic separator, **add 500 µL** of **fresh 80% ethanol** to each sample tube.
5. Incubate for 30 seconds at room temperature and then discard the 80% ethanol
6. Repeat 80% ethanol wash (**steps 5-6**).
7. Spin down, put the tube in a magnetic separator, and discard residual ethanol.

8. Dry the sample tube at room temperature for more than 2 minutes until residual ethanol has completely evaporated.
   * Important – Excessive drying of the bead pellets until cracks occur causes a decrease in elution efficiency. Proceed with step 10 before the crack appears.
9. **Add 12 µL of nuclease-free water** to the sample and mix well on a vortex mixer. Pulse-spin down and incubate the sample at room temperature for **5 minutes**.
10. Put the tube in the magnetic separator for **2 minutes** until the solution is clear.
11. **Transfer the supernatant (10 µL)** to a new 1.5 mL LoBind Tube.

Stop Point - If not immediately continuing to the next step, samples can be stored at -20°C.

Step 6. Pre-capture Pooling of samples

[Preparation]

CeleMag Clean-up Bead	Keep the bead at room temperature for at least 30 minutes before use. Store the bead at 4°C after use
80% ethanol solution	Using freshly made 80% ethanol solution is preferable

[Pre-capture pooling guide]

After the purification with CeleNM bead, the volume of each sample should be 10 µL. For the next step, we recommend pooling 4 samples into a single pooling library (see Table 17 below).

Table 17. Pooling Guide according to the Number of Samples

Number of samples	Total Volume (µL)
4	40 µL

[Procedure]

1. Thoroughly vortex CeleMag Clean-up Bead to achieve a homogeneous state.
2. **Add each DNA library sample (10 µL each)** to a new 1.5 mL LoBind tube according to Table 17. **Mix it with the same volume of CeleMag Clean-up Bead (See Table 18)**. Combine well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature **for 5 minutes**
   * Important – Avoid spinning down the bead too strongly to prevent precipitation.

   Table 18. Volumes of CeleMag Clean-up Bead (1x)

Reagent	Volume Ratio (Bead : Sample)
CeleMag Clean-up Bead	**1 : 1** (e.g., 40 µL : 40 µL)
3. Put the tube in the magnetic separator for **3-5 minutes** until the solution is clear.
4. Keep the tube in the magnetic separator and discard the supernatant
   * Important – During Steps 4 to 6, be careful not to touch the bead while you remove the supernatant.
5. Keeping the tube in the magnetic separator, **add 500 µL of fresh 80% ethanol** to each tube.
6. **Incubate for 30 seconds** at room temperature, then discard the ethanol.
7. Repeat wash steps 5-6 once.
8. Spin down, put the tube in the magnetic separator and discard residual ethanol.

9. **Dry the sample tube** at room temperature for **2 minutes** or **until residual ethanol has completely evaporated.**
   * Important – Excessive drying of the bead pellets until cracks occur causes a decrease in elution efficiency. Proceed with step 10 before the crack appears.
10. **Add 12 µL of nuclease-free water** to the sample and thoroughly mix on a vortex mixer. Pulse-spin down and incubate the sample at room temperature for **2 minutes**.
11. Put the tube in the magnetic separator for **2 minutes** until the solution is clear.
12. **Transfer the supernatant (10 µL)** to a new 1.5 mL LoBind Tube and keep it for the next step.

Stop Point - If not immediately continuing to the next step, samples can be stored at -20°C.