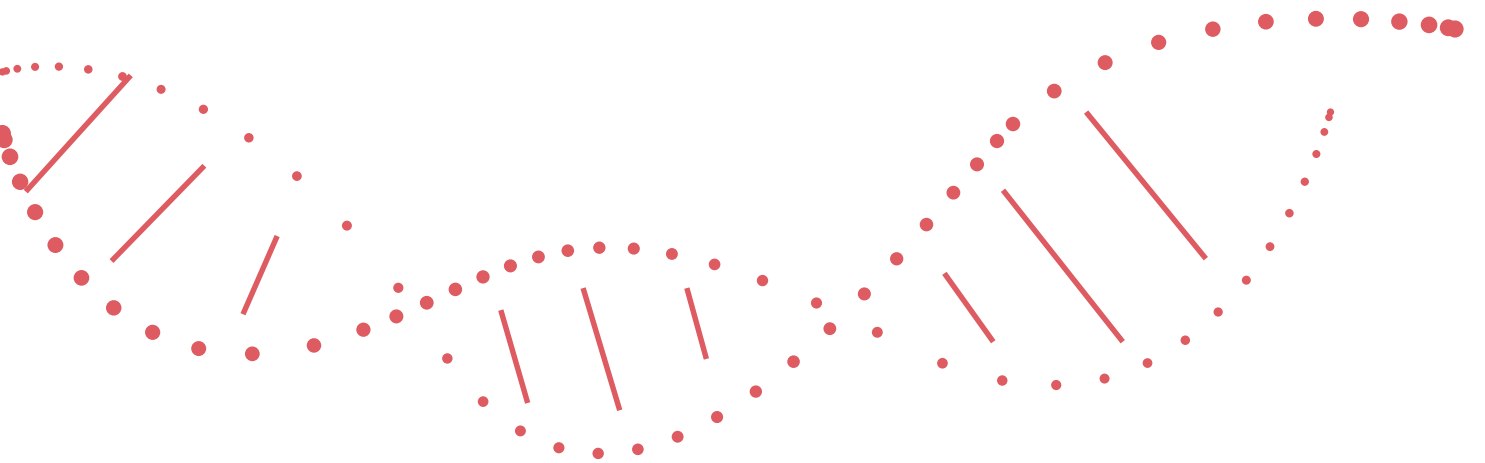
GeneMind

FASTASeq S

Sequencing Kit User Manual



GeneMind Biosciences Co., Ltd.

This user manual applies to the following kits:

Catalogue Number	Product Name	Product Specification
S000515	FASTASeq S Sequencing Kit V1.0 (FCL-D SE100-D)	FCL-D SE100-D
S000516	FASTASeq S Sequencing Kit V1.0 (FCL 100cycles)	FCL 100cycles
S000517	FASTASeq S Sequencing Kit V1.0 (FCL 300cycles)	FCL 300cycles
S000518	FASTASeq S Sequencing Kit V1.0 (FCM-D SE100-D)	FCM-D SE100-D
S000519	FASTASeq S Sequencing Kit V1.0 (FCM 100cycles)	FCM 100cycles
S000520	FASTASeq S Sequencing Kit V1.0 (FCM 300cycles)	FCM 300cycles

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Version History

Version	Revision Date
V1.0	July 10, 2025

Product Description

The use manual are applicable to the sequencing operations with the kits specified above and used with the FASTASeq S sequencing system only, covering the cartridge content, cartridge preparation guidelines for sequencing reagents and flow cells, preparation of libraries to be sequenced during the experiment, and starting the sequencing experiment, etc.

Intended Use

The product is a set of reagents and consumables for sequencing reaction intended to acquire nucleic acid sequence information from tested samples. It is designed for exclusively with the FASTASeq S sequencing system.
The product is used for research only.

Sequencing Technology

This product is used for sequencing based on the principle of Sequencing By Synthesis (SBS). The adapter sequences of the sample library hybridize with the complementary specific probes pre-fixed on the sequencing flow cells, provided within sequencing cartridges, for surface amplification to form molecular clusters. The addition of sequencing primers and the use of fluorescently-labelled reversible termination bases and the polymerase reaction system can achieve the synthesis and extension of only one base in each cycle of polymerization reaction; the fluorescent label is excited by a laser of a specific wavelength and imaged through a microscope; then the fluorophores are removed by chemical shear; then the next round of reaction proceeds. The process of “polymerization-acquisition of images-excision” is repeated. Each round generates images processed by specific analysis software, and finally, the nucleic acid sequence information of the tested sample is obtained.

Main Components

This product consists of two parts: box 1 of 2, box 2 of 2.
It is mainly composed of sequencing reagents cartridge (Box 1 of 2), library diluenting reagent (Box 1 of 2), library neutralizing reagent (Box 1 of 2), hybridization reagent (Box 1 of 2), 2mL centrifuge tube (Box 1 of 2), 2mL centrifuge cap (Box 1 of 2),and sequencing flow cell (Box 2 of 2).

Packing box	Component	Quantity
Box 1 of 2 (Sequencing Reagent)	Sequencing reagents cartridge	1 pcs
	Library Neutralizing Reagent	1 vial
	Library Diluenting Reagent	1 vial
	Hybridization Reagent	1 vial
	2mL Centrifuge Tube	1 pcs
	2mL Centrifuge Cap	1 pcs
Box 2 of 2 (Sequencing Flow Cell)	Sequencing Flow Cell	1 pcs

⚠ Note

- The specification for of “FCL 100cycles, FCL 300cycles, FCM 100 cycles and FCM 300 cycles” can be used for single-end and paired-end sequencing; The specification of “FCL-D SE100-D and FCM-D SE100-D” only can be used for single-end sequencing;
- Mixing of components from different batches of kits is strictly forbidden; each kit can be used for one time only.

Main Equipment And Materials Required for Running Sequencing Experiment But Not Provided Within The Cartridge

- Equipment: Mini centrifuge, vortex shaker, pipettes of various volume specifications, ice machine, freezer -20°C, 2-8°C refrigerator, micro-plate centrifuge, FASTASeq S sequencing system, etc.
- Materials: tips, ice box, powder-free rubber gloves, dust-free lens wiping paper and lint-free cloth, 2 mL centrifuge tube, compressed gas duster.
- Reagents: ultra-pure nuclease-free water, absolute ethanol, 0.2 M NaOH solution, 75% ethanol solution, Tween 20, NaClO solution.

Storage Conditions and Validity Period

- The box 1 of 2 is stored at -30°C to -15°C, the box 2 of 2 is stored at 2°C to 8°C.
- The validity period of this product are 9 months under the specified storage conditions. See the labels on the packing box for the production date and the expiry date.
- The box 1 of 2 should be stored at 2°C to 8°C after thawing, and used for sequencing within 24 hours.

Applicable Instrument

Product Name: FASTASeq S Sequencing System
Product Model : FASTASeq S

Sample Requirement

The recommend concentration is not less than 4 nM and the volume should be no less than 5 µL.

Sequencing Experiment

1.Preparation of sequencing reagent

- 1.1 Take out the box 1 of 2 from the storage environment (-30°C to -15°C), and box 2 of 2 (Sequencing Flow Cell) from the storage (2°C to 8°C), and discard the outer package.
- 1.2 Thaw the sequencing reagents cartridge from box 1 of 2 in the water tank filled with clean water at the temperature of 20-25°C, with the water level at approximately 2/3 of the height of the sequencing cartridge. Remove the waste liquid bottle of sequencing reagents cartridge before thawing the reagents.

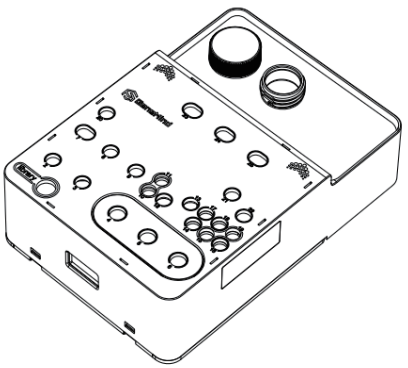


Figure 1 Sequencing Reagent Cartridge

- 1.3 Estimated thawing time of the sequencing cartridge components in the water tank.

Product Specification	Thawing Reference Time (Sequencing Reagents Cartridge)	Thawing Reference Time (Library Neutralizing Reagent, Library Diluenting Reagent and Hybridization Reagent)
FCL-D SE100-D FCM-D SE100-D	About 0.5 hours	About 10 minutes
FCL 100cycles FCM 100cycles	About 0.5 hour	
FCL 300cycles FCM 300cycles	About 1 hours	

- 1.4 Use the laboratory dust-free lens wiping paper to dry the sequence reagent cartridge surface and the aluminum sealing film of each reagent hole, and well mix the cartridge. Mixed operation: complete 180° upside down once in 1 second, and repeat the operation 15-20 times, to ensure that the reagent is thoroughly mixed . Before mixing the reagents, please check and confirm that all reagent components have completely thawed. If ice lumps are found, continue to thaw them in 20~25°C of clear water until fully thawed before proceeding with the mixing operation. Gently tap the reagent cartridge on the workbench to reduce bubbles at the bottom and ensure that individual tubes contain no noticeable bubbles. Put the waste liquid bottle to its original position in the reagent cartridge after mixing.
- 1.5 The sequencing reagents cartridge should be placed at 2-8°C and avoid light for storage before the sequencing experiment.

⚠ Note

After thawing of the sequencing reagents cartridge, the sequencing experiment should be started within 24 hours. There shall be no obvious water droplets on the RFID tag, otherwise the identification of RFID may be affected.

2.Preparation of sequencing flow cell

- 2.1 Take out box 2 of 2 (Sequencing Flow Cell) from the storage environment (2-8°C), and allow to stand for at least 30 minutes but not more than 12 hours at 20-25°C;
- 2.2 Open the aluminum foil bag and blister box. If you see small particles on the glass surface or back aluminum plate of the flow cell, blow them away with a nitrogen tank.

⚠ Note

Before preparing the flow cell, please change to new powder-free gloves to avoid contaminating the flow cell glass surface.

- 2.3 Carefully inspect the top and bottom surfaces of the flow cell for any damage (such as cracks or notches). If there are any notches or cracks, please consult technical support for handling suggestions. Carefully check if there are any particles around the flow cell gasket. If so, gently wipe them off with lens paper or blow the surface clean with a dust can.

- 2.4. If the stain is still not removed on the top or bottom surfaces of the flow cell, please use a dust-free lens cleaning paper folded in half three times and dip in a small amount of absolute ethanol. The last crease of the lens cleaning paper should face upwards. Press the middle-upper part of the lens cleaning paper with the pad of your finger (Figure 2). Then, wipe the stained area of the flow cell evenly in the same direction with force. Fold the lens cleaning paper every 1-2 times and wipe the next area upwards with the last crease (change the lens cleaning paper as needed) until there are no residues or marks on the surface of the flow cell.

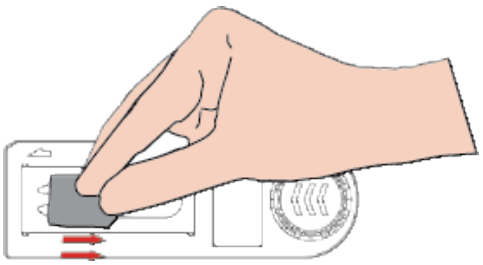


Figure 2 Wipe Flow Cell in One Direction

- 2.5 Check the holder platform of the instrument. If there are stains on the holder platform, take a piece of dust-free lens cleaning paper folded in half three times and dip in a small amount of absolute ethanol. Wipe the platform as described procedure in 2.3 until there is no residue on the holder platform.
- 2.6 Check the top and bottom surfaces of the flow cell under the light to ensure there are no visible stains, water stains, fingerprints, dust or other impurities remaining. Check if there is any liquid reflection on the surface of the flow cell and ensure that there is no residual wiping liquid on the surface of the chip. The flow cell should be in a clean and transparent state, with no obvious foreign substances adhering to it.
- 2.7 Confirm that there are no residues in the area where the holder platform contacts the flow cell.
- 2.8 Please put the prepared flow cell in a safe and clean place before sequencing start.

⚠ Note

- After unpacked the flow cell, sequencing needs to be started within 12 hours.
- If there are still stains on the surface of the Flow Cell after thorough wiping or if it is impossible to distinguish whether the stains are on the surface or inside the Flow Cell, please consult technical support for treatment suggestions.
- There shall be no obvious water droplets on the RFID tag, otherwise the identification of RFID may be affected.

3.Preparation of library to be sequenced

The FASTASeq S sequencing system only support automatic sample loading.

◆ 3.1 Dilute library (if applicable)

Pre-cool the library diluenting reagent and library neutralizing reagent from box 1 of 2 on ice or in fridge for later use. Dilute the library to 4 nM (if the concentration of the sample library is 4 nM, no further dilution is required) with pre-cooled library diluenting reagent, and calculate the volume of library and library diluening reagent needed according to the following formula:

$$\text{Library diluent}(\mu L) = \left(\frac{\text{Library concentration}(ng/uL) \times 10^6}{660 \times \text{Librarylength}(bp) \times 4\ nM} - 1 \right) \times \text{Library volume}(\mu L)$$

◆ 3.2 Automatic sample loading

3.2.1 Prepare fresh 0.2 M NaOH solution as a denaturing solution.

⚠ Note

- The 0.2 M NaOH solution needs to be used immediately after preparation (pH> 13, tested with pH test paper) and should not be stored for too long. NaOH solution will react with CO_2 in the air and become inefficient. The invalid denaturation solution is the most common cause of reduced sequencing throughput.

3.2.2 Preparation of the 20 pM library from the 4 nM library.

Denaturation of the library is completed directly by adding 0.2M NaOH solution by referring to the following table:

Reagent Component	Volume (μL)
NaOH solution was added to a centrifuge tube containing undenatured library	
0.2 M NaOH solution	5
4nM library	5
Mix well (vortex) and incubate to denaturize at room temperature for 5 minutes. After completion of denaturation, place the mixture on ice and add pre-cooled hybridization reagent.	
Hybridization Reagent	990
Total volume	1000

⚠ Note

- If the sample concentration is lower than 4nM but higher than 0.1 nM, denaturation can still be carried out; however, it should be noted that the denaturation of the library at a low concentration may affect the sequencing throughput. When the library is denatured at a low concentration, it is necessary to ensure the library: 0.2 M NaOH: library neutralizing reagent=1:1:1 (volume ratio).

3.2.3 Gently mix the sample well through the vortex oscillator, centrifuge quickly and place on ice before use.

3.2.4 Use the 2 mL clean centrifuge tube provided in the kit as the container for library loading.

⚠ Caution (Important)

- Since the library loading container should be placed in the library well of the sequencing reagents cartridge, be sure to used the 2 mL centrifuge tube provided in the kit as the library loading container so as to ensure that the library loading properly.Other brands or specifications may risk damaging the equipment.

3.2.5 Mix 20 pM library and use for quick centrifugation. The library dilution was calculated based on the desired loading concentration, and 500 µL library was prepared and diluted to the appropriate concentration using the pre-cooled Hybridization . The recommended final library concentration range is 2~8 pM.

The 20 pM library usage (µL) and the (µL) corresponding to the Final Library Concentration (pM) for loading can be obtained by the following formula:

$$V_{20\ \text{pM library}}\,(\mu L) = \frac{500 \times \text{Final library concentration(pM)}}{20\ \text{pM}}$$

$$\text{Hybridization Reagent}(\mu L) = 500 - \text{20 pM library usage}(\mu L)$$

3.2.6 Gently mix the sample well through the vortex oscillator, centrifuge quickly and place on ice before use.

3.2.7 Insert the library tubes into the corresponding wells of the kit and press down firmly.

⚠ Caution (Important)

- Avoid premature denaturation. Begin denaturation preparation only after the instrument and other reagents are ready, and perform denaturation approximately 30 minutes before loading onto the instrument. Denature the library immediately before use to ensure optimal performance. Do not store denatured libraries for extended periods.

4. Sequencing

- 4.1 Sequencing system Start-up and pre-run wash

  Refer to the FASTASeq S Sequencing System User Manual, start the sequencing system and software for pre-run wash.

- 4.2 Import information

  Refer to the FASTASeq S Sequencing System User Manual, set the basic experimental information and customized experimental information.

- 4.3 Load consumables

  Refer to the FASTASeq S Sequencing System User Manual, load the sequencing reagent cartridge and sequencing flow cell.

- 4.4 Sequencing and Post-run wash

  Refer to the FASTASeq S Sequencing System User Manual, select automatic wash in the sequencing interface and start sequencing. The instrument with perform automatic wash after sequencing is completed.

- 4.5 Check sequencing result

  After prompting completion of sequencing on the interface, a sequencing report has been generated. Check the sequencing results.

Requirements for Successful Experiment Result

1.To ensure the validity of sequencing experiment, it is necessary to meet the following conditions:

- The concentration and purity of the sequenced library meet the sequencing requirements.
- Refer to the User Manual of kit for the library construction regarding recommended sequencing parameters and settings.
- Conduct sequencing according to the process specified in the FASTASeq S Sequencing System User Manual.

2. The following situations should be eliminated before sequencing experiment since they may affect the sequencing experiment results:

- The kit has expired.
- The kit is not stored under specified temperature conditions.
- The kit is not mixed thoroughly after thawing.
- The kit is heated excessively when thawing.
- The duration from the thawing of reagents to the start of sequencing is more than 24 hours.
- The library preparation is not carried out according to the user manual.
- The 0.2 M NaOH solution for library denaturation is not prepared immediately prior to use; obsolete buffers are used during dilution and neutralization.
- The residual primers are not removed during sequencing library preparation. (Sequencing library clean-up is not part of this user manual, refer to your sequencing library prep guide)
- Library quality does not meet the requirements for NGS sequencing.
- The sequencing flow cell is not clean or not cleaned with absolute ethanol as required.
- The sequencing system liquid pathways (pipelines/tubing) are not washed as required.
- The temperature and humidity of the working environment of the sequencing system and its variation ranges are not within the product requirements.

Limitation of the Test

- The library sample applicable to this product is the output library of the library construction kit matching with the FASTASeq S Sequencing System, or relevant library samples matching NGS.
- This product should be used in conjunction with the FASTASeq S Sequencing System and cannot be used directly for nucleic acid sequence determination.

Product Performance Indicator

Sequencing was conducted using the enterprise reference. The performance indicators of products of different specifications are as follows.

Product Specification	Throughput	Data quality [1] (Q30)	Sequencing Error Rate[2]
FCL-D SE100-D	⩾20M Reads	⩾90%	⩽0.3%
FCL 100cycles	⩾20M Reads	⩾90%	⩽0.3%
FCL 300cycles	⩾20M Reads	⩾90%	⩽0.35%
FCM-D SE100-D	⩾40M Reads	⩾90%	⩽0.3%
FCM 100cycles	⩾40M Reads	⩾90%	⩽0.3%
FCM 300cycles	⩾40M Reads	⩾90%	⩽0.35%

- This performance parameter is obtained based on the average of multiple test results of the enterprise reference, the enterprise reference product library of Genemind; of which, the data output and high-quality data ratio are affected by factors such as sample type, sample quality, and effective utilization of flow cells. The actual performance parameters may be varied.
- The sequencing error rate is obtained by mapping the sequencing data with the reference genome E.coli_ATCC8739.

Matters Needing Attention

- This product is for in vitro diagnosis only.
- Please mix and vortex the reagent components thoroughly before use. Avoid mixing reagents from kits with different batch numbers.
- This product contains different chemical reagents that are potentially dangerous and should be avoided to contact with skin. Only the trained personnel with appropriate laboratory skills can use this kit and the test results should be reviewed by professionals. Samples may be biohazardous during operation. Wear appropriate laboratory clothes and disposable powder-free gloves. In case of accidental contact, please rinse it with running water or seek medical assistance.
- This product is disposable and must not be re-used after unpacking.
- Please read this user manual carefully before starting the sequencing experiment, or the preparation for it. All reagents in this kit are specially prepared for the above tests. Arbitrary replacement of any reagent or change of the operating procedures of the kit may affect the use effect.
- The used parts of the kits and samples are clinical wastes and should be disposed of according to regulations.
- The box 2 of 2 contains desiccant. Don't eat it by mistake.
- If the box 1 of 2 condenses into ice at low temperature, it should be thawed at room temperature and mixed by vortex before use.
- Confirm whether the product components are intact before use. Don't use a kit with damaged or leaked reagent components.
- This product should be used in a common clean laboratory environment.

Markings

	Keep away from sunlight		Consult instructions for use
	Keep dry		Manufacturer
	Do not re-use		Date of manufacture
	Caution		Use-by date
	Temperature limit	LOT	Batch code
UDI	Unique device identifier	REF	Catalogue number

Contact Information and Product Support

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