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Discover-sc WTA Kit V2

N711

Vazyme

Instruction for Use

Version 22.1

Contents

01/Product Description

Discover-sc WTA Kit V2 is capable of obtaining sufficient samples for sequence analysis through first-strand cDNA synthesis and amplification using 1 - 1,000 cells or 10 pg - 10 ng of total RNA as templates, thus overcoming the technical difficulty that the conventional mRNA-seq method cannot be used for sequence analysis of trace samples such as single cells due to the low RNA content.

The kit employs Oligo dT Primer as the reverse transcription primer for cDNA synthesis. The kit also utilizes the template-switching activity of Discover-sc Reverse Transcriptase to add an adapter sequence at the 3' end of the cDNA, based on which subsequent PCR amplification is performed to obtain full-length cDNA amplification products, effectively avoiding 3' end preferences and rRNA contamination during cDNA synthesis.

Discover-sc WTA Kit V2 is an upgraded version developed on the basis of Discover-sc WTA Kit. The upgraded version features substantially improved detection sensitivity and volume compatibility, and is more suitable for the detection of low-abundance genes and low-concentration templates. Generally, one reaction can output 2 - 20 ng of cDNA amplification products.

02/Components

	Components	N711-01 (12 rxns)	N711-02 (24 rxns)	N711-03 (96 rxns)
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 ddH_2O	1 ml	1 ml	2 × 1 ml

▲Colored bullets indicate the cap of the reagent to be added.

03/Storage

BOX 1: Store at -85 ~ -65℃ and ship on dry ice.

BOX 2: Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

The kit employs Oligo dT Primer to amplify RNA with a poly(A) sequence and is compatible with multiple sample types:

◇1 - 1,000 mammalian cells or other eukaryotic cells without cell walls.

◇10 pg - 10 ng of purified total RNA with the poly(A) sequence.

◇Incompatible with prokaryotic cells.

◇Incompatible with fixed cells.

05/Self-prepared Materials

Magnetic beads for purification:
VAHTS DNA Clean Beads (Vazyme #N411);
AMPure XP Beads (Beckman Coulter #A63881) or equivalents.
RNA analysis: Agilent RNA 6000 Pico Kit (Agilent #5067-1513).
DNA analysis: High Sensitivity DNA Chip (Agilent #5067-4626).

Other materials:
80% ethanol (freshly prepared with Nuclease-free ddH_2O); RNase-free PCR tubes, low binding EP tubes (Eppendorf #022431021). Agilent Technologies 2100 Bioanalyzer or equivalents, PCR instrument, magnetic stand, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

◇ The kit employs Oligo dT Primer to amplify RNA with a poly(A) sequence. Please make sure that the system is free of DNA with a poly(A) sequence.

◇ The cell lysis method of this kit cannot effectively lyse the cell wall. For eukaryotic cells with cell walls, please lyse the cell wall before using this kit, or use purified RNA as the template. Mammalian cells can be lysed and amplified directly according to this protocol.

◇ This kit is incompatible with fixed cells.

◇ When using RNA as the starting template, please make sure that the RNA is intact and free of contamination.

◇ The kit has high detection sensitivity. The experimental operations should be done on a positive-pressure ultra-clean workbench instead of common PCR operation platforms.

◇ All components of this product should be stored in a nucleic-acids-free and nuclease-free environment so as to avoid experimental failure.

◇ All components in this kit have been specifically designed to work together and are optimized for this protocol. Please do not make any substitutions.

◇ If you are carrying out this protocol for the first time, it is recommended to perform positive and negative controls to verify that the kit components are working properly.

07/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 to lessen the possible impact on the reaction system.

07-1/Cell Sample Preparation

Number of cells: The kit can take 1 - 1,000 cells as the starting template for amplification; excessive cells may inhibit the reaction.

Cell collection method: Since the gene expression of cells keeps changing, the cell type, viability, and cycle can all significantly affect the final cDNA yield. Please determine the effect of the collection method on cell viability. It is recommended that the cell viability should be identified after each sample collection because dead cells undergo obvious RNA degradation, leading to reaction failure. It is recommended to start the reaction immediately after finding that the cell viability is acceptable because improper storage conditions may also lead to RNA degradation in the cells.

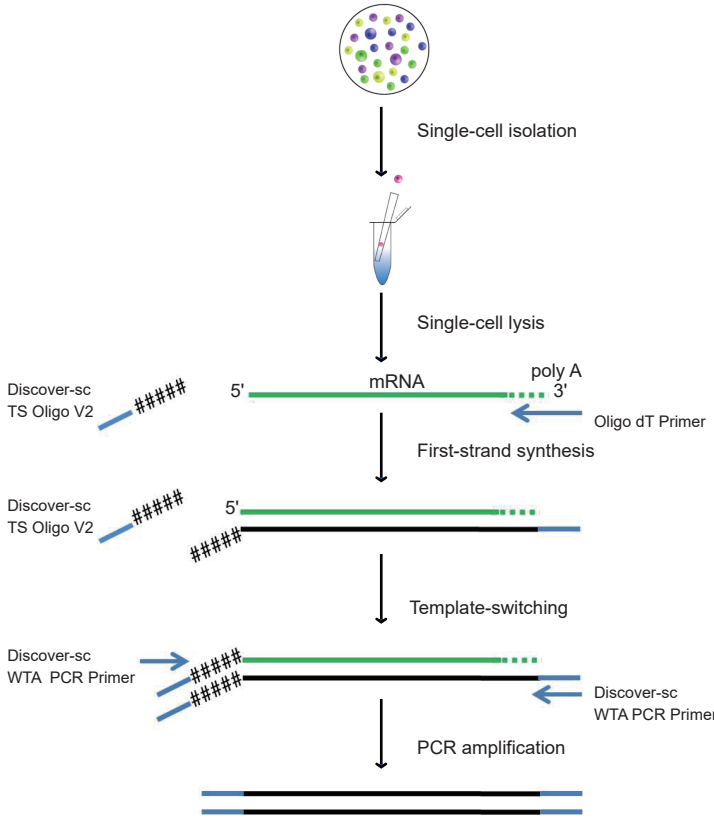
Storage method: If you need to store the cells for a period of time before performing the reaction, please refer to 09-1/First-strand cDNA Synthesis/Step 2 and 3 for the operations to take. The prepared cell samples should be stored at -70℃ or lower temperature and subjected to amplification immediately after being taken out. Isolated living cells may also be cryopreserved in cell cryopreservation solutions and recovered when needed. If such a preservation protocol is used, please check whether the cells stay alive before the reaction because dead cells undergo obvious RNA degradation, which will lead to reaction failure.

Cell culture medium test: For cultured cells, test whether the culture medium has any inhibitory effect on the reaction before performing the experiment. The culture medium expected to be included in the cell sample may be added to the control RNA reaction system to check whether the culture medium inhibits cDNA synthesis. If the effect of the culture medium on the reaction cannot be confirmed, resuspend the cells in PBS before operations. Cells resuspended in PBS have verified yields when processed according to this protocol.

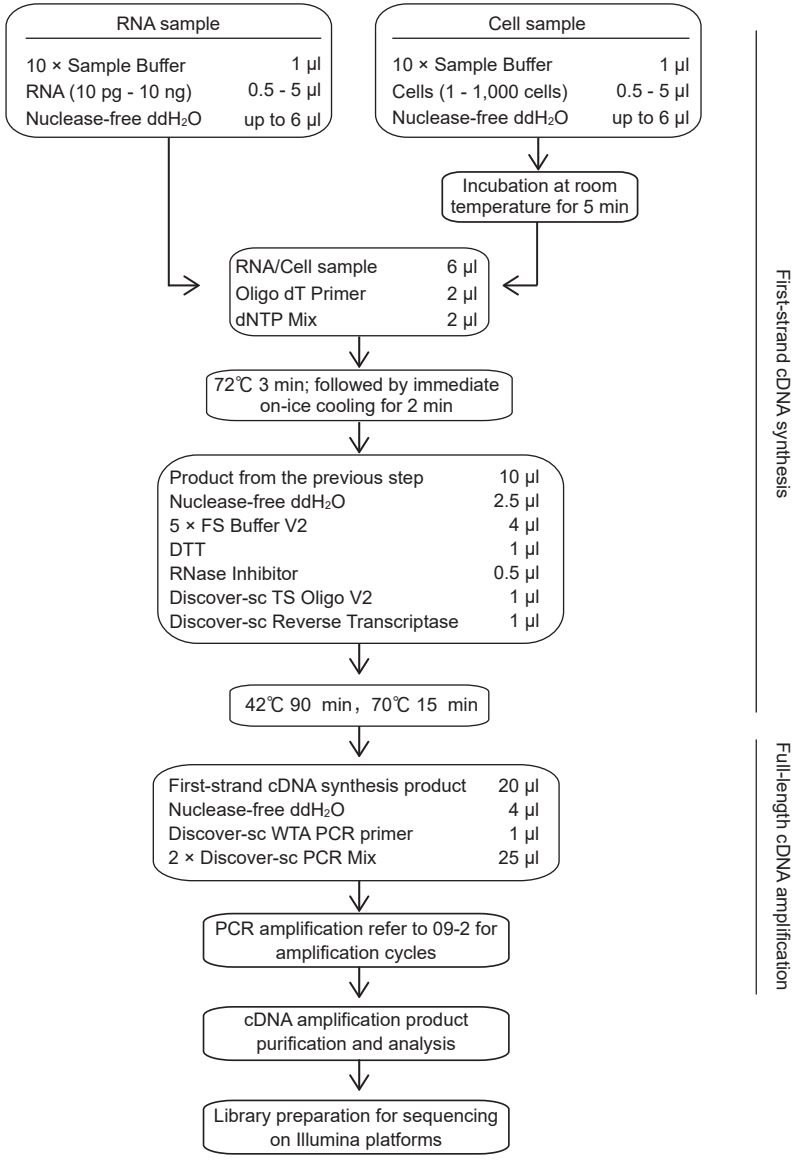
07-1/Cell Sample Preparation

For purified RNA samples, it is recommended to evaluate the RNA integrity using the Agilent RNA 6000 Pico Kit prior to the reaction. Using incomplete RNA as the starting template impacts the cDNA yield and product size distribution, which may lead to reaction failure.

08/Mechanism & Workflow



Mechanism of Full-length cDNA Synthesis Based on Template-switching



Workflow of Single-cell Transcriptome Library Preparation Using Discover-sc WTA Kit V2

09/Experiment Process

Please read the manual carefully before the experiment. The kit is suitable for cDNA synthesis using 1 - 1,000 cells or 10 pg - 10 ng of total RNA as templates. This manual applies to Discover-sc WTA Kit V2. If you are using a Discover-sc WTA Kit, please refer to its corresponding manual.

09-1/First-strand cDNA Synthesis (please operate on an ultra-clean workbench)

In this step, purified total RNA or total RNA in cells are used as templates for first-strand cDNA synthesis. The kit employs Oligo dT Primer as the reverse transcription primer, and utilizes the template-switching activity of Discover-sc Reverse Transcriptase to link an adapter to the 3' end of the cDNA.

1. Take out the components required for first-strand cDNA synthesis except for Discover-sc Reverse Transcriptase and Discover-sc TS Oligo V2, dissolve them on ice, mix well by shaking and briefly centrifuge to collect them to the bottom of the tube, and then place them on ice.

   ▲ Precipitates may form in 5 × FS Buffer V2. Please shake the buffer before use to thoroughly dissolve the precipitates.

2. Prepare 10 × Sample Buffer according to the following table.

Components	Volume	
10 × Lysis Buffer V2	19 μl	■
RNase Inhibitor	1 μl	■
Total	20 μl	

   ▲ Mix the components by gently pipetting up and down and briefly centrifuge to collect them to the bottom of the tube. Avoid bubbles during this procedure.

3. Sample preparation:

   RNA sample: Add 0.5 - 5 μl of purified sample RNA into a 0.2 ml RNase-free PCR tube. Use Nuclease-free ddH_2O to make up the volume to 5 μl if needed. Add 1 μl of 10 × Sample Buffer.

   Cell sample: Add 0.5 - 5 μl of isolated sample cells into a 0.2 ml RNase-free PCR tube. Use Nuclease-free ddH_2O to make up the volume to 5 μl if needed. Add 1 μl of 10 × Sample Buffer. Flick the tube wall to mix the components well, briefly centrifuge to collect them to the bottom of the tube, and incubate at room temperature for 5 min. For other cell samples, refer to 07-1/Cell Sample Preparation.

   Set the control and test samples according to the following table and prepare the following reaction systems.

Components	Negative Control	Positive Control	Test Sample	
10 × Sample Buffer	1 μl	1 μl	1 μl	
Nuclease-free ddH_2O	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	

▲ The concentration of the Control Total RNA provided in the kit is 1 μg/μl. Please dilute it to the required concentration with the provided Nuclease-free ddH_2O before use.

▲ Some of the components in the cell culture medium and the sample may inhibit the reaction. Please try to reduce unnecessary sample volume to lessen the possible impact on the reaction system. If you need to store the cells for a period of time before amplification, the cell sample prepared in this step should be stored at -70℃ or lower temperature. If the samples need to be transported, please ship them on dry ice.

Components	Volume
RNA/Cell sample from Step 3	6 μl
Oligo dT Primer	2 μl
dNTP Mix	2 μl
Total	10 μl

5. Mix the solution thoroughly by gently pipetting up and down, briefly centrifuge to collect it to the bottom of the tube, and place it on ice.
6. Preheat the PCR instrument with a heated lid function to 72℃.
7. Run the following program.

Temperature	Time
72℃	3 min
Put on ice immediately	2 min

▲ Preheat the PCR instrument to 72℃ in advance. Take the tube out immediately after the reaction and place it on ice for at least 2 min.

8. Preheat the PCR instrument to 42℃ for later use.
9. Prepare the reaction system according to the following table.

Components	Volume
Product from Step 7	10 μl
Nuclease-free ddH_2O	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

▲ For Discover-sc Reverse Transcriptase and Discover-sc TS Oligo V2, flick the tube wall to mix well (do not mix by vortexing) before use.

10. Mix the solution thoroughly by gently pipetting up and down, briefly centrifuge to collect it to the bottom of the tube, and place it on ice.
11. Place the PCR tube in a PCR instrument preheated to 42℃ and run the following program.

Temperature	Time
42℃	90 min
70℃	15 min
4℃	Hold

12. Place the product on ice until the next reaction is ready.

The reaction product can be stored at 4℃ overnight but for no longer than 12 h.

09-2/Full-length cDNA Amplification (please operate on an ultra-clean workbench)

In this step, the first-strand cDNA synthesis product is amplified by PCR.

1. Take out the reagents required for PCR, dissolve them on ice, mix well by shaking and briefly centrifuge to collect them to the bottom of the tube, and then place them on ice.
2. Prepare the reaction system according to the following table.

Components	Volume	
First-strand cDNA synthesis product	20 μl	
Nuclease-free ddH_2O	4 μl	☐
Discover-sc WTA PCR Primer	1 μl	
2 × Discover-sc PCR Mix	25 μl	
Total	50 μl	

3. Mix the solution thoroughly by gently pipetting up and down, briefly centrifuge to collect it to the bottom of the tube, and place it on ice.

▲Perform the subsequent steps in a normal experimental environment.

4. Run the following program on the PCR instrument.

Temperature	Time	Cycles
98℃	1 min	
98℃	10 sec	x
65℃	15 sec	
72℃	6 min	
72℃	5 min	
4℃	Hold	

Number of amplification cycles 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

▲ The number of PCR cycles varies greatly among amplification reactions with different cells as the starting template. The reference amplification cycles in the above table are obtained with 293T cells as the test sample. Please adjust the cycles according to the RNA level in different cells. When testing a type of cell for the first time, it is recommended to determine the optimal number of amplification cycles by referring to 10/FAQ & Troubleshooting.

5. Place the product on ice after the reaction.

The reaction product can be stored at 4℃ overnight but for no longer than 12 h.

09-3/cDNA Amplification Product Purification and Analysis

In this step, the cDNA amplification products are purified using magnetic beads, and the product quality is analyzed using the Agilent 2100 Bioanalyzer.

cDNA amplification product purification

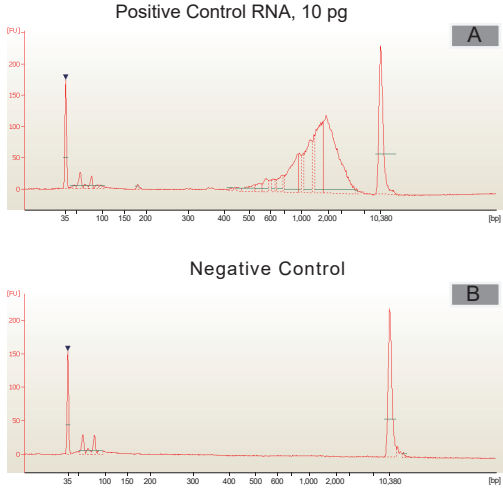
Aliquot VAHTS DNA Clean Beads into 1.5 ml EP tubes as needed. Before use, mix the VAHTS DNA Clean Beads thoroughly by shaking, and place them at room temperature for at least 30 min. Prepare 80% ethanol (freshly prepared with Nuclease-free ddH_2O) (about 400 μl for each sample).

1. Mix the VAHTS DNA Clean Beads well by vortexing, add 50 μl of VAHTS DNA Clean Beads to the above cDNA amplification reaction system, and mix the system thoroughly by pipetting up and down at least 10 times.
   ▲The beads are sticky. When mixing, pipette up a sufficient volume of beads and pipette it down slowly.
2. Incubate the mixture at room temperature for 8 min to allow the cDNA to bind to the beads.
3. Centrifuge the reaction tube briefly and place it on a magnetic separation device to separate the beads from the solution.
4. Keep the PCR tube on the magnetic separation device. After the solution becomes clear (about 5 min), carefully remove the supernatant without disturbing the beads.
5. Keep the PCR tube on the magnetic separation device. Add 200 μl of 80% ethanol (freshly prepared with Nuclease-free ddH_2O) without disturbing the magnetic beads. Incubate the tube at room temperature for 30 sec and carefully remove the supernatant.
6. Repeat Step 5 for a second rinse.
7. Centrifuge the PCR tube briefly to collect the sample to the bottom of the tube, place it on the magnetic separation device for 30 sec, and remove the residual liquid with a pipette.
8. Uncap the tube to air dry for 3 - 5 min.
   ▲ Ensure the beads are dried properly. The properly dried beads look not glossy. If the beads are not dried completely, the residual ethyl ethanol in the sample can decrease the cDNA elution efficiency and may interfere with downstream reactions. If the beads are over-dried, it is recommended to prolong the incubation time in Step 10 to rehydrate the beads fully; otherwise, the cDNA elution effect may be reduced, ultimately decreasing the cDNA yield.
9. After the magnetic beads are dried, remove the PCR tube from the magnetic separation device, add 17 μl of Elution Buffer to immerse the beads, and mix the beads thoroughly by pipetting up and down.
10. Incubate the mixture at room temperature for 2 min. If the beads are over-dried and cracked, prolong the incubation time.
11. Centrifuge the PCR tube briefly and place it on the magnetic separation device to separate the beads from the solution until it becomes clear (about 5 min).
    ▲ If a small number of beads are no longer adsorbed on the magnetic separation device resuspend the beads by pipetting the supernatant and then continue to incubate until no beads are left in the supernatant.

12. Carefully transfer 15 μl of the supernatant into a new low-adsorption EP tube and store it at -20℃.

cDNA amplification product analysis

The yield and size distribution are important indicators of qualified cDNA amplification products. The Agilent 2100 Bioanalyzer is recommended for the analysis. Analyze 1 μl of purified cDNA amplification product using the Agilent 2100 Bioanalyzer and the High Sensitivity DNA Chip. Refer to the IFU of the High Sensitivity DNA Chip for the operating instructions. In general, depending on the starting template used, one reaction can output 2 - 20 ng of amplification products, distributing between 400 - 10,000 bp with a library peak located at about 2,000 bp (Figure A); the negative control containing no template yields no product (Figure B).



10/FAQ & Troubleshooting

◇ Is this kit applicable to plant cell samples for direct amplification?

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

◇ Is this kit applicable to fixed cells for direct amplification?

No. After treatment with formaldehyde or acetone, the quality of RNA in tissues or cells declines significantly, causing amplification failure.

◇ Can cell samples be amplified with this kit directly without being rinsed with PBS?

For cultured cells, please test whether the culture medium has any inhibitory effect on the reaction before the experiment. The culture medium expected to be included in the cell sample can be added to the control RNA to check whether the culture medium inhibits cDNA synthesis. If the culture medium does not contain components that inhibit the synthesis of cDNA, the cells can be directly pipetted and used. If the effect of the culture medium on the reaction cannot be confirmed, it is recommended to resuspend the cells in PBS before lysis and reaction. For other types of cells, test whether the components in the cell sample inhibit the reaction as described above.

◇ Can I preserve the cell samples if I cannot perform amplification in time after taking them out? How should I process the cell samples?

If you need to store the cells for a period of time before performing the reaction, please refer to 09-1/First-strand cDNA Synthesis/Step 2 and 3 for the operations to take. The prepared cell samples should be stored at -70℃ or lower temperature and subjected to amplification immediately after being taken out. Isolated living cells may also be cryopreserved in cell cryopreservation solutions and recovered when needed. If such a preservation protocol is used, please check whether the cells stay alive before the reaction because dead cells undergo obvious RNA degradation, which will lead to reaction failure.

◇ How can I determine the number of amplification cycles when amplifying full-length cDNA by PCR?

The selected optimal number of amplification cycles should ensure that the amplification remains in the exponential phase. If the yield no longer increases with further amplification cycles, the reaction has reached the plateau phase. Over-amplified cDNA may lead to compromised cDNA library quality, while insufficient amplification may result in decreased cDNA yield. The number of amplification cycles should be minimized as long as enough yield is ensured. It is recommended to set up several parallel reactions with a different number of amplification cycles to determine the optimal number. For example, set up three parallel reactions, one carried out per the recommended number of amplification cycles and the other two with 2 - 3 cycles fewer or more (for example, 100 cells undergo 12, 10, and 8 amplification cycles, respectively).

◇ What is the yield of single-cell transcriptome amplification? How are the amplification products prepared into libraries for sequencing?

Since the gene expression of cells keeps changing, the cell type, viability, and cycle can all significantly affect the final cDNA yield. Generally, Discover-sc WTA Kit V2 can output 2 - 20 ng of cDNA amplification products.

It is recommended to prepare libraries for sequencing using transposases.