Discover-sc WTA Kit V2

Catalog # N711

Version 6.2

Vazyme biotech co., ltd.

1. Introduction

Discover-sc WTA Kit V2 is designed for whole transcriptome amplification of total RNA or mRNA from single cells. Discover-sc WTA Kit V2 can achieve enough amount of full-length cDNA for sequence analysis through synthesis and amplification from 1-1000 of cells or 10 pg-10 ng of total RNA as template, which makes it ideal for sample preparation from single cells or trace RNA samples.

Discover-sc WTA Kit V2 takes Oligo dT as reverse transcription primer to perform cDNA synthesis, and takes advantage of a template-switching activity of reverse transcriptase to link an adapter to the 3'-end of reverse transcribed full length cDNA. This adapter sequence can be used to amplify the full-length cDNA, effectively avoiding the 3'-preferences and rRNA contamination.

Discover-sc WTA Kit V2 is an updated version based upon Discover-sc WTA Kit. Compared with the previous version, its detection sensitivity and volume compatibility has been significantly improved, which makes it more suitable for the detection of templates of low abundance and low concentration. In general, one reaction can produce 2 ng-20 ng of cDNA amplification products.

2. Contents of Kits

	Components	N711-01 (12 rxn)	N711-02 (24 rxn)	N711-03 (96 rxn)
Box 1	Discover-sc TS Oligo V2	12 μl	24 μl	96 μl
	Control Total RNA (1 μg/μl)	5 μl	5 μl	5 μl
Box 2	10 × Lysis Buffer V2	230 μl	460 μl	2 × 920 μl
	RNase Inhibitor	20 μl	40 μl	160 μl
	Oligo dT Primer	24 μl	48 μl	192 μl
	dNTP Mix	24 μl	48 μl	192 μl
	DTT	12 μl	24 μl	96 μl
	5 × FS Buffer V2	48 μl	96 μl	384 μl
	Discover-sc Reverse Transcriptase	12 μl	24 μl	96 μl
	2 × Discover-sc PCR Mix	300 μl	600 μl	4 × 600 μl
	Discover-sc WTA PCR Primer	12 μl	24 μl	96 μl
	Elution Buffer	1 ml	1 ml	2 × 1 ml
	Nuclease-free H2O	1 ml	1 ml	2 × 1 ml

3. Storage

Box 1: Store at -70°C.
Box 2: Store at -20°C.

4. Applicable Samples

This kit, using Oligo dT as primers to amplify RNA with poly A sequence, is applicable to many kind of samples:

◇ 1-1000 of mammalian cells, or other eukaryotic cells without cell wall;
◇ 10 pg -10 ng of purified total RNA with poly A sequence;
◇ Not suitable for prokaryotic cells;
◇ Not suitable for fixed cells.

5. Additional Materials Required

Beads for Purification: VAHTS DNA Clean Beads (Vazyme, #N411), AMPure® XP Beads (Beckman Coulter, #A63881) or other similar products;

RNA Evaluation: Agilent RNA 6000 Pico Kit (Agilent, #5067-1513);

DNA Evaluation: High Sensitivity DNA Chip (Agilent, #5067-4626);

Library Preparation Kit:

TruePrep DNA Library Prep Kit V2 for Illumina® (Vazyme, #TD503) or other similar products;

TruePrep Index Kit V2/V3 for Illumina® (Vazyme, #TD202/TD203) or other similar products;

Other Materials Required: 80% ethyl alcohol, low adsorption EP tubes (Eppendorf, #022431021), Agilent Technologies 2100 Bioanalyzer and other similar Bioanalyzer, PCR instrument, magnetic stand.

6. Application Notes

- ◇ This kit uses Oligo dT Primers to amplify RNA containing poly A sequence, please make sure there is no DNA contamination containing poly A;
- ◇ The lysis buffer in this kit cannot lyse cell wall efficiently. For eukaryotic cells with cell walls, please lyse the cell wall before using this kit, or use purified RNA as template. Mammalian cells can be directly applied with this kit;
- ◇ This kit is not suitable for fixed cells;
- ◇ When using RNA as starting template, please make sure that RNA is intact and purified;
- ◇ This kit is of high detection sensitivity, please perform experiments in a positive pressure super clean workbench.
- ◇ All components in this kit should be stored in nucleic acids-free and nuclease-free environment.
- ◇ All components in this kit had been optimized, please do not change the reaction systems.
- ◇ If you are using this protocol for the first time, it is recommended to set up a positive and a negative control to verify whether the reaction system is working normally.

7. Sample Preparation

Some of the components in the cell culture medium or the sample may inhibit the reaction, please try to reduce unnecessary sample volume in order to reduce the possible influence on the reaction system.

7-1. Cell Sample Preparation

Cell Number: The starting template is 1-1000 cells. Excess cells may inhibit the reaction.

Obtaining Method: Gene expression in different cells changes instantaneously, therefore, different cell type, activity, and cycle will significantly influence the final cDNA yield. In order to verify the influence on cell activity by different cell obtaining methods, it is recommended to identify cell activity after each samples collection. Obvious RNA degradation will occur in cell death and leads to the failure of the experiment. It is also recommended to start the reaction immediately after certification, because improper preservation conditions may lead to RNA degradation in cells.

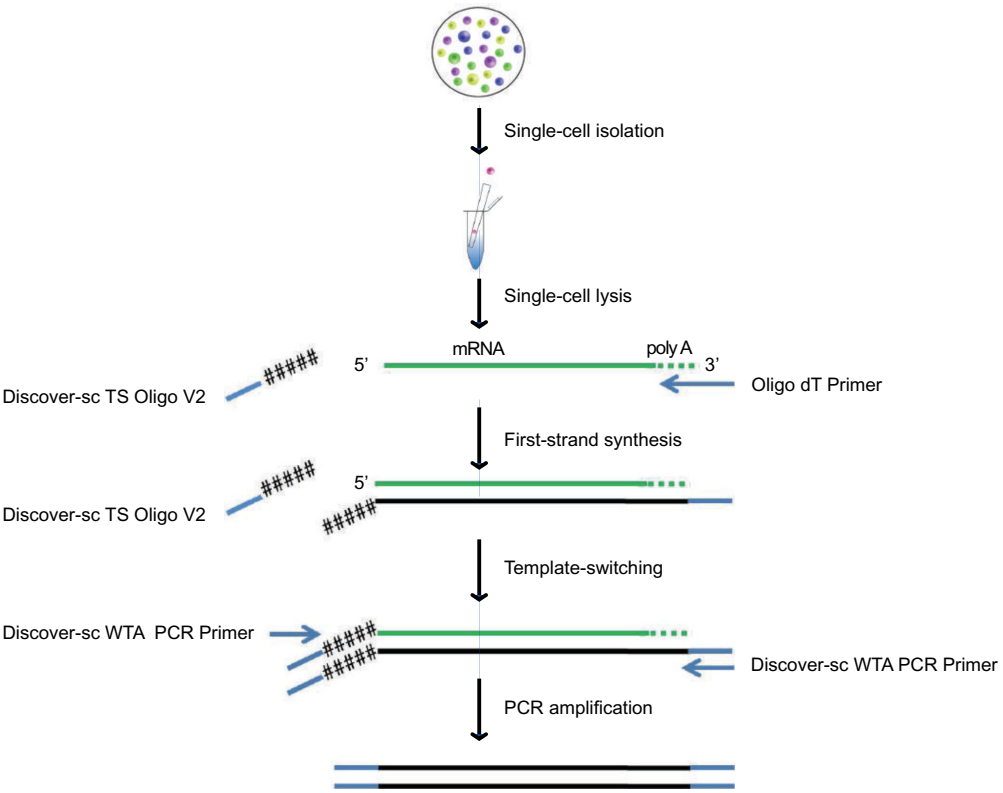
Storage Condition: If your sample needs to be stored for a certain period, please refer to the **Section 9-1/9-2** for operation. The prepared cell samples should be stored at -70°C or lower temperature, and please start the reaction immediately after taking out from refrigerator. The isolated living cells could be stored in freezing medium and recovered before use. Please check if the cells stay alive before reaction, because dead cells showing obvious RNA degradation may cause the failure of the experiment.

Cell Culture Medium Detection: For cultured cells, please confirm if the culture medium has inhibitory effect on the reaction. The medium can be added to the RNA control reaction to check whether the medium inhibits the cDNA synthesis. If it is hard to identify, please re-suspend the cells in PBS before starting the reaction.

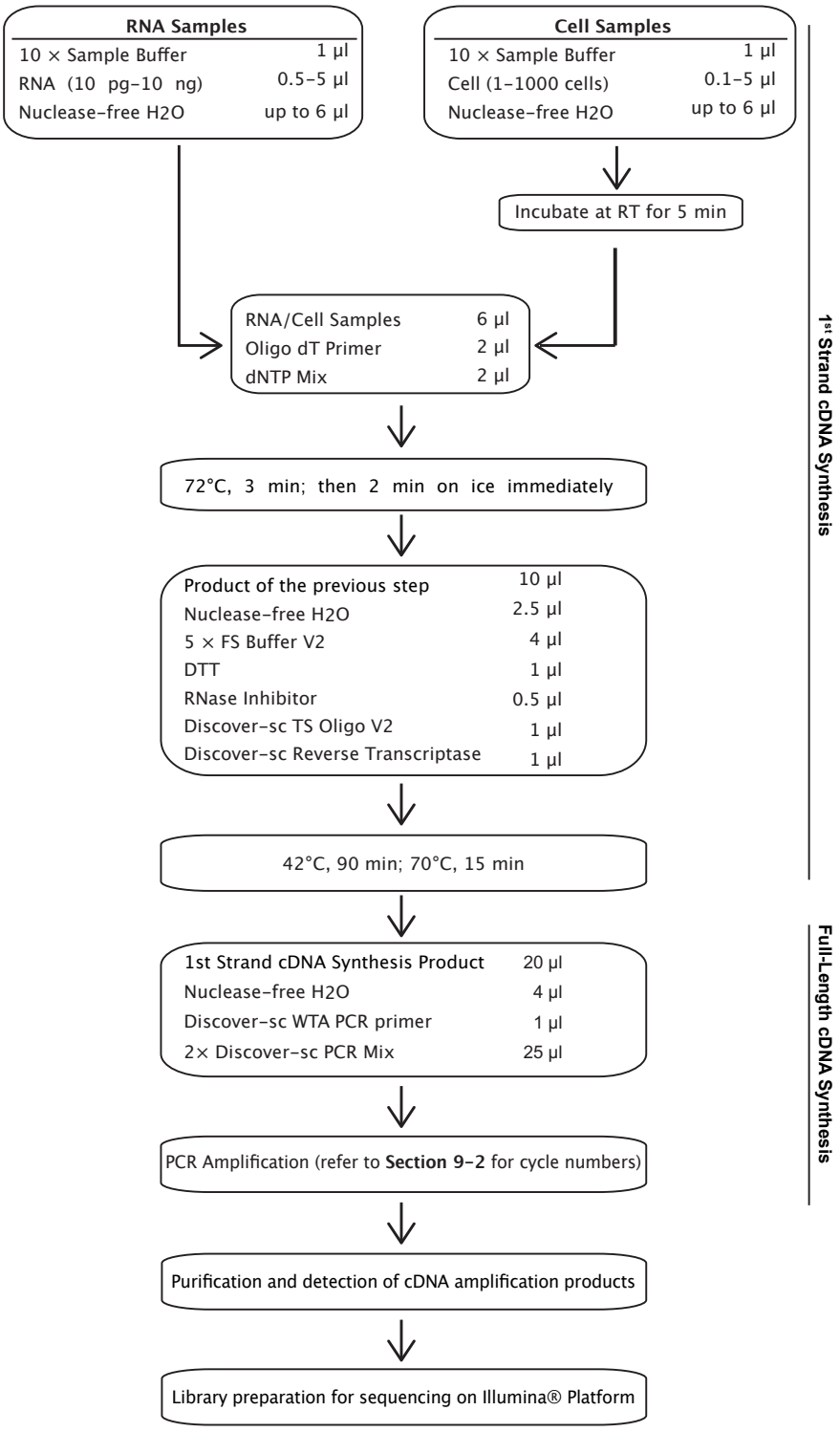
7-2. RNA Sample Preparation

For purified RNA samples, it is recommended to evaluate the integrality of the RNA using Agilent RNA 6000 Pico Kit before reaction. Taking intact RNA as starting template will affect the cDNA yield and product size distribution, and may lead to the failure of the experiment.

8. Mechanism and Workflow Overview



cDNA synthesis mechanism based on template-switching.



Workflow overview of Discover-sc WTA Kit V2.

9. Protocol

Please read this manual carefully before experiment. This kit is applicable to starting template of 1-1000 cells or 10 pg-10 ng of total RNA.

9-1. First Strand cDNA Synthesis (Please operate in an Ultra-Clean workbench)

The 1st strand cDNA was synthesis from purified RNA or total RNA of cells as template at this step. Take Oligo dT as reverse transcription primer, and use the template-switching activity of Discover-sc reverse transcriptase to link an adapter to the 3'-end of full-length cDNA.

1. Take out all of the components for 1st strand cDNA synthesis with the exception of Discover-sc Reverse Transcriptase and Discover-sc TS Oligo V2, dissolve them on ice, mix by vortex and briefly centrifuge to collect them to the bottom of the tube, and then place on ice.

Note: There may be precipitates in 5 × FS Buffer V2, please mix by vortex and dissolve thoroughly before use.

2. Prepare 10 × Sample Buffer as follows:

10 × Lysis Buffer	19 μl	■
RNase Inhibitor	1 μl	■
Total	20 μl	

Note: Mix thoroughly by gently pipetting, and briefly centrifuge to collect them to the bottom of the tube. Avoid bubbles during this procedure.

3. Sample Preparation:

For RNA Sample: Add 0.5-5 μl of purified RNA into a 0.2 ml RNase-free PCR tube. Use Nuclease-free H_2O to make up the volume to 5 μl if needed. Then, add 1 μl of 10 × Sample Buffer.

For Cell Sample: Add 0.1-5 μl of isolated cell samples into a 0.2 ml RNase-free PCR tube. Use Nuclease-free H_2O to make up the volume to 5 μl if needed. Then, add 1 μl of 10 × Sample Buffer, flick the tube to mix, briefly centrifuge to collect them to the bottom of the tube and incubate at room template for 5 min. For other cell samples, please refer to **Section 7-1**.

Set up control and test sample reactions as follows:

Components	Negative Control	Negative Control	Sample	
10 × Sample Buffer	1 μl	1 μl	1 μl	
Nuclease-free H2O	5 μl	0-4.5 μl	0-4.5 μl	□
Diluted Control Total RNA	–	0.5-5 μl	–	
RNA/Cell Sample	–	–	0.5-5 μl	
Total	6 μl	6 μl	6 μl	

Note: The concentration of the control Total RNA in the kit is 1 μg/μl, please dilute with Nuclease-free H_2O before use.

Note: Some of the components in the cell culture medium may inhibit the reaction, please try to reduce unnecessary sample volume in order to reduce the possible influence on the reaction system. If your sample needs to be stored for a certain time, it should be stored at -70°C or lower temperature. If the samples need to transport, please transport in dry ice.

4. Place the sample on ice, and prepare the reaction system as follows:

RNA/Cell Sample from **Step 3**	6 μl	
Oligo dT Primer	2 μl	■
dNTP Mix	2 μl	■
Total	10 μl	

5. Mix thoroughly by gently pipetting, briefly centrifuge, and place on ice.

6. Preheat the PCR instrument (with hot-lid) to 72°C.

7. Run the program as follows:

72°C	3 min
Place on ice immediately	2 min

8. Preheated the PCR instrument to 42°C.

9. Prepare the reaction system as follows:

Step 7	10 μl	
Nuclease-free H2O	2.5 μl	□
5 × FS Buffer V2	4 μl	■
DTT	1 μl	■
RNase Inhibitor	0.5 μl	■
Discover-sc TS Oligo V2	1 μl	■
Discover-sc Reverse Transcriptase	1 μl	■
Total	20 μl	

Note: Gently flick the tube to mix Discover-sc Reverse Transcriptase and Discover-sc TS Oligo V2 before use. DO NOT mix by vortex.

10. Mix thoroughly by gently pipetting, briefly centrifuge, and place on ice.

11. Place the PCR tube to the preheated (42°C) PCR instrument, run the following program:

42°C	90 min
70°C	15 min
4°C	Hold

12. Place the products on ice until next step.

Note: The products of this step can be store at 4°C overnight, but do not exceed 12 hours.

9-2. Full-length cDNA Amplification (Please operate in an Ultra-Clean workbench)

The 1st Strand cDNA synthesized during the previous step is amplified through PCR in this step.

1. Take out the components for PCR, dissolve them on ice, mix thoroughly by vortex and briefly centrifuge to collect them to the bottom of the tube, and then place on ice.

2. Prepare the reaction system as follows:

1 st strand cDNA	20 µl	
Nuclease-free H2O	4 µl	☐
Discover-sc WTA PCR Primer	1 µl	■
2 × Discover-sc PCR Mix	25 µl	■
Total	50 µl	

3. Mix thoroughly by gently pipetting, briefly centrifuge and place on ice.

Note: After this step, all of the following steps can be performed under the normal experimental environment.

4. Run the following program in a PCR instrument:

Temperature	Time	Cycles
98°C	1 min	
98°C 65°C 72°C	10 sec 15 sec 6 min	X (Refer to the following table)
72°C	5 min	
4°C	Hold	

Cycle numbers for different starting templates for reference:

Total RNA	Cells	Cycles
10 ng	1,000 cells	7-8
1 ng	100 cells	10-11
100 pg	10 cells	14-15
10 pg	1 cell	17-18

Note: PCR cycle numbers varies between starting templates of different cells types. The cycle numbers in the above table are obtained from 293T cells as test sample. Please adjust the cycles according to the RNA amount in different cells. It is recommended to refer to **Section 10. FAQs** to determine the optimal cycle number.

5. Place the products on ice after reaction.

Note: The products of this step can be store at 4°C overnight, but do not exceed 12 hours.

9-3. Purification and Detection of cDNA Amplification Products

The cDNA amplification products are purified by beads in this step, and the qualities are detected by Aglient 2100 Bioanalyzer.

Purification of cDNA Amplification Products

Aliquot VAHTS DNA Clean Beads into 1.5 ml EP tubes accordingly. Mix the VAHTS DNA Clean Beads by vortex and incubate at room temperature for 30 min. Prepare fresh 80% ethyl alcohol (approximately 400 µl for each sample).

1. Mix VAHTS DNA Clean Beads thoroughly by vortex, add 50 µl of VAHTS DNA Clean Beads to the cDNA amplification products, pipette 10 times to mix the reaction system thoroughly.

Note: The beads are sticky, please make sure to absorb enough volume of the beads and push out it slowly.

2. Incubate at room temperature for 8 min to let the cDNA bind on the beads.

3. Briefly centrifuge the tube and place it on a magnetic stand to separate the beads from the solution.

4. Keep the PCR tube on the magnetic stand. Wait until the solution clarified (about 5 min), carefully remove the supernatant without disturbing the beads.

5. Keep the PCR tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethyl alcohol without disturbing the beads. Incubate at room temperature for 30 sec, the carefully remove the supernatant.

6. Repeat **Step 5** (rinse the beads twice in total).

7. Briefly centrifuge to collect the sample to the bottom of the PCR tube, and keep it on the magnetic stand for 30 sec. Remove the entire residual solution using a pipette.

8. Open the tube and air-dry the beads for 3-5 min.

Note: Please make sure that the beads are just dry (they will look without burnish).

Note: If the beads do not dry completely, the residual ethyl alcohol can reduce the elution rate of the cDNA, which may interfere with downstream reactions.

Note: If the beads are over-dried, it is recommended to prolong the incubation time to fully rehydrate them in **Step 10** to avoid the reduce in elution efficiency and yield of the cDNA.

9. Take the sample out of the magnetic stand. Add 17 µl of Elution Buffer to cover the beads, mix them by pipetting.

10. Incubate at room temperature for 2 min. If the beads are over-dried and cracking, please extend the incubation time.

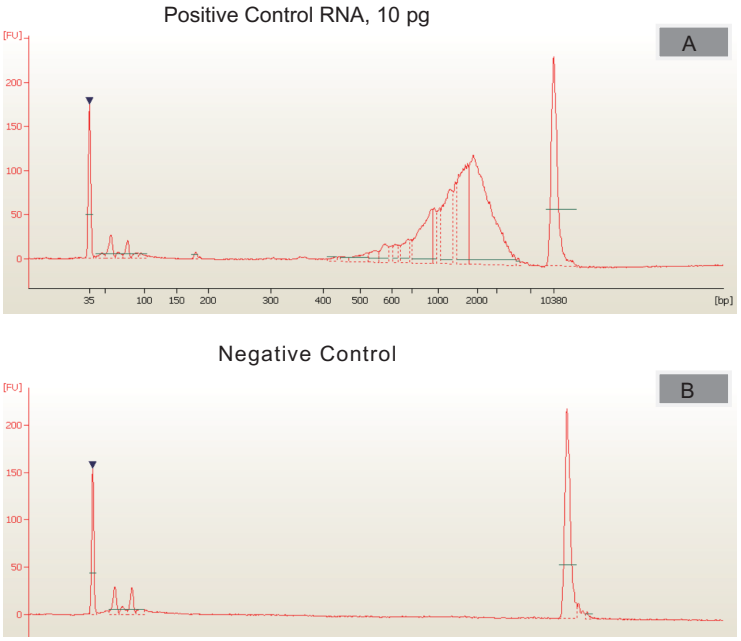
11. Briefly centrifuge the PCR tube and then place on the magnetic stand to separate the beads and solution until the solution are clarified (about 5 min).

Note: If there are beads that fail to bind to the magnetic stand after separation, please re-suspend the beads by pipetting supernatant, and then incubate on the magnetic stand until there is no beads suspend in the supernatant.

12. Carefully transfer 15 µl of supernatant to a new low adsorption EP tube. Store at -20°C.

Detection of cDNA Amplification Products

The yield and size distribution are important indicator of qualified cDNA amplification product. It is recommended to use an Agilent 2100 Bioanalyzer for detection. Analyze 1 μl of amplified cDNA using an Agilent Technologies 2100 Bioanalyzer and High Sensitivity DNA Chip. Under normal circumstances, one reaction can output 2-20 ng of amplified cDNA (depending on the initial template), distributing between 400-10000 bp with a library peak located at about 2000 bp (**Fig. A**). Negative control containing no template yields no product (**Fig. B**).



9-4. Library Preparation for Sequencing on Illumina® Platforms

At this step, it is recommended to take 1 ng of cDNA as starting material, and using TruePrep DNA Library Prep Kit V2 for Illumina® (Vazyme, #TD503) or Nextera® XT DNA Library Preparation Kit (Illumina, #FC-131-1024) for library construction.

10. FAQs

◇ Is this kit applicable to plant cell samples?

No. The lysis buffer in this kit cannot lyse cell wall efficiently. For eukaryotic cells with cell walls, please lyse the cell wall before using this kit, or use purified RNA as template.

◇ Is this kit applicable to fixed cells?

No. After treatment with formaldehyde or acetone, the quality of RNA in tissue or cells declined significantly, cause the failure of amplification. So this kit is not applicable to fixed cells.

◇ Are cell samples can be operated with this kit directly without rinsed with PBS solution?

For cultured cells, please confirm if the culture medium has inhibitory effect on the reaction. It is recommended to add the medium to the RNA control reaction to check whether the medium inhibits the cDNA synthesis. If it is hard to identify, please re-suspend the cells in PBS before starting the reaction.

◇ Could the cell samples be stored for a certain time, when we can't conduct amplification immediately after the samples are prepared?

If your sample needs to be stored for a certain time, please refer to the **Section 9-1/9-2** for operation. The prepared cell samples should be stored at -70°C or lower temperature, and please start the reaction immediately after taking out from refrigerator. The isolated living cells could be stored in freezing medium, and recovered before use. Please check if the cells stay alive before reaction, because dead cells showing obvious RNA degradation may cause the failure of the experiment.

◇ How to confirm the cycle number when amplify full-length cDNA through PCR?

Select the optimal cycle number to ensure that the amplification is still in the exponential phase. When the expansion cycle number increases, while cDNA yield no longer increase accordingly, the amplification has reached a plateau. Over-amplified cDNA will lead to a decline in quality of cDNA library. However, low cycle number will cause a decline in cDNA yield. Therefore, please set the cycle number as low as possible on the premise of yielding enough products. It is recommended to set several parallel reactions with different cycle numbers to determine the optimal cycle number. For example, set up three reactions, one reaction carried out in accordance with the recommended cycle number, the other two reactions with 2-3 less cycles and 2-3 more cycles respectively.

◇ How is the single-cell transcriptome amplification yield? And how to prepare the library for sequencing?

The gene expression in different cells varies instantaneously. Cell type, activity, and cycle will significantly influence the final cDNA yield. Under normal circumstances, Discover-sc WTA Kit V2 can output 2-20 ng of cDNA library. It is recommended to prepare library using transposase method: take 1 ng of cDNA as starting material, and use TruePrep DNA Library Prep Kit V2 for Illumina® (Vazyme, #TD503) or Nextera® XT DNA Library Preparation Kit (Illumina, #FC-131-1024) to prepare library for sequencing.