CELEMICS

Kit User Manual

Whole Exome Sequencing Target Enrichment

Enhanced Hybridization Kit

Illumina Platform

Version 1.0.2

Research Use Only

CELEMICS

Table of Contents

Materials Provided

Table 1. Target Capture Solution #1 **Storage condition: -20℃**

Box No.	Product Name	1 rxn (µL)	Cat No. TI3004 (4 rxns)	Cat No. TI3012 (12 rxns)	Cat No. TI3024 (24 rxns)
TI30	Block #1	2.5	TI300104	TI300112	TI300124
	Block #2	2.5	TI300204	TI300212	TI300224
	Block #3 (D)	0.6	TI300404	TI300412	TI300424
	Target Capture Probe #2	1.0	TI300504	TI300512	TI300524
	Post Capture PCR Forward Primer	2.5	TI300604	TI300612	TI300624
	Post Capture PCR Reverse Primer	2.5	TI300704	TI300712	TI300724

Table 2. Target Capture Solution #2 **Storage condition: RT**

Box No.	Product Name	1rxn (µL)	Cat No. TC4004 (4 rxns)	Cat No. TC4012 (12 rxns)	Cat No. TC4024 (24 rxns)
TC40	Wash Buffer #1	800.0	TC400104	TC400112	TC400124
	Wash Buffer #2	500.0	TC400204	TC400212	TC400224
	Wash Buffer #3	3500.0	TC400304	TC400312	TC400324
	Hyb Buffer	24.0	TC400704	TC400712	TC400724
	Hyb Buffer Enhancer	3.0	TC400804	TC400812	TC400824

Table 3. Target Capture Solution #3 **Storage condition: -80℃**

Box No.	Product name	1rxn (µL)	Cat No. BO5004 (4 rxns)	Cat No. BO5012 (12 rxns)	Cat No. BO5024 (24 rxns)
BO50	Target Capture Probe #1 (TC#1)	6.0	YYMMPDNNN	YYMMPDNNN	YYMMPDNNN

Additional Purchasable Materials

Table 4. Additionally Provided Materials List

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

Materials Required (Not Provided)

Table 5. 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	-
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

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 Workflow

	Workflow	Time
Hybridization & Amplification	Prepare libraries and reagents for hybridization	**1 hr**
	Hybridize target capture probes with DNA library	**16 hr or 4 hr**
	Selection of target captured library by using streptavidin bead	**1.5 hr**
	Post-capture PCR amplification, Purification, and QC	**1.5 hr**
Sequencing on an Illumina platform		

Hybridization and Washing

Recommendation

We recommend that you perform a pilot test to determine if experimental conditions are suitable for hybridization.

* Prepare PCR plates or tubes suitable for your thermal cycler. After sealing the PCR plates or tubes with sealing tape or PCR caps, incubate 27 µL of nuclease-free water at 65˚C for 2 hours (lid temperature is 105˚C).
* Ensure that there is no extensive evaporation. If the evaporation volume does not exceed 3.4 µL, the hybridization can be performed with the materials you used in the pilot test.

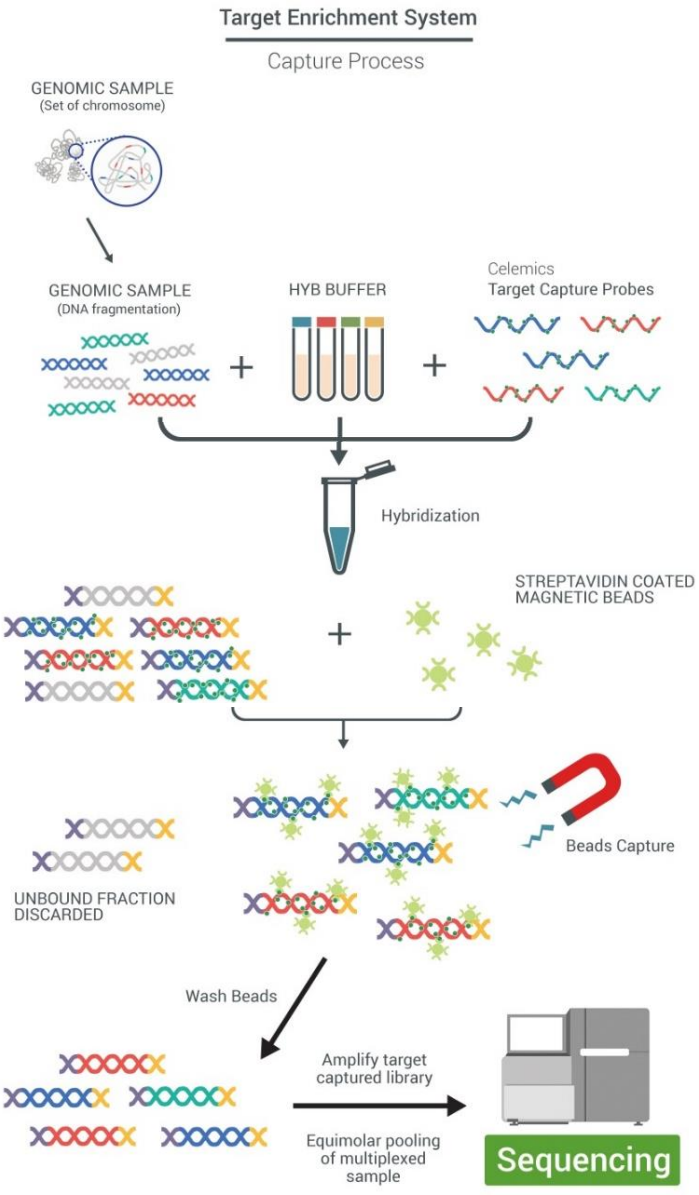

Figure 1. The Process of target captured enrichment system

Step 1. Enhanced Hybridization of the Library

[Note]

- Thaw the Target Capture Probe #1 on ice slowly.
- Do not vortex Target Capture Probe #1 (tapping or pipetting is allowed).
- Hyb Buffer should be pre-warmed at 37°C before use.
- Maintain the sample at 65°C during the whole hybridization process.

[Preparation]

Target Capture Solution Box #1	**Block #1**	Thawing on Ice Keep cool on ice (or cooling block)
	Block #2	
	Block #3 (D)	
	Target Capture Probe #2	
Target Capture Solution Box #2	**Hyb Buffer**	Pre-warmed at 37°C until the solution is clear
	Hyb Buffer Enhancer	Keep on room temperature
Target Capture Solution Box #3	**Target Capture Probe #1**	Thawing on Ice Keep cool on ice (or cooling block)

[Target Capture Probe preparation]

- For Celemics Whole Exome Sequencing kits, the target capture probe is supplied in powder form. Please check your reaction number and add the appropriate volume of nuclease-free water as described below

▶ **Target Capture Probe; resuspension of the powdered probe**

The powdered target capture probe must be dissolved with nuclease-free water before use.

Add **Nuclease-free water** (**8.4 µL** per hybridization reaction) to **Target Capture probe #1 (TC#1)** as shown below and mix well by pipetting **(Do not use Vortex Mixer)** to dissolve the reagent.

* **Keep** the eluted Target Capture probe #1 (TC#1) **on ice**.
* Once you dissolve the powder target capture probe, store the remaining probe solution at -80°C after use.
* Each pre-capture pooled library mix will be treated as 1 Hyb. reaction.

- For **12** Hyb. reaction kit
  Add **100.8 µL of nuclease-free water** into the tube.
- For **24** Hyb. reaction kit (composed of 12 reactions x 2 tubes)
  Add **100.8 µL of nuclease-free water** into each tube.

[Procedure]

Hybridization needs three reagents: **1) Block Mix with DNA library**, **2) Hybridization Buffer Mix**, **and 3) Capture Library Mix**. The three reagents are sequentially incubated in the thermal cycler and mixed for a hybridization reaction.

1. Set the program into a thermal cycler (see the details in Table 9).
2. **Preparation of Block Mix with DNA library.**
   a. Take **10 µL of pre-capture pooled sample** to a **new 8-strip tube** (or 1st column of 96-well PCR plate, see figure 2).
   b. Prepare Block Mix as shown in Table 6.

Table 6. Block Mix

Reagent	Volume for 1 Library (µL)
Block #1	2.5
Block #2	2.5
Block #3 (D)	0.6
Total Volume	**5.6**

   c. Add 5.6 µL of Block Mix to each sample tube. (The total volume is 15.6 µL)
   d. Mix well, Seal, and Spin down the 8-strip PCR tube (96-well PCR plate).
   e. Keep the PCR tube (or plate) on ice before hybridization.
3. **Preparation of Hybridization Buffer Mix.**
   a. Confirm the Hyb Buffer had pre-warmed at 37°C before use. If any precipitates are remains, keep incubating at 37°C until the solution is clear.
   b. Prepare Hyb Buffer Mix as shown in Table 7 and load it into a new 8-strip PCR tube. Mix-well gently pipetting and seal the tube completely and spin down.
      * Do not place the Hyb Buffer tube on ice. Keep the tube at RT.
      * When using 96-well plate, do not put the Hybridization Buffer Mix on 96-well plate at this step.
      * When Hyb buffer and Hyb Buffer Enhancer are mixed, it turns into a cloudy white solution, however, this does not affect your final capture product.

Table 7. Hyb Buffer Mix

Reagent	Volume for 1 capture (µL)
Hyb Buffer	24
Hyb Buffer Enhancer	3
Total Volume	**27**

4. **Preparation of Capture Library Mix.**

> *** Caution**
> - Do not vortex Target Capture Probe #1 and Capture Library Mix

a. Prepare Capture Library Mix as shown in Table 8. Mix well with gentle pipetting.

Table 8. Capture Library Mix

Reagent	Volume for 1 capture (µL)
Target Capture Probe #1 (TC#1)	6
Target Capture Probe #2	1
Total Volume	**7**

b. **Put 7 µL of Capture Library Mix** into a new 8-strip PCR tube and seal it completely. (Spin down if needed)

c. **Keep** the Capture Library Mix tube **on ice**.
* When using 96-well plate, do not put the Capture Library Mix on 96-well plate at this step.

5. Perform hybridization reaction on a thermal cycler described in Table 9.

a. Input the following program into a thermal cycler (**lid temperature: 105°C**).

Table 9. Hybridization PCR Program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	65°C	Hold

b. Put the **Block Mix with DNA library (from step 1)** tube (or plate) in the thermal cycler and start the Hybridization PCR program in Table 9.
* Important – Keep the tube on thermal cycler until 'step i'

c. Once the temperature reaches **65°C**, **put the Hyb Buffer Mix (from step 2) tube** into the thermal cycler and **incubate at 65°C for 3 minutes**.
*When using a 96-well PCR plate, transfer Hyb Buffer to an empty column (wells without Block Mix, see figure 2) and quickly seal the PCR plate.
*Important – Keep the tube on thermal cycler until 'step i'

d. After 3 minutes (incubation of Hyb Buffer at 65°C), **put the Capture Library Mix (from step 3) PCR tube** into the thermal cycler and **incubate at 65°C for 2 minutes**.
* When using a 96-well PCR plate, transfer 7 µL Capture Library Mix to an empty column (wells without Block Mix and Hyb Buffer Mix, see figure 2) and completely seal the PCR plate quickly.

e. After **step 4-d**, there should be three PCR tubes (or 3 lanes of reagents in a plate) in the thermal cycler.

IMPORTANT

Following processes must proceed very quickly and efficiently.
The high reaction temperature can cause the evaporation of reagents.
It will decrease the hybridization efficiency.

For reducing the reagents evaporation, please prepare

1) A new cap (or a new sealing film) and set the volume of a pipette (or a multi pipette) to 16 µL.
2) See **Figure 2** before proceeding. When using the 96-well PCR plate, transfer reagents into the well (or lane) containing **Capture Library Mix.**

f. **Open** the thermal cycler and remove the three PCR tube caps (or sealing film).

g. **Slowly pipette Hyb Buffer Mix up and down 2-3 times and transfer 18 µL** into **Capture Library Mix** PCR tube (or PCR plate) and mix well by pipetting up and down 2-3 times (Figure 2. ①).

h. **Transfer 15.6 µL (entire amount) of Block Mix with DNA library** into **Capture Library Mix** PCR tube (or well) that contains regents (Hyb Buffer and Capture Library Mix) and mix well by pipetting up and down 2-3 times (Figure 2. ②).

i. **Quickly seal** the PCR tube (or PCR plate) with a new cap (or a new sealing film) completely and **incubate at 65°C** as described **in Table 10.**
* In this step, remove empty PCR tubes from this step**.**

Table 10.The Reaction Temperature and Time by the Hybridization Method

Hybridization method	Temperature	Time
Standard	65°C	16 hours
One-day	65°C	4 hours

j. Make sure that the PCR tube (or PCR plate) is completely sealed and the lid temperature is 105°C.
*** The volume of the hybridization mixture** will be **30 to 32 µL,** depending on the degree of evaporation during incubation.

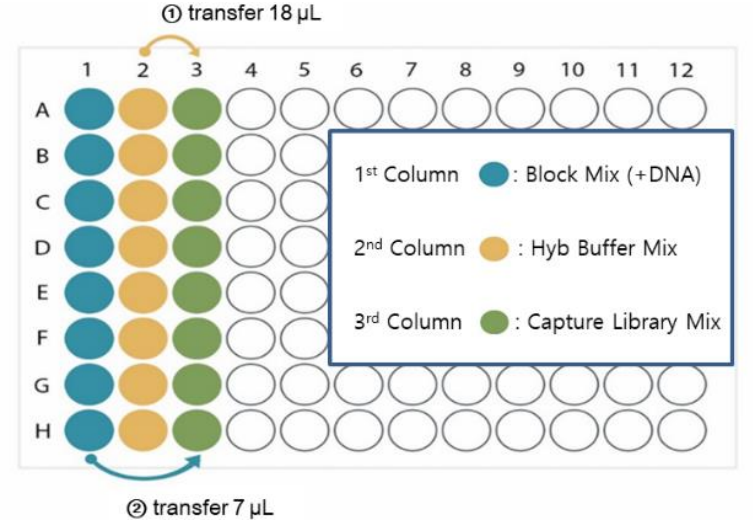

Figure 2. Mix all reagents in the 96-well PCR plate

Step 2. Preparation of CeleMag Streptavidin Bead

[Preparation]

Target Capture Solution Box #2	**Wash Buffer #1**	Keep on Room temperature
	Wash Buffer #3	Pre-heat at 70°C
	CeleMag Streptavidin bead	Store at 4°C after use.

[Procedure]

1. **Pre-heat Wash Buffer #3 at 70°C in a heat block** for the next step, **Step 3. Selection of the Target Captured Library**.
2. Vortex CeleMag Streptavidin bead on a vortex mixer to achieve a homogeneous state.
3. Prepare a new 1.5 mL LoBind tube and add 50 µL of the Streptavidin bead to the tube.
4. Wash the Streptavidin bead as follow:
    a. **Add 200 µL of Wash Buffer #1** to the tube containing 50 µL of the streptavidin bead.
    b. Mix well on the vortex mixer and spin down briefly.
    c. Put the tube in the magnetic separator until the solution is clear.
    d. Keep the tube in the magnetic separator and discard the supernatant (be careful not to touch the bead while you remove the supernatant).
    e. Repeat **steps a - d** two more times (**total 3 times**).
    f. **Add 200 µL of Wash Buffer #1** to the bead.

IMPORTANT - Proceed with the next step right away.

Step 3. Selection of the Target Captured Library

[Preparation]

Target Capture Solution Box #2	**Wash Buffer #2**	Keep on Room temperature
	Wash Buffer #3	Pre-heat at 70°C

[Procedure]

1. Keep the sample-hybridization mixture from **Step 1. Enhanced Hybridization of the Library** in a thermal cycler at 65°C.
2. Gently unseal the PCR tube (or PCR plate) in the thermal cycler and **immediately transfer the sample-hybridization mixture** to the bead solution (**from Step 2. Preparation of CeleMag Streptavidin Bead**).
3. Invert the tube 3-5 times to mix them thoroughly and **rotate the sample** with a rotator (25 rpm) for 30 minutes at room temperature.
   * Important - Do not vortex the tube vigorously, as it may damage the Target Capture Probe.
4. Spin down briefly.
5. Put the tube in a magnetic separator until the solution is clear. **Carefully remove the supernatant**.
6. Take the tube out of the magnetic separator and **add 500 µL (180 µL for PCR tube or 96-well plate) of Wash Buffer #2** to each tube. Mix well on a vortex mixer and pulse-spin down.
7. Incubate the sample for **15 minutes** at room temperature.
8. Briefly mix the sample on the vortex mixer and pulse-spin down. Put the tube in the magnetic separator until the solution is clear and **remove the supernatant**.
9. Rinse the bead with Wash Buffer #3 (pre-heated **from 2.2 Preparation of Magnetic Bead**).
   a. Add **500 µL (180 µL for PCR tube or 96-well plate) of pre-heated Wash Buffer #3** to each tube and mix well on a vortex mixer. Spin down briefly.
   b. Incubate the solution for 10 minutes at **70°C** in a heat block.
   c. Briefly mix the sample on the vortex mixer and pulse-spin down.
   d. Put the tube in the magnetic separator until the solution is clear. **Carefully remove the supernatant**.
   e. Repeat steps a-d twice with Wash Buffer #3.
      * Important – Rinse the bead with Wash Buffer #3 3 times.

f. Spin down the tube for 3-5 seconds and put the tube in the magnetic separator to remove residue.
* Important - Wash Buffer #3 must be completely removed.

g. **Add 30 µL of nuclease-free water** to each tube and mix well on a vortex mixer. Spin down briefly.

h. **On-Bead Capture Pool** is prepared.

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

Amplification of Target Captured Library

Step 1. Amplification of the Captured Library

[Preparation]

CLM Polymerase	**CLM Polymerase**	Keep cool on ice (or cooling block)
Target Capture Solution Box #1	**Post Capture PCR Forward Primer**	Thawing on ice
	Post Capture PCR Reverse Primer	Keep cool on ice (or cooling block)

[Procedure]

1. Set the program into a thermal cycler (see the details in Table 13).
2. Prepare new 1.5 mL tube and make PCR Master Mix (CLM Polymerase, Nuclease-free water, Post Capture PCR Forward Primer, Post Capture PCR Reverse Primer) as described in Table 11 (add 5% of the additional amount of reagents for multiple libraries).

Table 11. Composition of PCR Master Mix

Reagent	Volume for 1 Library (µL)
CLM Polymerase	25
Nuclease-free water	5
Post Capture PCR Forward Primer	2.5
Post Capture PCR Reverse Primer	2.5
Total Volume	**35**

3. Add 35 µL of the PCR master mix to each PCR tube.
   * Important – Combine all reagents on ice.
4. Thoroughly mix the **On-Bead Capture Pool** to a homogeneous state and **add 15 µL of the On-Bead Capture Pool** to the PCR tube.

Table 12. Composition of PCR Mix

Reagent	Volume for 1 Library (µL)
PCR Master Mix	35
On-Bead Capture Pool	15
Total Volume	**50**

5. Mix well and spin down briefly.
6. Run the thermal cycler with the PCR program shown in Table 13. (see the number of PCR cycles in Table 14).

Table 13. PCR program

Step	Temperature	Time
Step 1	98°C	45 seconds
Step 2	98°C	15 seconds
Step 3	60°C	30 seconds
Step 4	72°C	1 minute
Step 5		Repeat step 2 to step 4 (Total PCR cycles, refer to Table 14.)
Step 6	72°C	10 minutes
Step 7	4°C	Hold

Table 14. The Number of PCR Cycles

Target Size	Total Cycles
Target Size ≥ 1 Mb	14

IMPORTANT - Proceed with the next step right away.

Step 2. Purification of Sample Using 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 90 µL CeleMag Clean-up Bead** to a new 1.5 ml LoBind Tube and **add 50 µL of amplified captured DNA library** 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 15. Volumes of CeleMag Clean-up Bead (1.8x)

Reagent	Volume Ratio (Bead : Sample)
CeleMag Clean-up Bead	**1.8 : 1** (e.g., 90 µL : 50 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 32 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 (30 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 3. Assess Quality and Quantity of Each Library

Check the quality and quantity of the captured library with available instruments.

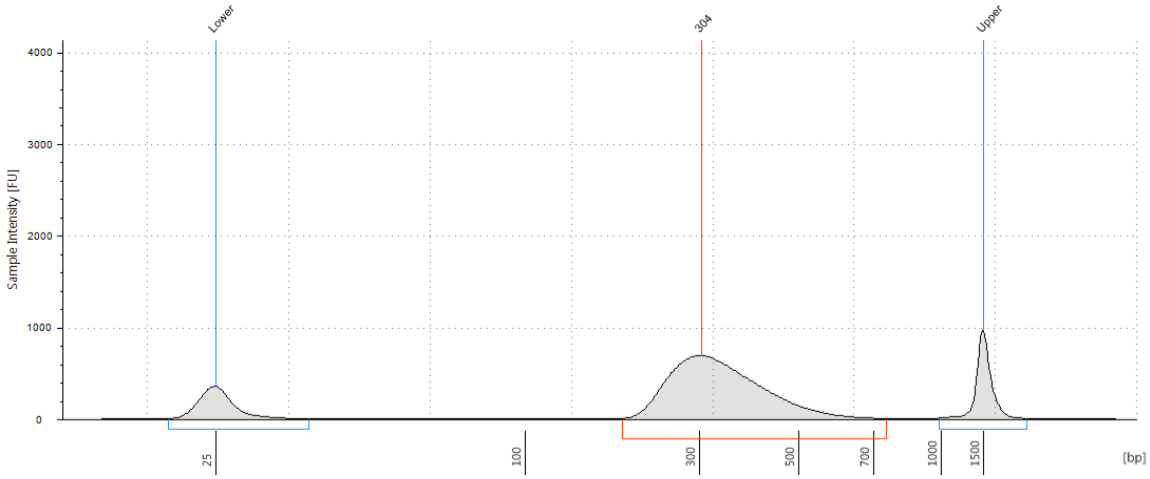

Figure 3. The example electrophoresis diagram of the captured library.

- Make sure that the size of the **amplified captured DNA library** is **120-150 bp larger** than the initial fragmented DNA.

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Sequencing Library Pooling

In this step, all target captured pre-pooled samples are pooled in one tube.
The index of each sample must not overlap each other.
Consider target size, the quantity of data needed, and the concentration of every sample.

1. Create the spreadsheet as shown below in Table 16.
2. Enter values of each sample in columns 1, 2, 3, 4, 5, and 6 of the spreadsheet.
3. Calculate Column 7 by dividing 100* (100 ng is recommended. Please refer to the note at the bottom of Table 16 for more information) by the concentration of each pre-capture pooled sample indicated in Column 5.
4. Mix each pool with calculated volumes
5. After pooling, perform sequencing according to the running protocol of the sequencing equipment.

Table 16. Example of a Spreadsheet for Sample Pooling

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
Sample name	**Containing samples**	**i5 Index**	**i7 Index**	**Conc (ng/µL)**	**Volume of Sample (µL)**	**Vol. for Pooling (µL)**	**Needed Data (Gb)**
Pre-capture pool 1	Pool1-A	501	701	10.5	30	9.52	5
	Pool1-B	502	702				
	Pool1-C	503	703				
	Pool1-D	504	704				
Pre-capture pool 2	Pool2-E	501	705	8.85	30	11.3	5
	Pool2-F	502	706				
	Pool2-G	503	707				
	Pool2-H	504	708				
Pre-capture pool 3	Pool3-I	505	701	13.2	30	7.58	5
	Pool3-J	506	702				
	Pool3-K	507	703				
	Pool3-L	508	704				
Pre-capture pool 4	Pool4-M	505	705	19.3	30	5.18	5
	Pool4-N	506	706				
	Pool4-O	507	707				
	Pool4-P	508	708				

* The particular example in Table 16 indicates that the volume for pooling (Column 7) is calculated by dividing 100 ng by each Pre-capture pool concentration (Column 5). However, 100 ng is a recommendation and the ng(amount) can be changed according to the user's convenience.