CELEMICS

Kit User Manual

Target Enrichment

Enhanced Hybridization

Illumina Platform

Version 2.0

Research Use Only

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Materials Provided

Table 1. Target Capture Solution #1

Storage condition: -20℃

Box No.	Product Name	1 rxn (µL)	Cat No. TC3016 (16 rxn)	Cat No. TC3024 (24 rxn)	Cat No. TC3048 (48 rxn)	Cat No. TC3096 (96 rxn)
TC30	Block #1	2.5	TP300116	TP300124	TP300148	TP300196
	Block #2	2.5	TP300216	TP300224	TP300248	TP300296
	Block #3 - Single (Single Index)	0.6	TI300316	TI300324	TI300348	TI300396
	Block #3 - Dual (Dual Index)	0.6	TI300416	TI300424	TI300448	TI300496
	Target Capture Probe #2	1.0	TP300516	TP300524	TP300548	TP300596
	Post Capture PCR Forward Primer	2.5	TC300616	TC300624	TC300648	TC300696
	Post Capture PCR Reverse Primer	2.5	TC300716	TC300724	TC300748	TC300796

Table 2. Target Capture Solution #2

Storage condition: RT

Box No.	Product Name	1rxn (µL)	Cat No. TC4016 (16rxn)	Cat No. TC4024 (24rxn)	Cat No. TC4048 (48rxn)	Cat No. TC4096 (96rxn)
TC40	Wash Buffer #1	800.0	TC400116	TC400124	TC400148	TC400196
	Wash Buffer #2	500.0	TC400216	TC400224	TC400248	TC400296
	Wash Buffer #3	3500.0	TC400316	TC400324	TC400348	TC400396
	Hyb Buffer	25.0	TC400716	TC400724	TC400748	TC400796
	Hyb Buffer Enhancer	2.5	TC400816	TC400824	TC400848	TC400896

Table 3. Target Capture Solution #3

Storage condition: -80℃

Box No.	Product name	1rxn (µL)	Cat No. BO5016 (16rxn)	Cat No. BO5024 (24rxn)	Cat No. BO5048 (48rxn)	Cat No. BO5096 (96rxn)
TC50	Target Capture Probe #1 (TC#1)	6.0	YYMMPDNNN	YYMMPDNNN	YYMMPDNNN	YYMMPDNNN

Additional Purchasable Materials

Table 4. Additional Purchasable 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

Product Name	Suggested Supplier	
Reagents & Consumables		
Nuclease-free water (not DEPC-treated)	General lab supplier	-
Universal Human RNA	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
Vacuum concentrator	General lab supplier	-
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. DNA extracted from FFPE tissue is highly variable in quality due to chemical damage and fragmentation, and often is available only in small amounts. There is a possibility that these steps will not lead to success with DNA extracted from FFPE samples. 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

Hybridization Target Enrichment Workflow	Time
Prepare libraries and reagents for hybridization	1 hour
Hybridize capture probes with DNA library	4-16 hours
Bind hybridized targets to streptavidin beads	1.5 hour
Post-capture PCR amplify, purify, and perform QC	1.5 hour
STOPPING POINT	
Sequencing on an Illumina platform	-

Hybridization and Washing

Step 1. Hybridization of the Library 9

Step 2. Preparation Magnetic Beads (CeleMag Streptavidin Beads Washing) 14

Step 3. Selection of the Target Captured Library 15

* This step is for the hybridization of capture probe and sample library.

Recommendation

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

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* Prepare PCR plates or tubes suitable for a thermal cycler. After sealing the PCR plates or tubes with sealing tape or PCR caps, incubate 27 μL of nuclease-free water 65°C for 2 hours (lid temperature is 105°C).

* Ensure that there is no extensive evaporation. In the event that the volume of evaporation doesn't exceed 3.4 μL, the hybridization experiment can be performed with the materials you used in the pilot test.

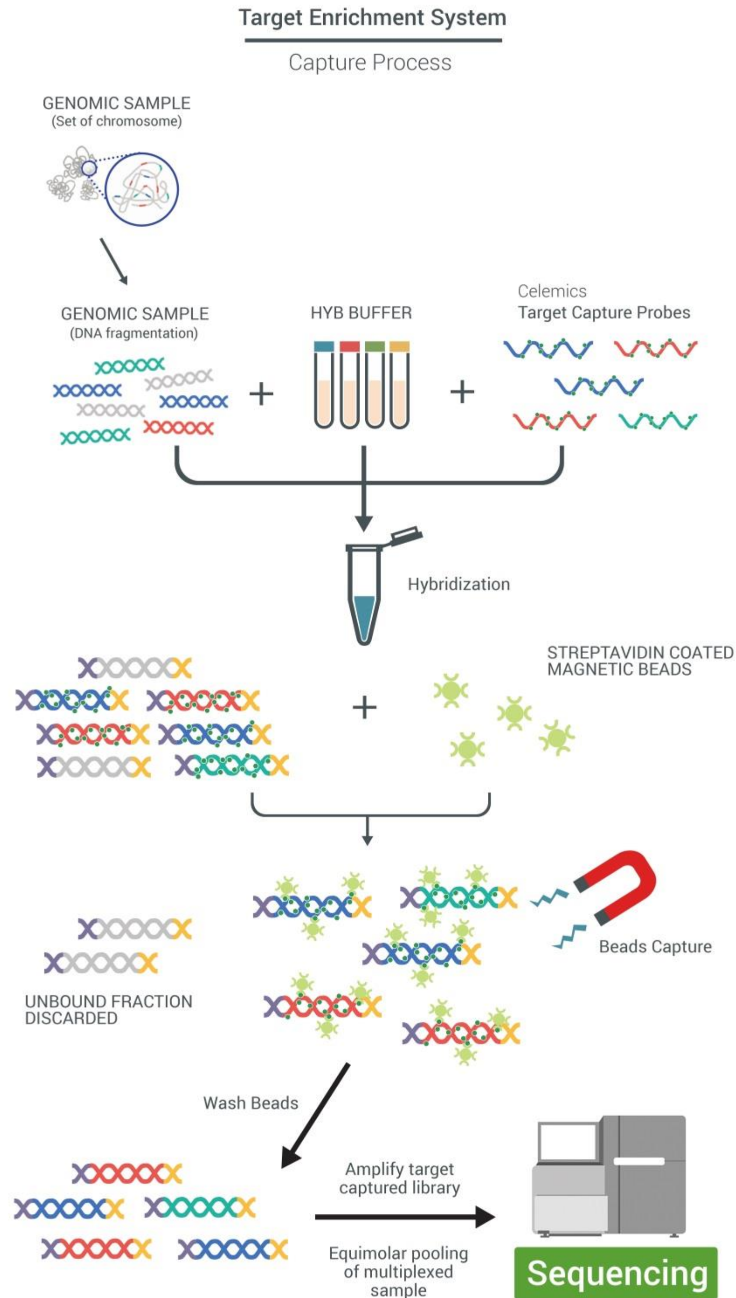

Figure 1. The Process of target captured enrichment system

Step 1. Hybridization of the Library

[Note]

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

[Preparation]

- Vacuum concentrator (e.g. SpeedVac Vacuum Concentrator)
- Target Capture Solution Box #1
  → Block #1, Block #2, Block #3, Target Capture Probe #2
- Target Capture Solution Box #2
  → Hyb Buffer, Hyb Buffer Enhancer
  * Important - Hyb Buffer should be pre-warmed at 37°C before use.
- Target Capture Solution Box #3
  → Target capture probe #1

[Pre-capture pooling guide]

If the samples need to be pooled, refer to the Table 6 below based on the amount of 2.4µg and proceed with pooling.

* If total amount is less than 2.4 µg, pool each sample to the maximum amount with same amount.

Table 6. Pooling guide according to the number of samples

Number of samples	1 sample amount (ng)	Total amount after pooling (ng)
4	600	2400
8	300	2400
12	200	2400

[Procedure]

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

1. Preparation of Block Mix with amplified adapter-ligated DNA library.

   a. The required amount of DNA for hybridization is **500 or 750 ng**.

      * **Pooled sample**: Needed amount for hybridization refer to the Table 6.

b. Based on the concentration of the sample from Pre-PCR, add the volume of **500 or 750 ng or needed amount (pooled sample, refer to the Table 6) of each amplified DNA library** in a new 1.5 mL Micro tube.

c. Prepare Block Mix as shown in Table 7.

Table 7. Block Mix

Reagent	Volume for 1 Library (µL)
Block #1	2.5
Block #2	2.5
Block #3 (Single or Dual)	0.6
Total volume	5.6

d. **Add 5.6 µL of Block Mix** to each sample tube.

e. **Dry** the amplified DNA library and Block Mix using a vacuum concentrator at ≤ 45°C.

f. **Add 7 µL of nuclease-free water** in the tube (with completely dried sample).

g. Resuspend the dried amplified DNA library and Block mix. Spin down the tube.

h. **Transfer the entire Block Mix with DNA library (7 µL)** into a new 8 strip PCR tube (or 96-well PCR plate) and **seal the PCR tube (or plate) completely** and spin down.

i. Keep the PCR tube (or plate) **on ice** before hybridization.

2. Preparation of Hybridization Buffer Mix.

a. Hyb Buffer should be pre-warmed at 37°C before use.

* This step allows any precipitation to be removed in Hyb Buffer. Make sure Hyb Buffer is homogeneous status without any precipitation by pre-warming.

b. Prepare Hyb Buffer Mix and load into a new 8 strip PCR tube (or 96-well PCR plate) as shown in Table 8. Mix-well gently pipetting and **seal the PCR tube (or plate)** completely and spin down.

Table 8. Hyb Buffer Mix

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

* Do not place the Hyb Buffer Mix tube on ice. Keep the tube at RT.

* When Hyb buffer and Hyb Buffer Enhancer are mixed, it turns into a cloudy white solution; however, this does not affect to your final capture product.

3. Preparation of Capture Library Mix.

Caution
* Thaw Target Capture Probe #1 on ice slowly.
* Do not vortex Target Capture Probe #1 and Capture Library Mix (tapping and pipetting are allowed).

[Powder-probe elution]

Powder probe must be dissolved with nuclease-free water before use.

Add **Nuclease-free water** (**8.4 µL** per reaction) to **Target Capture probe #1 (TC#1)** as shown below and mix well by pipetting to dissolve the reagent.

* **Keep** the eluted Target Capture probe #1 (TC#1) **on ice**.

* 1 Hyb. reaction means 1 pre-capture pooling of 8 sample libraries.

- For **1** Hyb. reaction kit (sample test)
  Add **8.4 µL of nuclease-free water** into the tube.
- For **2** Hyb. reaction kit [16 sample kit]
  Add **16.8 µL of nuclease-free water** into the tube.
- For **8** Hyb. reaction kit [64 sample kit]
  Add **67.2 µL of nuclease-free water** into the tube.
- For **12** Hyb. reaction kit [96 sample kit]
  Add **100.8 µL of nuclease-free water** into the tube.
- For **16** Hyb. reaction kit [128 sample kit]
  Add **134.4 µL of nuclease-free water** into the tube.
- For **48** Hyb. reaction kit [384 sample kit] (composed of 16 reaction x 3 tube)
  Add **134.4 µL of nuclease-free water** into each tube.
- For **96** Hyb. reaction kit [768 sample kit] (composed of 16 reaction x 6 tube)
  Add **134.4 µL of nuclease-free water** into each tube.

[Solution-probe]

Thaw the Target Capture Probe #1 on ice slowly and keep the probe (Target Capture probe #1) on ice before use.

* Store the remaining probe solution at -80°C after use.

a. Prepare Capture Library Mix as shown in Table 9. Mix-well by pipetting.

Table 9. 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 (or 96-well PCR plate) and **seal the PCR tube (or plate)** completely and spin down.

c. **Keep** the Capture Library Mix tube **on ice**.

4. Perform hybridization reaction on a thermal cycler.

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

Table 10. 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 tube (or plate) in the thermal cycler and start the Hybridization PCR program.

* After this step, **do not remove the tube** from the thermal cycler until '**step i**'.

c. Once the temperature of the thermal cycler reaches **65°C**, **put the Hyb Buffer Mix tube** into the thermal cycler and **incubate at 65°C for 3 minutes**.

*When using a 96-well PCR plate, transfer 27 μL Hyb Buffer Mix to an empty column (wells without Block Mix) and completely seal the PCR plate quickly.

* After this step, do not remove the tube from the thermal cycler until '**step i**'.

d. After step c (incubation of Hyb Buffer Mix at 65°C), **put the Capture Library Mix 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 empty column (wells without Block Mix and Hyb Buffer Mix) and completely seal the PCR plate quickly.

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

* Subsequent processes must proceed very quickly and efficiently from the moment the cap (or plate sealing film) is opened because high temperature causes evaporation of reagents.

* **Prepare a new cap (or a new sealing film)** and set the volume of a pipette (or a multi pipette) to **18 µL**.

*When using the 96-well PCR plate, transfer reagents into the well (or lane) containing Captured Library Mix. See **Figure 2** before proceeding.

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** of the PCR tube (or PCR plate) and mix well by pipetting up and down 2-3 times (Figure 2. ①).

h. **Transfer 7 µL (entire amount) of Block Mix with DNA library** into the PCR tube (or well) that contains regents (Hyb Buffer Mix 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 for 4-16 hours.**

*** In this step, remove empty PCR tubes from this step.**

j. Make sure that the PCR tube (or PCR plate) is completely sealed and the lid temperature is 105°C.

*** The volume of hybridization mixture** will be **30 to 32µL,** depending on the degree of evaporation during incubation.

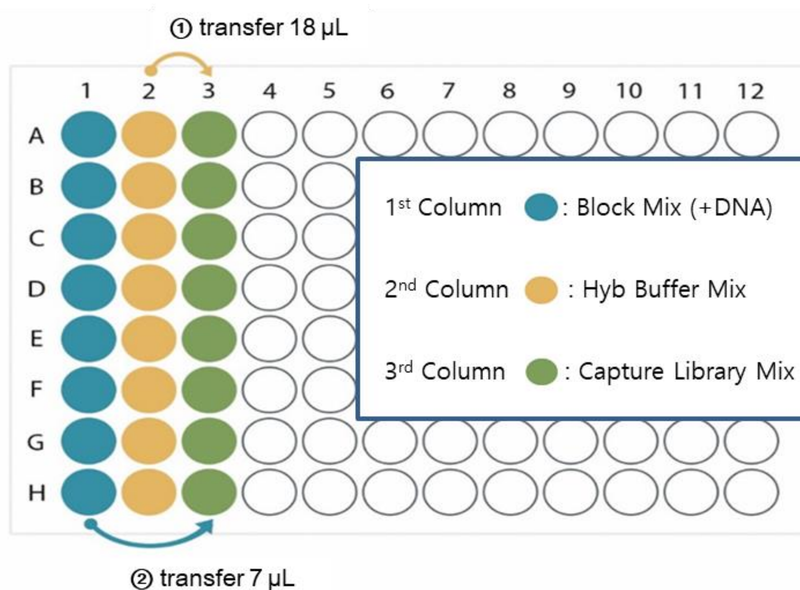

Figure 2. Mix all reagents in the 96-well PCR plate

Step 2. Preparation of Magnetic Beads (CeleMag Streptavidin Beads Washing)

[Preparation]

- Target Capture Solution Box #2

  → Wash Buffer #1, Wash Buffer #2, Wash Buffer #3

- CeleMag Streptavidin Beads (CMSB)
- Nuclease-free water

[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 Beads on a vortexer to achieve a homogeneous state.
3. Prepare a new 1.5 mL LoBind tube and add 50 μL of CeleMag Streptavidin Beads to the tube.
4. Wash CeleMag Streptavidin Beads as follows:
   a. **Add 200 μL of Wash Buffer #1** to the tube containing 50 μL of CeleMag Streptavidin Beads.
   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 beads while you remove the supernatant).
   e. Repeat **step a - d** two more times (**total 3 times**).
   f. **Add 200 μL of Wash Buffer #1** to the beads.

Step 3. Selection of the Target Captured Library

[Procedure]

1. Keep the sample-hybridization mixture from **step 1. 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.
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 RNA 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 vortexer and pulse-spin down.
7. Incubate the sample for **15 minutes** at room temperature.
8. Briefly mix the sample on the vortexer and pulse-spin down. Put the tube in the magnetic separator until the solution is clear and **remove the supernatant**.
9. Rinse the beads with Wash Buffer #3 (pre-heated from Step 2. Preparation of Magnetic Beads).
   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 vortexer and pulse-spin down.
   d. Put the tube in the magnetic separator until the solution is clear. **Carefully remove the supernatant**.
   e. Repeat step a-d twice with Wash Buffer #3.

      * Important – The total wash number with Wash Buffer #3 is 3.
   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-beads captured library is prepared.

Amplification of Target Captured Library

Step 1. Amplification of the Captured Library 17

Step 2. Purification of Sample Using Clean-up Beads 19

Step 3. Assess Quality and Quantity of Each Library..20

Step 1. Amplification of the Captured Library

[Preparation]

- Target Capture Solution Box #1
  → Post Capture PCR Forward Primer, Post Capture PCR Reverse Primer
- CLM Polymerase
- 0.2 mL PCR tubes and caps

*Important - In this step, half of on-beads captured DNA will be amplified.
The remainder should be stored at -20°C.

[Procedure]

1. For **1** library
   a. Prepare a new 0.2 mL PCR tube and PCR mix, as described in Table 11.
   b. **Add 35 µL of the PCR mix** to each PCR tube.
   c. Thoroughly mix on-bead captured library to a homogeneous state and **add 15 µL of on-beads captured library** to PCR tube.
   d. Mix PCR mix and on-beads captured library well.

      * Important – Combining all reagents on ice.

   e. Run the thermal cycler with the PCR program shown in Table 12 (see the number of PCR cycles in Table 13).
2. For **multiple** libraries
   a. Prepare new 0.2 mL PCR tubes and PCR mix as described in Table 11 (add **5% additional amount** of reagents for multiple libraries).
   b. **Add 35 µL of the PCR mix** in each PCR tube.
   c. Thoroughly mix on-bead captured library to a homogeneous state and **add 15 µL of on-beads captured library** to PCR tube.
   d. Mix well and spin down briefly.

      * Important – Combining all reagents on ice.

   e. Run the thermal cycler with the PCR program shown in Table 12 (see the number of PCR cycles in Table 13).

Table 11. Composition of PCR mix

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

Table 12. 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, see in Table 13)
Step 6	72°C	10 minutes
Step 7	4°C	Hold

Table 13. PCR Cycle

Target Size	Total Cycles	Pooling sample (Optional)
Targets Size < 1 Mb	16	9 Cycles
Targets Size ≥ 1 Mb	14	

Important

Proceed with the next step right away.

Step 2. 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 90 μL CeleMag Clean-up Beads** to a new 1.5 mL LoBind tube and **add 50 μL amplified captured DNA library** 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 (1.8X)

Reagent	Volume Ratio (Beads : Sample)
CeleMag Clean-up Beads	**1.8 : 1** 90 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 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 solution is clear.
12. **Transfer the supernatant (30 μL)** to a new 1.5 mL LoBind Tube.

Stop Point

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

Step 3. Assess Quality and Quantity of Each Library

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

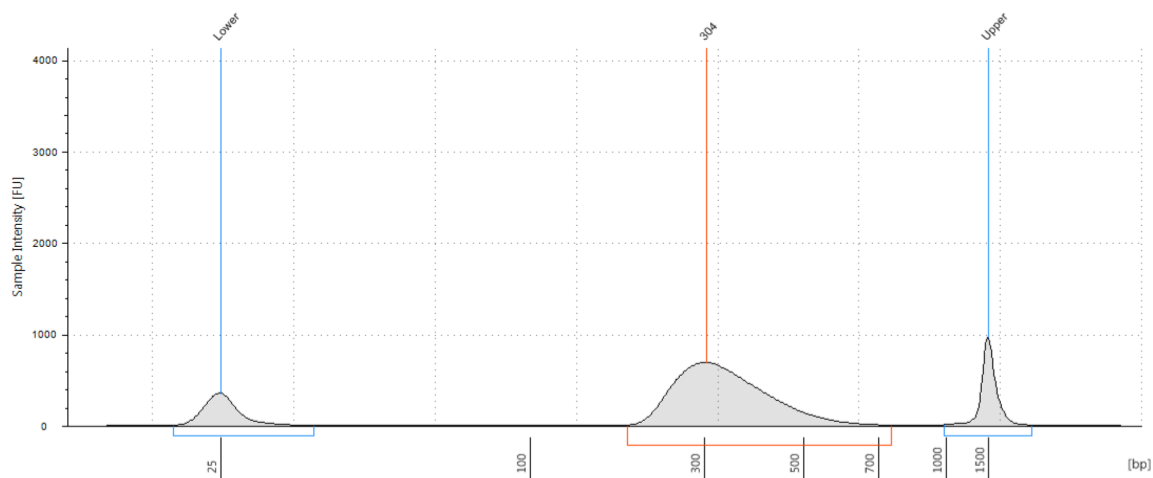

Figure 3. The electrophoresis diagram shows a peak in the region of 300 bp of the captured library.

- Make sure that the size of **amplified captured DNA library** is **120-150 bp larger** than the initial fragmented RNA (check the peak size close to the peak size shown in the 'Assess Quality and Quantity of Each Index-tagged Library' step).

Sequencing Library Pooling

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Sequencing Library Pooling

In this step, all target captured samples are pooled.

* Caution - It is important to maintain a unique index for each sample.

The index of each sample must not overlap each other.

Consider target size, quantity of data needed and concentration of every sample.

1. Create the spread sheet as shown below in Table 15.
2. Enter values of each sample or formula in column 1, 2, 3, 4, 5 and 8 of the spread sheet.
3. Select one standard sample, such as sample No. 5 by considering the value of columns 5 and 8 in Table 15 (in case of pooling every sample in equimolar, select a standard sample whose concentration after capture is the lowest).
4. Calculate 'Amount for Sequencing (amol)' of each sample and fill in the value in column 6.
5. Calculate the volume (µL) of each sample and fill in the value in column 7.

Table 15. Example of spread sheet for sample pooling

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
Sample	**Index**	**Conc. After Post PCR (pmol/L)**	**Volume of Sample (µL)**	**Total Amount (amol)**	**Amount for Sequencing (amol)**	**Vol. for Sequencing (µL)**	**Needed Data (Gb)**
A	XXXXXX	B	C	D=B*C	E	F=E/B	G
1	ATCACG	648	30	19440	7760	12.0	2
2	CGATGT	292	30	8760	7760	26.6	2
3	TTAGGC	7190	30	215700	5820	0.8	1.5
4	TGACCA	725	30	21750	5820	8.0	1.5
5	ACAGTG	388	30	11640	3880	10.0	1
6	GCCAAT	540	30	16200	3880	7.2	1
7	CAGATC	1230	30	36900	1940	1.6	0.5
8	ACTTGA	675	30	20250	1940	2.9	0.5