# *Trans*NGS® Single Cell Full Length cDNA Synthesis&Amplification Kit

**Please read the manual carefully before use.**

**Cat. No.** KC901

**Version No.** Version 3.0

**Storage:** Single cell TSO should be stored at -70°C or below for one year. The other components should be stored at -18°C or below for one year.

## Description

*Trans*NGS® Single Cell Full Length cDNA Synthesis&Amplification Kit is a reagent kit capable of obtaining amplified full-length cDNA from single cell. It is suitable for amplifying and constructing cDNA libraries from 1-500 cells or 10 pg-10 ng total RNA. The kit uses Single Cell Oligo (dT) as the reverse transcription primer and employs a reverse transcriptase with high synthesis efficiency and template-switching activity to add a special sequence to the 3' end of the cDNA, thereby obtaining full-length cDNA products. The kit is compatible with various cell types and tissue types, suitable for cell materials with varying RNA content. Typically, a single-cell library can yield 10-60 ng of amplified cDNA products.

## Feature

• Strong compatibility with different cell types, suitable for cells with low RNA content (such as immune cells).

• Strong compatibility with different tissue types, suitable for tissues that are difficult to dissociate (such as brain tissue).

• High yield in constructing single-cell libraries, excellent peak shapes, high efficiency in gene detection (FPKM > 1).

## Suitable sample types

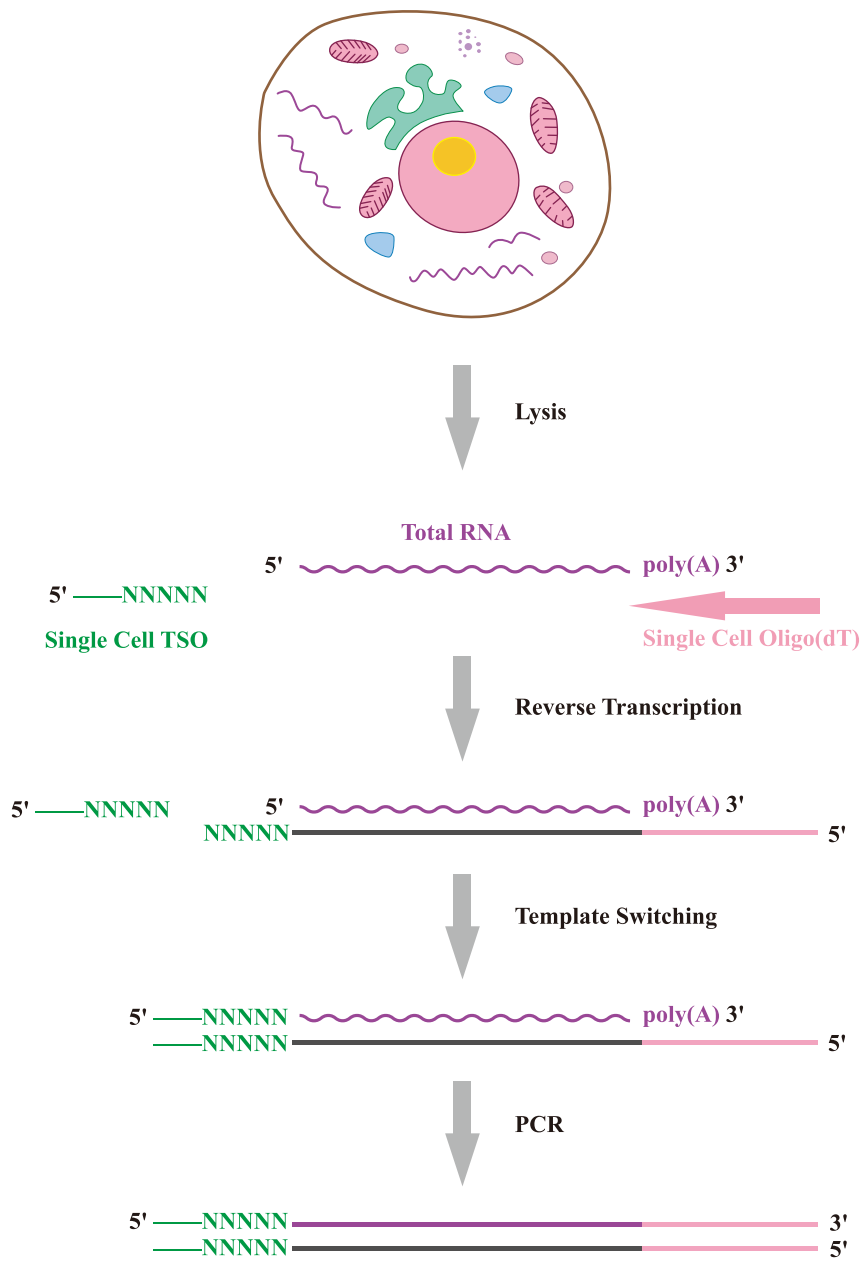
• 1-500 mammalian cells or eukaryotic cells without cell wall structures (such as protoplasts).

• 10 pg-10 ng total RNA (including mRNA with poly(A) sequences).

## Kit contents

| Component | KC901-01 (12 rxns) | KC901-02 (24 rxns) | KC901-03 (96 rxns) |
|---|---|---|---|
| ● Single Cell Lysis Buffer | 120 µl | 240 µl | 960 µl |
| ● Single Cell Oligo(dT) | 12 µl | 24 µl | 96 µl |
| ● dNTP Mix | 12 µl | 24 µl | 96 µl |
| ● Ribonuclease Inhibitors | 24 µl | 48 µl | 192 µl |
| ● Single Cell RT Buffer II | 24 µl | 48 µl | 192 µl |
| ● Single Cell TSO | 6 µl | 12 µl | 48 µl |
| ● Single Cell Reverse Transcriptase II | 12 µl | 24 µl | 96 µl |
| ● 2×Single Cell Amplification SuperMix II | 300 µl | 600 µl | 3×800 µl |
| ● Single Cell PCR Primer | 12 µl | 24 µl | 96 µl |
| ○ RNase-free Water | 1 ml | 2 ml | 5 ml |
| ○ Library Elution Buffer | 300 µl | 600 µl | 2.4 ml |

## Experimental principle schematic diagram



**Initial sample preparation**

- **For Cell Sample**

After cell collection, it is recommended to resuspend the cells in 1× PBS to remove the culture medium, as it may interfere with subsequent reactions. Cell samples should be assessed for viability using methods such as trypan blue staining.

For cells to be sorted, please label them with a viability dye for flow cytometry (such as FC301 from the recommended reagents or self-prepared calcein dye, etc.). After dye labeling, wash and resuspend the cells in PBS to avoid affecting sorting quality. Sort cells into reaction wells pre-loaded with lysis buffer using methods such as fluorescence-activated cell sorting (FACS). After sorting, centrifuge the plates as soon as possible to ensure the sorted single cells are fully immersed in the lysis buffer. Since the volumes of both the lysis buffer and the liquid carried by the single cells are very small, immediate centrifugation can prevent cells sorted near the edge of the well from drying on the wall, thereby improving the success rate of library preparation. The volume of the buffer carried by the sorted single cells is negligible. The sorted cell samples can be stored at low temperatures (<-70°C) and shipped on dry ice. It is recommended to store them for no more than half a month.

Note: Low cell viability or impure cellular environments leading to RNA degradation will reduce cDNA yield, result in smaller peak sizes, and severely impact library preparation efficiency.

- **For RNA Sample**

Please assess RNA integrity using agarose gel electrophoresis or the Agilent RNA 6000 Pico Kit before proceeding. Degraded RNA will result in reduced cDNA yield, smaller peak sizes, and significantly impact library construction efficiency.

**Operational steps:**

1.For N samples, prepare N+1 lysis & reverse transcription pre-reaction systems:

| Component | Volume |
|---|---|
| Single cell Lysis Buffer | 1 μl × **(N+1)** |
| Ribonuclease Inhibitors | 0.2 μl × **(N+1)** |
| Single cell oligo(dT) | 1 μl × **(N+1)** |
| dNTP Mix | 1 μl × **(N+1)** |

Add RNA or sort the cell samples into each reaction system according to the table below (total volume: 4 μl each):

| Component | Volume |
|---|---|
| Lysis & Reverse Transcription Pre-reaction System | 3.2 μl |
| Cell/RNA Sample | ≤0.8 μl |
| RNase-free Water | up to 4 μl |

(2) Gently mix the samples, incubate at 72°C for 3 minutes in a thermal cycler, after then immediately place the PCR tube on ice for 2 minutes.

**2. cDNA first-strand synthesis (perform in a clean bench)**

(1) During ice incubation, prepare the first-strand cDNA synthesis mix (total volume **6 μl**) and place it on ice.

| Component | Volume |
|---|---|
| Single Cell RT Buffer II* | 2 μl |
| Single Cell TSO | 0.5 μl |
| Single Cell Reverse Transcriptase II | 1 μl |
| RNase-free Water | 2.5 μl |

*Single Cell RT Buffer II should be completely thawed **at room temperature** and **thoroughly vortexed** before use.

(2) Add 6 µl of the first-strand cDNA synthesis mix to the step 1 samples that have completed the 2-minute ice incubation (operate on ice), gently mix with a pipette, and centrifuge briefly, reaching a total volume of 10 µl.

(3) Run the following program in a thermal cycler (with a heated lid set at 85°C).

| Temperature | Time |
|---|---|
| 42°C | 90 min |
| 85°C | 5 min |
| 4°C | Hold |

### 3. Full-length cDNA amplification (perform in a clean bench)

(1) Add the following components to the samples that have completed the step 2.(3) (operate on ice), with a total volume of **50 µl**.

| Component | Volume |
|---|---|
| 2×Single Cell Amplification SuperMix II | 25 µl |
| Single Cell PCR Primer | 1 µl |
| RNase-free Water | 14 µl |

(2) Vortex the sample thoroughly and centrifuge briefly. Run the following program in a thermal cycler machine (with a heated lid set at 105°C).

| Temperature | Time | Cycle |
|---|---|---|
| 98°C | 3 min | 1 |
| 98°C<br>67°C<br>72°C | 15 sec<br>20 sec<br>3 min | X* |
| 72°C | 5 min | 1 |
| 4°C | Hold | 1 |

*The recommended amplification number of cycles are as follows, and they can be adjusted accordingly based on the specific cell type.

| Cell count | Total RNA | Cycles |
|---|---|---|
| 1 cell | 10 pg | 17-18 |
| 10 cells | 100 pg | 13-14 |
| 100 cells | 1 ng | 10-11 |
| 500 cells | 10 ng | 7-8 |

### 4. Purification of full-length cDNA products

It is recommended to use 0.6× *MagicPure*® Size Selection DNA Beads (Cat. No. EC401) for the purification of full-length cDNA products. The specific steps are as follows:

(1) Remove the magnetic beads from 2-8°C and stay still for 30 minutes at room temperature before use.

(2) Vortex the beads thoroughly, pipet **30 µl** of beads (**0.6×**) to 50 µl of PCR products.

(3) Pipet to mix well, stay still for 5 minutes at room temperature.

**Note:** Insufficient mixing will significantly affect experimental results.

(4) Place the centrifuge tube on the magnetic stand and stay still at room temperature until the solution is clear (about 5 minutes). Make sure the beads settle to the magnet completely. Discard the supernatant.

**Note:** Spin down briefly before put on magnetic stand if there is liquid on the wall. Make sure the beads settle to the magnet completely. Be careful not to disturb the beads, otherwise will affect the final yield.

(5) Keep the centrifuge tube on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to the centrifuge tube, do not pipet the beads, stay still for 30 seconds at room temperature, and discard the supernatant.

**Note:** Be sure to use freshly prepared ethanol, otherwise it will affect the experimental results.

(6) Repeat step (5) once.

(7) Air dry the beads at room temperature while the centrifuge tube is on the magnetic stand.

**Note:** Do not heat dry the beads, as it may affect the final yield.

(8) Remove the centrifuge tube from the magnetic stand, add 22 µl of Library Elution Buffer. Mix by pipetting or vortexing, stay still for 3 minutes at room temperature.

(9) Place the centrifuge tube on the magnetic stand and stay still at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

**Note:** Spin down briefly before put on magnetic stand if there is liquid on the wall. Incubation time can be extended to 5 minutes at room temperature.

(10) Carefully pipet 20 µl of cDNA and transfer it to a clean centrifuge tube for yield and peak identification.

(11) It is recommended to use 1 ng of purified cDNA for Tn5 sequencing library preparation, and the remaining samples can be stored long-term at -20°C. It is recommended to use *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina® (for 1ng DNA) (Cat. No. KP111) and *Trans*NGS® Tn5 Index Kit for Illumina® (Cat. No. KI101).

## Appendix

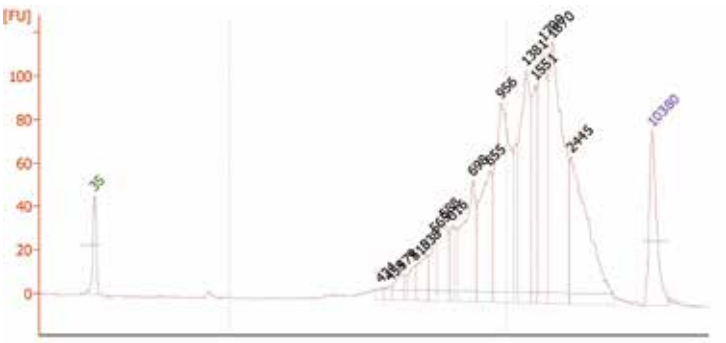



Figure 1: Peak shape of full-length cDNA product from a 293T single cell



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