CELEMICS

Kit User Manual

# NGS Library Preparation

## Sonication based DNA Fragmentation

### Illumina Platform

### Single / Dual Index

### Adapter (S)

Version 2.0

Research Use Only

CELEMICS

# Table of Contents

# Materials Provided

## Table 1. Library Preparation #1

**Storage condition: -20**°C

| Box No. | Product Name | 1 rxn (µL) | Cat No. LI1016 (16 rxn) | Cat No. LI1024 (24 rxn) | Cat No. LI1048 (48 rxn) | Cat No. LI1096 (96 rxn) |
|---|---|---|---|---|---|---|
| LI10 | 5X ER/A-Tailing Enzyme Mix | 10 | LI100416 | LI100424 | LI100448 | LI100496 |
| | 10X ERA Buffer | 5 | LI100516 | LI100524 | LI100548 | LI100596 |
| | WGS Ligase | 10 | LI100616 | LI100624 | LI100648 | LI100696 |
| | 5X Rapid Ligase Buffer | 20 | LI100716 | LI100724 | LI100748 | LI100796 |

## Table 1-1. Library Preparation #2 (Single Index)

**Storage condition: -20°C**

| Box No. | Product Name | 1 rxn (µL) | Cat No. LI2016 (16 rxn) | Cat No. LI2024 (24 rxn) |
|---|---|---|---|---|
| LI20 | PCR Index Primer 1-24 | 2.5 | LI200116 -LI201616 | LI200124 -LI202424 |
| | Pre Capture PCR Forward Primer | 2.5 | LI202716 | LI202724 |
| | Adapter (S) | 10 | LI205716 | LI205724 |

## Table 2-2. Library Preparation #2 (Dual Index)

**Storage condition: -20°C**

| Box No. | Product Name | 1 rxn (µL) | Cat No. LI2016 (16 rxn) | Cat No. LI2024 (24 rxn) | Cat No. LI2048 (48 rxn) | Cat No. LI2096 (96 rxn) |
|---|---|---|---|---|---|---|
| LI20 | Dual Index 501-513 | 2.5 | LI202816 - LI204016 | LI202824 - LI204024 | LI202848 - LI204048 | LI202896 - LI204096 |
| | Dual Index 701-716 | 2.5 | LI204116 - LI205616 | LI204124 - LI205624 | LI204148 - LI205648 | LI204196 - LI205696 |
| | Adapter (S) | 10 | LI205716 | LI205724 | LI205748 | LI205796 |

# Additional Purchasable Materials

### Table 3. Additional Purchasable Materials List

| Product Name | Cat No. | Storage Condition (°C) | Comment |
|---|---|---|---|
| Celemics DNA purification bead (CeleMag Clean-up Bead) | CMCB50 (50 mL)<br>CMCN500 (500 mL) | 4°C | Included in All-in-one kit |
| Celemics Streptavidin bead (CeleMag Streptavidin Bead) | CMSB02 (2 mL)<br>CMSB10 (10 mL) | 4°C | |
| CLM Polymerase Amplification Kit PCR Ready Mix only | CMPFX.XX<br>(X.XX: amount, mL) | -20°C | |

# Materials Required (Not Provided)

### Table 4. Not Provided Materials List

| Product Name | Suggested Supplier | |
|---|---|---|
| **Reagents & Consumables** | | |
| NEBNext® dsDNA Fragmentase® (DNA fragmentation - option 2) | NEB | p/n: M0348S or M0348L |
| Nuclease-free water (not DEPC-treated) | General lab supplier | - |
| Ethanol (200 proof) | 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, 250 pieces | Eppendorf | p/n: 022431021 or equivalent |
| Microtubes 1.5ml Clear, Sterile | AXYGEN | p/n: MCT-150-C-S or equivalent |
| Eppendorf™ twin.tec™ 96 Well LoBind PCR Plates, Skirted | Eppendorf | p/n: 0030129512 or equivalent |
| Microseal 'B' adhesive seals | Bio-Rad | p/n: MSB-1001 or equivalent |
| **Equipment** | | |
| TapeStation Instrument | Agilent Technologies, Inc. | p/n: G2991BA or equivalent |
| Magnetic separator | Invitrogen | p/n: 12321D or equivalent |
| Thermal Cycler | General lab supplier | - |
| Micro-centrifuge | General lab supplier | - |
| Vortex mixer | General lab supplier | - |
| Timer | 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. For fragmented or damaged samples, such as DNA extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue, experimental results cannot be guaranteed. Therefore, it is recommended to use high-quality DNA 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/RNA extracted from bio-specimens such as blood, saliva, FFPE, and fresh tissue should be used as sample specimens.
4. Reagents should be used in accordance with 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. Consult an authorized management company for proper management of used and expired reagents in accordance with the relevant laws.
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

| NGS Library Preparation workflow | Time |
|---|---|
| DNA Fragmentation | 0.5 hour |
| End-repair & A-tailing / Adapter ligation / Purification using CeleMag Clean-up Beads | 2 hours |
| **STOPPING POINT** | |
| Amplification of the Adapter-Ligated Library / Purification | 1.5 hour |
| **STOPPING POINT** | |
| Assess Quality and Quantity / (Optional) Remove Adapter-Dimer | 0.5-1 hour |

# Sample Preparation

## Step 1. DNA Fragmentation

[Note]

Genomic DNA (gDNA) from biospecimen such as blood, saliva, FFPE and fresh tissue can be used for NGS sample preparation.

- Choose one of the two options below:

[Option 1] DNA fragmentation using sonication

1. Ensure the appropriate quality and quantity of gDNA. Make sure the **DNA integrity number (DIN)** value of gDNA is higher than **6.5**.
2. Fragment the gDNA into 100-500 bp fragments so that **the main peak of the gDNA is 150-200 bp**. Check fragmented DNA size through available instruments such as TapeStation, Bioanalyzer, etc..

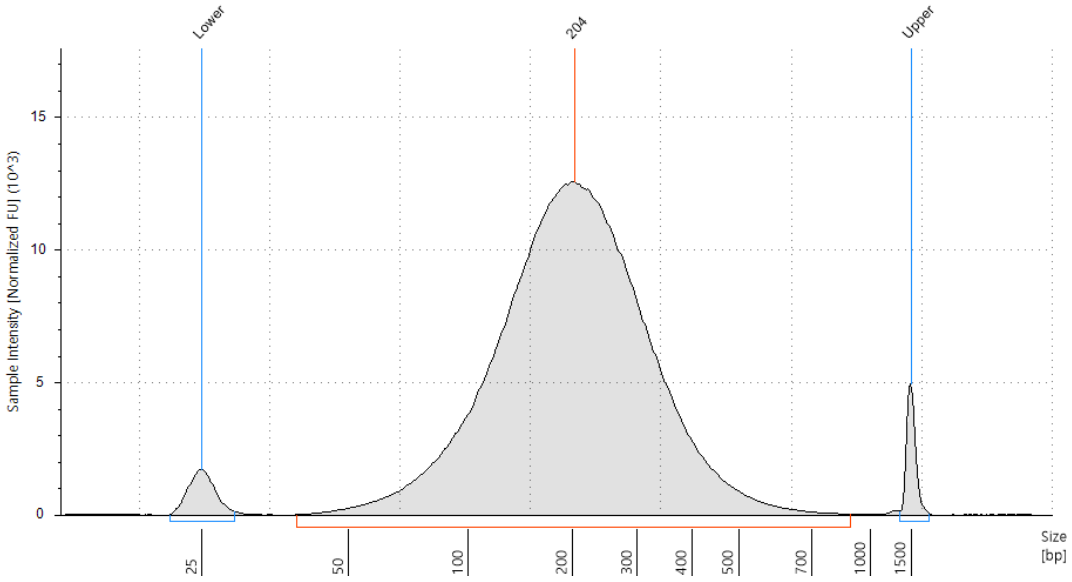


Figure 1. The electrophoresis diagram shows main peak size between 150 to 200 bp.

[Option 2] DNA fragmentation using enzyme

1. An enzyme based fragmentation kit (e.g. NEBNext® dsDNA Fragmentase®) can also be used for DNA fragmentation.
2. **Adjust the enzyme treatment time** to fragment the gDNA into 100-500 bp fragments so that the main peak of the gDNA is 150-200 bp.
3. Check fragmented DNA size through available instruments such as TapeStation, Bioanalyzer, etc.

## Step 2. (Optional) Purification of the DNA Sample Using Clean-up Beads

[Note]

If the amount of fragmented DNA is less than 100 ng, this step may be omitted.

[Preparation]

- CeleMag Clean-up Beads
  Keep the beads at room temperature for at least 30 minutes before use.
  Store CeleMag Clean-up Beads at 4°C after use.
- 80% ethanol solution
- Nuclease-free water

[Procedures]

1. Vortex CeleMag Clean-up Beads in order to achieve a homogeneous state.
2. **Add 180 µL CeleMag Clean-up Beads** to a new 1.5 ml LoBind Tube and **transfer the sheared DNA (100 µL)** to the tube. Mix well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for **5 minutes** (*avoid spinning down the CeleMag Clean-Up Beads too strongly to prevent precipitation*).

   Table 5. Volumes of CeleMag Clean-up Beads (1.8X)

| Reagent | Volume Ratio (Beads : Sample) |
|---|---|
| CeleMag Clean-up Beads | **1.8 : 1**<br>(180 µL : 100 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 *(be careful not to touch the beads 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) fresh 80% ethanol** to each sample.
6. **Incubate for 30 seconds** at room temperature, then discard the ethanol *(be careful not to touch the beads while you remove the supernatant).*
7. Repeat the 80% ethanol wash (**step 5-6**).
8. Spin down and put the tube in magnetic separator and discard residual ethanol.
9. **Dry the sample tube** at room temperature for **2 minutes** or **until residual ethanol has completely evaporated** (*avoid drying beads until pellets appear cracked, as excessive drying decreases elution efficiency*).
10. **Add 37 µL of nuclease-free water** to the sample and thoroughly mix on a vortexer. 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 solution is clear.
12. **Transfer the supernatant (35 µL)** to a new 1.5 mL LoBind Tube and keep the supernatant for the next step.

**Stop Point**

If not continuing to the next step, the sample can be stored at -20℃.

## Step 3. Repair the Ends and A-tailing

[Preparation]

- Library Preparation Box #1 → 5x ER/A-Tailing Enzyme Mix, 10x ERA buffer
- 0.2 mL PCR tubes and caps
- Ice bucket with ice or cooling block
- (Optional) Nuclease-free water

[Procedure]

1. Prepare **ERA buffer mix** in a PCR tube as described Table 6.
   * Important – Combine all reagents on ice and mix well by pipetting.

   Table 6. Composition of ERA buffer mix

| Reagent | 1 Reaction (µL) |
|---|---|
| 10x ERA buffer | 5 |
| Eluted DNA sample | 35 |
| Total volume | 40 |

2. **Add 10 µL of 5x ER/A Enzyme Mix** to each reaction tube and gently mix well.
   * Important – Combine it on ice and mix well by pipetting
   * Important – The final reaction volume is 50 µl
3. Spin down the sample tube and keep it on ice
4. Input the following program into a thermal cycler (**lid temperature: 70°C**).

   Table 7. Thermal cycler program

| Step | Temperature | Time |
|---|---|---|
| 1 | 4°C | 1 min |
| 2 | 20°C | 30 min |
| 3 | 65°C | 30 min |
| 4 | 4°C | Hold |

5. Transfer the sample tube to the thermocycler. Run the thermal cycler program.
6. When the reaction is finished and block temperature reaches at 4°C, transfer the End repaired and A-tailed sample **to ice**.

**Important**
Proceed with the next step right away.

## Step 4. Adapter Ligation

[Preparation]

- Library Preparation Box #1 → 5x Rapid Ligase Buffer, WGS Ligase
- Library Preparation Box #2 → Adapter (S)

  * Tip. Thaw the Adapter on ice in advance.

- 0.2 mL PCR tubes and caps
- Nuclease-free water

[Procedure]

1. **Add 10 µL of Adapter (S)**directly into the PCR tube where End repaired and A-tailed reaction was performed. Mix well by pipetting.
2. Prepare a ligation reaction mix in a separate tube on ice and mix well with vortexing (add 5% of additional amount of reagents for multiple samples).
   * Important – Combine all reagents on ice

   Table 8. Composition of ligation reaction mix

| Reagent | 1 Reaction (µL) |
|---|---|
| 5x Rapid Ligase Buffer | 20 |
| WGS Ligase | 10 |
| Nuclease-free water | 10 |
| Total volume | 40 |

3. **Add 40 µL of the ligation reaction mix** to the sample from Procedure 1.
   * Important – The final reaction volume is 100 µl
4. Incubate the ligation reaction mix at 20°C for 15 minutes.

   * Lid temperature: 40°C

**Important**
Proceed with the next step right away.

## Step 5. Purification of Sample Using Clean-up Beads

[Preparation]

- CeleMag Clean-up Beads
  Keep the beads at room temperature for at least 30 minutes before use.
  Store CeleMag Clean-up Beads at 4°C after use.
- 80% ethanol solution
- Nuclease-free water

[Procedure]

1. Thoroughly vortex CeleMag Clean-up Beads in order to achieve a homogeneous state.
2. **Add 100 µL CeleMag Clean-up Beads** to a new 1.5 mL LoBind tube and **add 100 µL adapter ligated sample** to the tube. Combine well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes (*avoid spinning down the CeleMag Clean-Up Beads too strongly to prevent precipitation*).

   Table 9. Volumes of CeleMag Clean-up Beads (1X)

| Reagent | Volume Ratio (Beads : Sample) |
|---|---|
| CeleMag Clean-up Beads | **1 : 1**<br>100 µL : 100 µL |

3. Place the tube in a magnetic separator for **3-5 minutes** until the solution is clear.
4. Keep the tube in the magnetic separator and discard the supernatant *(be careful not to touch the beads 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) fresh 80% ethanol** to each sample tube.
6. Incubate for 30 seconds at room temperature and then discard the 80% ethanol *(be careful not to touch the beads while you remove the supernatant)*.
7. Repeat 80% ethanol wash (**step 5-6**).
8. Spin down and put the tube in a magnetic separator and discard residual ethanol.
9. **Dry the sample tube** at room temperature for **2 minutes** or **until residual ethanol has completely evaporated** (*avoid drying beads until pellets appear cracked, as excessive drying decreases elution efficiency*).
10. **Add 22 µL of nuclease-free water** to the sample and mix well on a vortexer. 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 solution is clear.
12. **Transfer the supernatant (20 µL)** to a new 1.5 mL LoBind Tube.

**Stop Point**

If do not continuing to the next step, samples can be stored at -20℃.

## Step 6-1. Amplification of the Adapter-Ligated Library (Single Index)

[Note]

Determine the appropriate index primers for each sample (Table 13-1).

Use different index if the samples are sequenced in the same lane.

[Preparation]

- Library Preparation Box #2 → Pre Capture Forward Primer, PCR index primer
- CLM Polymerase
- 0.2 mL PCR tubes and caps

[Procedure]

1. For **1** library
    a. Prepare 0.2 mL PCR tube and PCR mix as described in Table 10-1. Mix **PCR mix (30 µL)** and **Adapter-ligated DNA library (20 µL)**.

    * Important – Combine all reagents on ice.

    b. Run the PCR program as described in Table 11-1 using a thermal cycler (see the number of PCR cycles in Table 12-1).

2. For **multiple** libraries
    a. Prepare new 0.2 mL PCR tubes and PCR mix as described in Table 10-1 (add 5% additional amount of reagents for multiple libraries).

    b. **Add 25 µL of the CLM Polymerase** to each tube.

    c. **Add 2.5 µL of PCR Index Primer and 2.5 µL of Pre Capture PCR Forward Primer** to each tube.

    d. **Add 20 µL of Adapter-ligated DNA library** to each PCR tube, mix well and spin down.

    *** Important** – Combine all reagents **on ice**.

    e. Run the thermal cycler with the PCR program shown in Table 11-1 (see the number of PCR cycles in Table 12-1).

Table 10-1 (Single Index). Composition for PCR mix

| Reagent | Volume for 1 Library |
|---|---|
| PCR Index Primer (select one) | 2.5 µL |
| Pre Capture PCR Forward Primer | 2.5 µL |
| CLM Polymerase | 25 µL |
| Total Volume | 30 µL |

Table 11-1 (Single Index). 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 12-1) |
| Step 6 | 72°C | 10 minutes |
| Step 7 | 4°C | Hold |

Table 12-1 (Single Index). The number of PCR cycles

| Fragmented DNA amount (ng), from page 8 - Step 1 | Total PCR cycles |
|---|---|
| 500 ng ≤ fragmented DNA amount | 6 |
| 100 ng ≤ fragmented DNA amount < 500 ng | 8 |
| 50 ng ≤ fragmented DNA amount < 100 ng | 10 |
| fragmented DNA amount < 50 ng | 12 |

* The quality of gDNA could affect PCR efficiency.

Table 13-1 (Single Index). Index sequences

| Index | Sequence | Index | Sequence |
|---|---|---|---|
| PCR Index Primer 1 | ATCACG | PCR Index Primer 13 | AGTCAA |
| PCR Index Primer 2 | CGATGT | PCR Index Primer 14 | AGTTCC |
| PCR Index Primer 3 | TTAGGC | PCR Index Primer 15 | ATGTCA |
| PCR Index Primer 4 | TGACCA | PCR Index Primer 16 | CCGTCC |
| PCR Index Primer 5 | ACAGTG | PCR Index Primer 17 | GTCCGC |
| PCR Index Primer 6 | GCCAAT | PCR Index Primer 18 | GTGAAA |
| PCR Index Primer 7 | CAGATC | PCR Index Primer 19 | GTGGCC |
| PCR Index Primer 8 | ACTTGA | PCR Index Primer 20 | GTTTCG |
| PCR Index Primer 9 | GATCAG | PCR Index Primer 21 | CGTACG |
| PCR Index Primer 10 | TAGCTT | PCR Index Primer 22 | GAGTGG |
| PCR Index Primer 11 | GGCTAC | PCR Index Primer 23 | ACTGAT |
| PCR Index Primer 12 | CTTGTA | PCR Index Primer 24 | ATTCCT |

## Step 6-2. Amplification of the Adapter-Ligated Library (Dual Index)

[Note]

Determine the appropriate index primers for each sample (Table 13-2).

Use different index primers if the samples are sequenced in the same lane.

[Preparation]

- Library Preparation Box #2 → Dual Index
- CLM Polymerase
- 0.2 mL PCR tubes and caps

[Procedure]

1. For **1** library
    a. Prepare 0.2 mL PCR tube and PCR mix as described in Table 10-2. Mix **PCR mix (30 µL)** and **Adapter-ligated DNA library (20 µL)**.
    b. Run the thermal cycler with the PCR program shown in Table 11-2 (see the number of PCR cycles in Table 12-2).
2. For **multiple** libraries
    a. Prepare new 0.2 mL PCR tubes and PCR mix as described in Table 10-2 (add 5% of additional amount of reagents for multiple libraries).
    b. **Add 25 µL of the CLM Polymerase** to each tube.
    c. **Add 2.5 µL of each dual index PCR Primers** to individual tube.

    * Important – Check the appropriate of dual index combination Table13-2.

    d. **Add 20 µL of Adapter-ligated DNA library** to each PCR tube, mix well and spin down.

    * Important – Combine all reagents on ice.

    e. Run the thermal cycler with the PCR program shown in Table 11-2 (see the number of PCR cycles in Table 12-2).

Table 10-2 (Dual Index). Composition for PCR mix

| Reagent | Volume for 1 Library |
|---|---|
| Dual index primer 501-513 (select one) | 2.5 µL |
| Dual index primer 701-716 (select one) | 2.5 µL |
| CLM Polymerase | 25 µL |
| Total Volume | 30 µL |

Table 11-2 (Dual Index). 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 12-2) |
| Step 6 | 72°C | 10 minutes |
| Step 7 | 4°C | Hold |

Table 12-2 (Dual Index). The number of PCR cycles

| Fragmented DNA amount (ng), from page 8 - Step 1 | Total PCR Cycles |
|---|---|
| 500 ng ≤ fragmented DNA amount | 6 |
| 100 ng ≤ fragmented DNA amount < 500 ng | 8 |
| 50 ng ≤ fragmented DNA amount < 100 ng | 10 |
| fragmented DNA amount < 50 ng | 12 |

* The quality of gDNA can affect PCR efficiency.

Table 13-2 (Dual Index). 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 714 primer | CATTCCAG |
| - | - | Dual index 715 primer | GGCAGGAT |
| - | - | Dual index 716 primer | CAACAACA |

**Important**

Proceed with the next step right away.

## Step 7. Purification of Sample Using Clean-up Beads

[Preparation]

- CeleMag Clean-up Beads
  Keep the beads at room temperature for at least 30 minutes before use.
  Store CeleMag Clean-up Beads at 4°C after use.
- 80% ethanol solution
- Nuclease-free water

[Procedure]

1. Thoroughly vortex CeleMag Clean-up Beads in order to achieve a homogeneous state.
2. **Add 50 µL CeleMag Clean-up Beads** to a new 1.5 mL LoBind tube and **add 50 µL amplified adapter-ligated sample** to the tube. Combine well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes (*avoid spinning down the CeleMag Clean-Up Beads too strongly to prevent precipitation*).

   Table 14. Volume of CeleMag Clean-up Beads (1X)

| Reagent | Volume Ratio (Beads : Sample) |
|---|---|
| CeleMag Clean-up Beads | **1 : 1**<br>50 µL : 50 µL |

3. Place the tube in a magnetic separator for **3-5 minutes** until the solution is clear.
4. Keep the tube in the magnetic separator and discard the supernatant *(be careful not to touch the beads 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) fresh 80% ethanol** to each sample tube.
6. Incubate for 30 seconds at room temperature and then discard the 80% ethanol *(be careful not to touch the beads while you remove the supernatant)*.
7. Repeat 80% ethanol wash (**step 5-6**).
8. Spin down and 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** (*avoid drying beads until pellets appear cracked, as excessive drying decreases elution efficiency*).
10. **Add 32 µL of nuclease-free water** to the sample and mix well on a vortexer. 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 (30 µL)** to a new 1.5 mL LoBind Tube.

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

## Step 8. Assess Quality and Quantity of Each Index-Tagged Library

1. Determine the quality and quantity of the sample with the appropriate instrument.

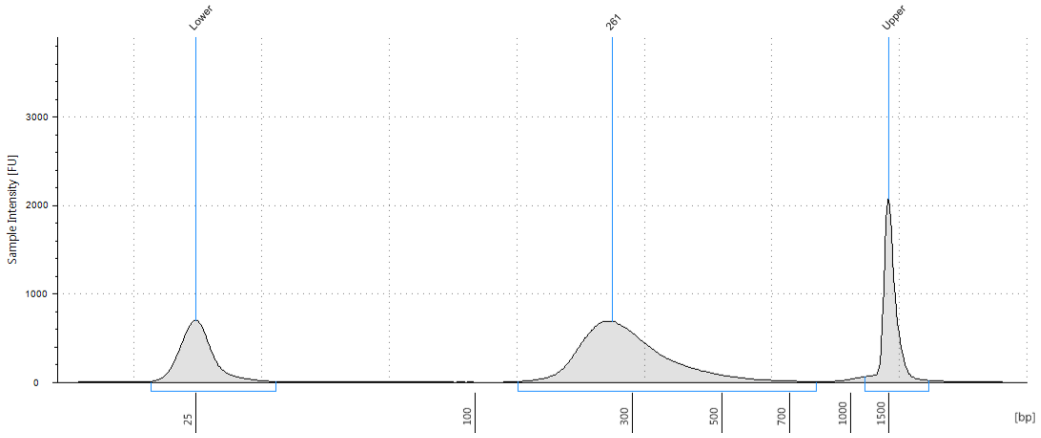


Figure 2. Electrophoresis diagram detected by TapeStation.

- The diagram shows the peak size, which is approximately 200-400 bp.
- Make sure that the size of amplified adapter-ligated library is **120-150 bp larger** than the initialfragmented DNA.

## Step 9. (Optional) Removal of Adapter-Dimer

[Note]

Identifying of adapter-dimer in pre-capture amplified library can be detected by gel-electrophoresis based instrument such as TapeStation and Bioanalyzer.

1. In the event that an adapter-dimer peak appeared, of which concentration is over 3.5 ng/µL, remove the adapter-dimer.

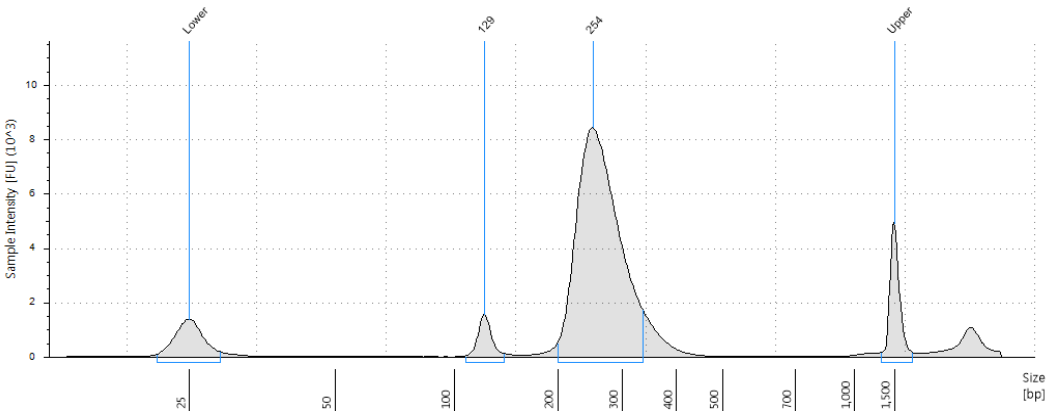


Figure 3. The electrophoresis diagram shows the adapter-dimer in 129 bp.

2. Purify the sample using the following procedure shown in **Step 7**, with the amount of bead shown in Table 15.

Table 15. Amount of beads ratio

| Amount of beads ratio |
|---|
| Beads:DNA sample = **0.8:1** |

3. Confirm the removal of adapter-dimer through available instrumentation

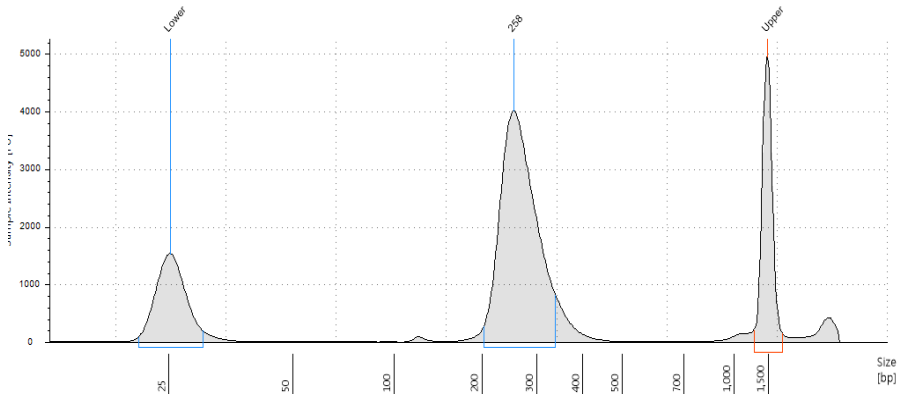


Figure 4. The electrophoresis diagram shows removal of the adapter-dimer in 129 bp.