

Liquid-phase nonTS/TAS-Seq using a reverse transcriptase having terminal transferase activity

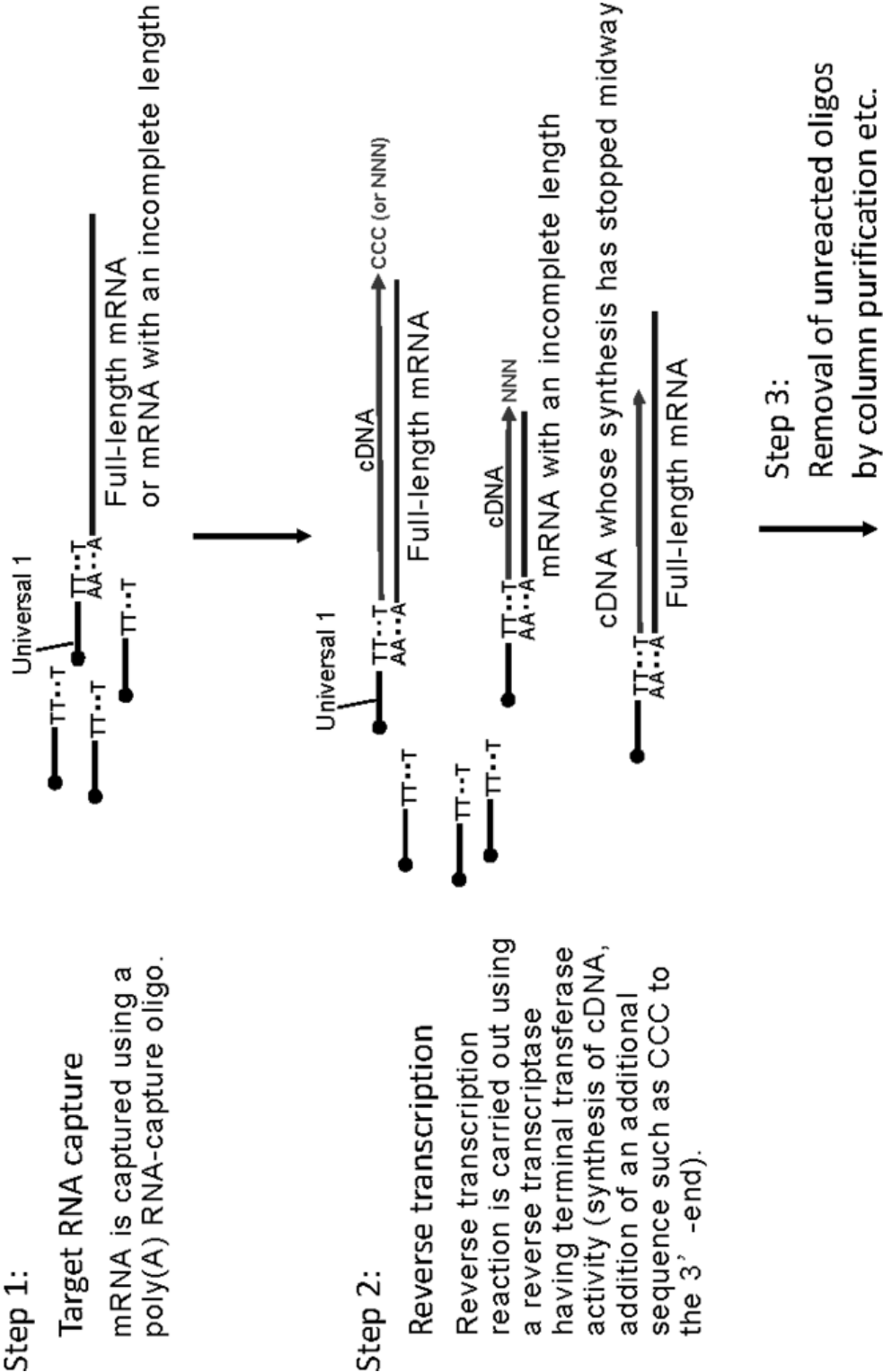


Fig.1-1

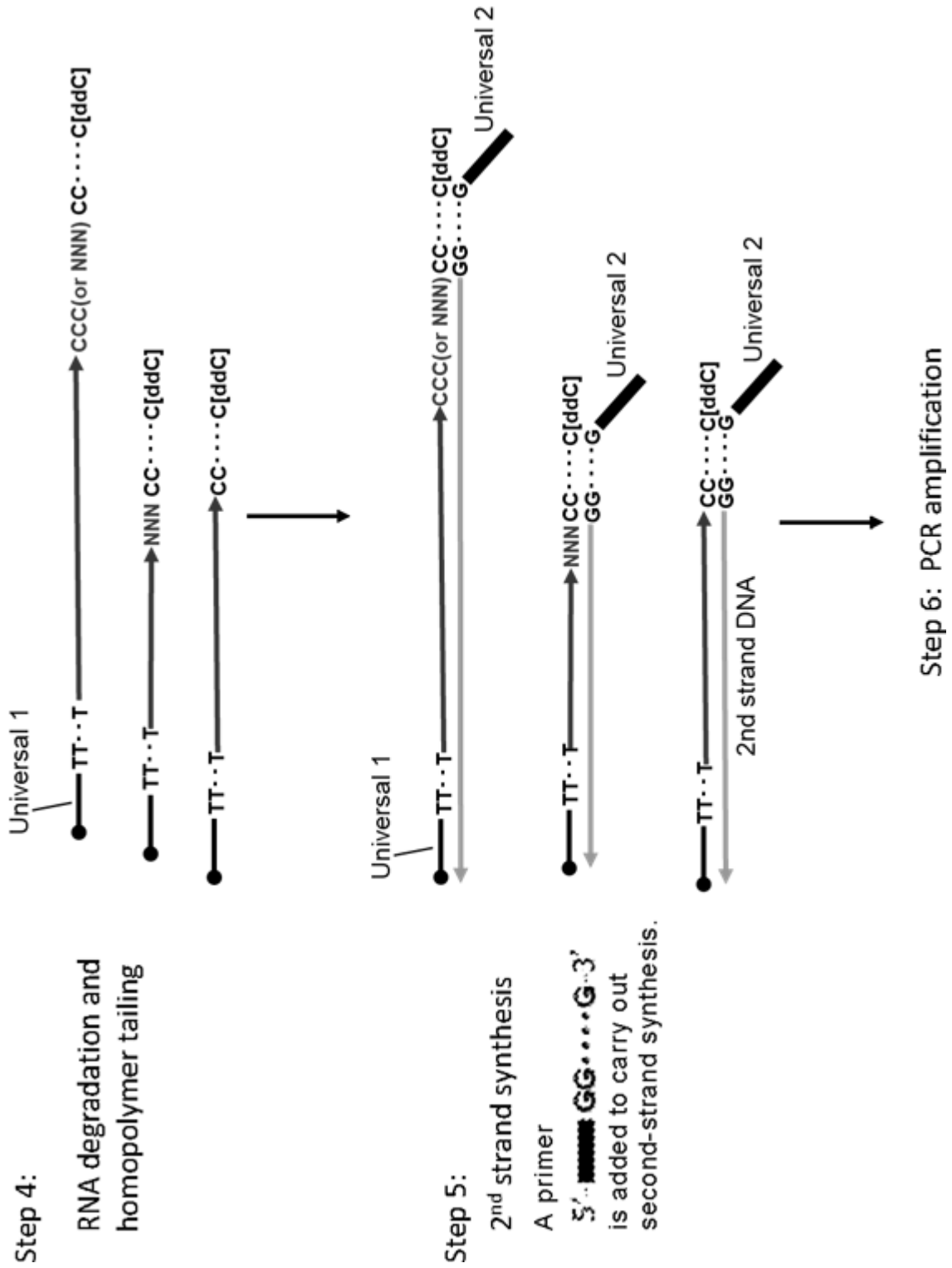


Fig.1-2

TS/TAS-Seq (solid-phase system) using a reverse transcriptase having terminal transferase activity but having no strand displacement activity

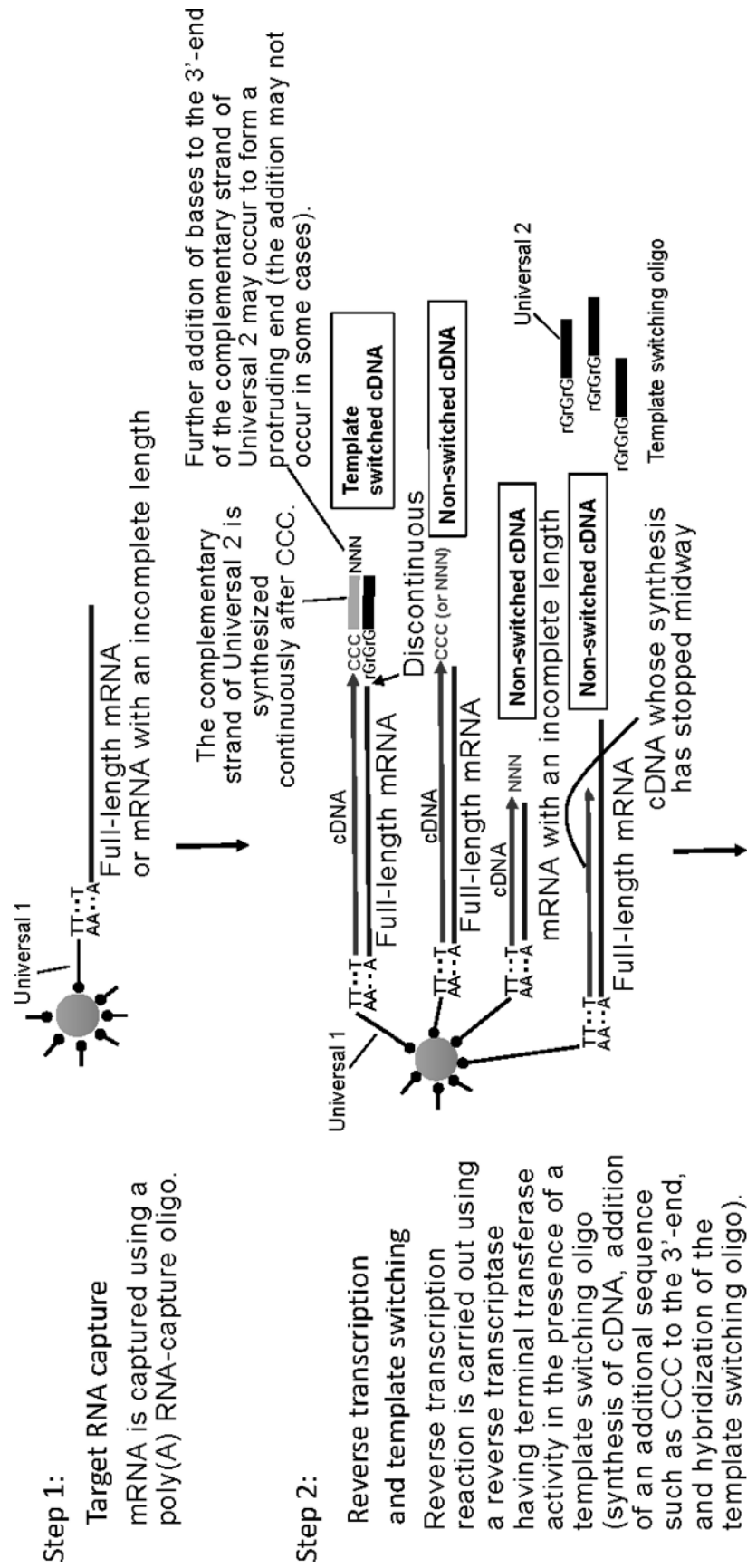


Fig.2-1

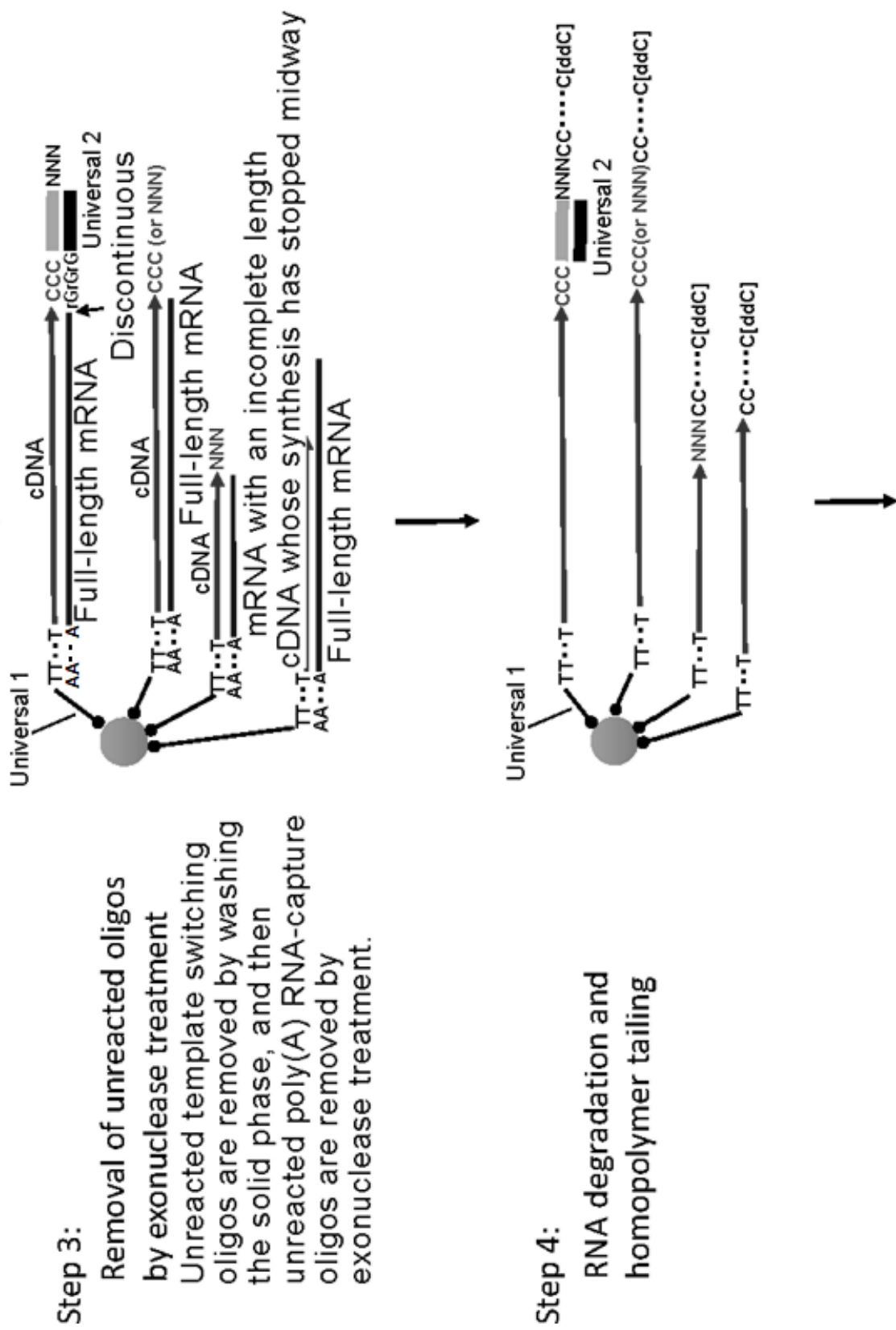


Fig.2-2

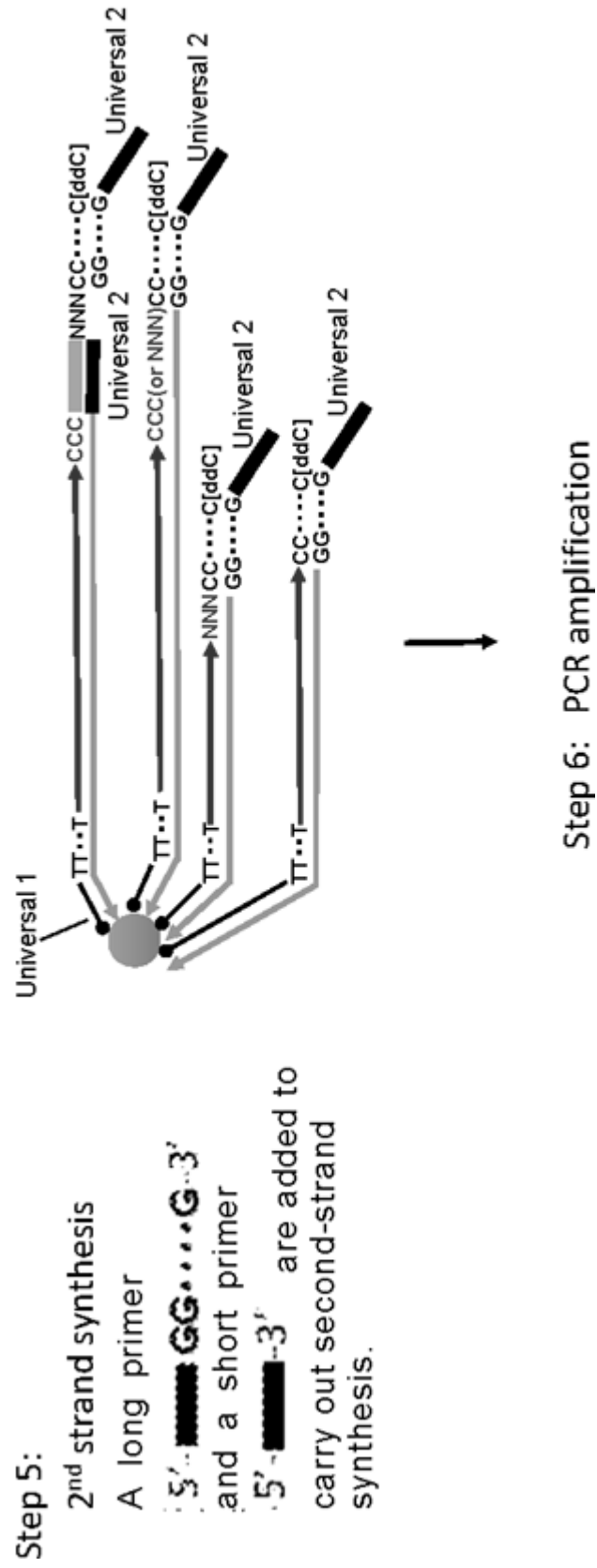


Fig.2-3

TS/TAS-Seq (solid-phase system) using a reverse transcriptase having terminal transferase activity and strand displacement activity

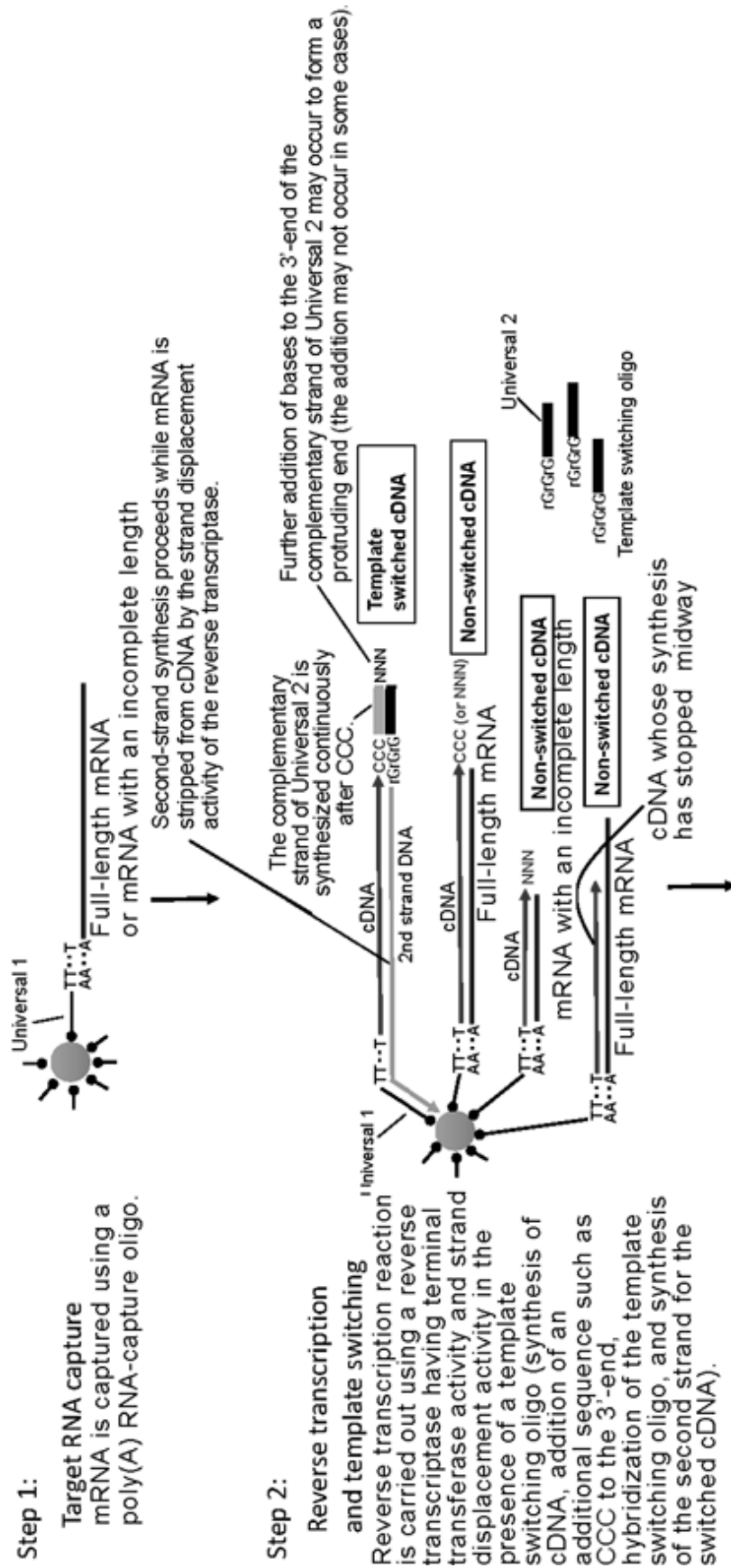


Fig.3-1

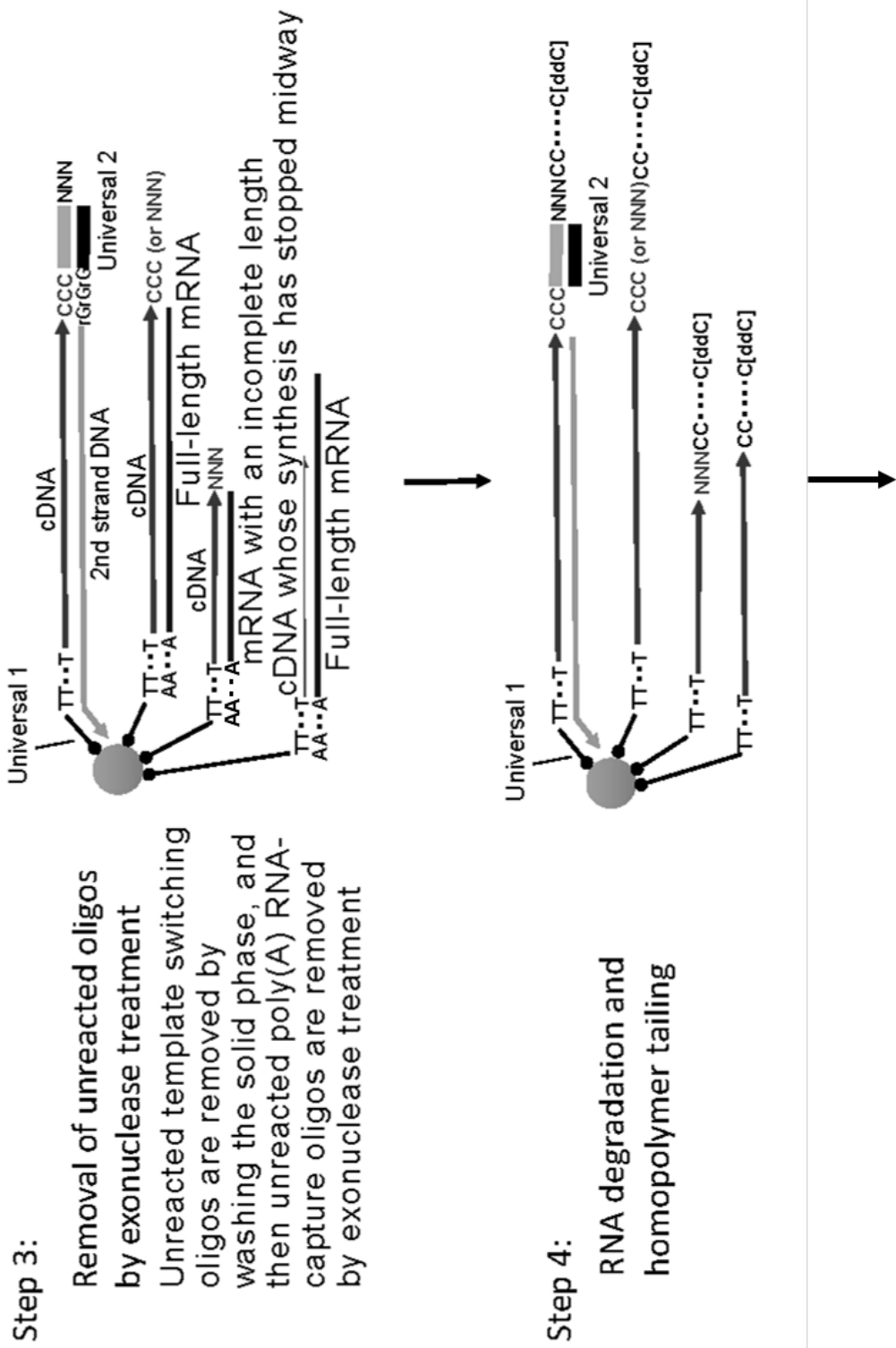


Fig.3-2

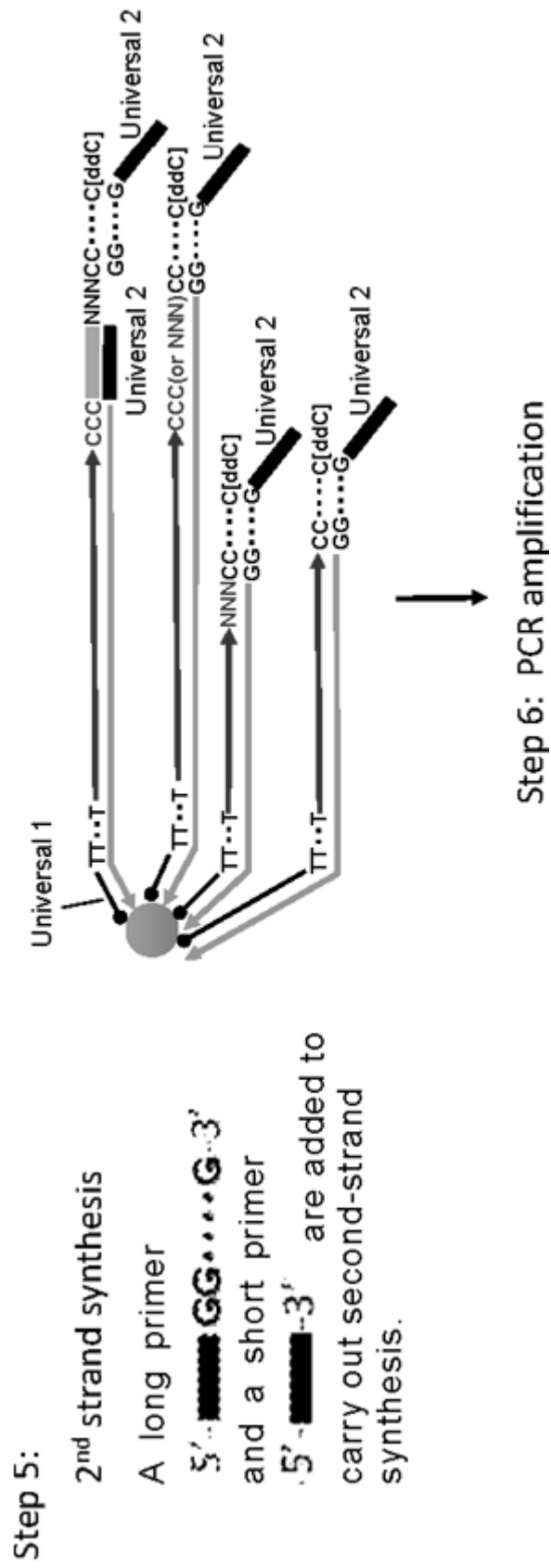


Fig.3-3

TS/TAS-Seq (liquid-phase system) using a reverse transcriptase having terminal transferase activity but having no strand displacement activity

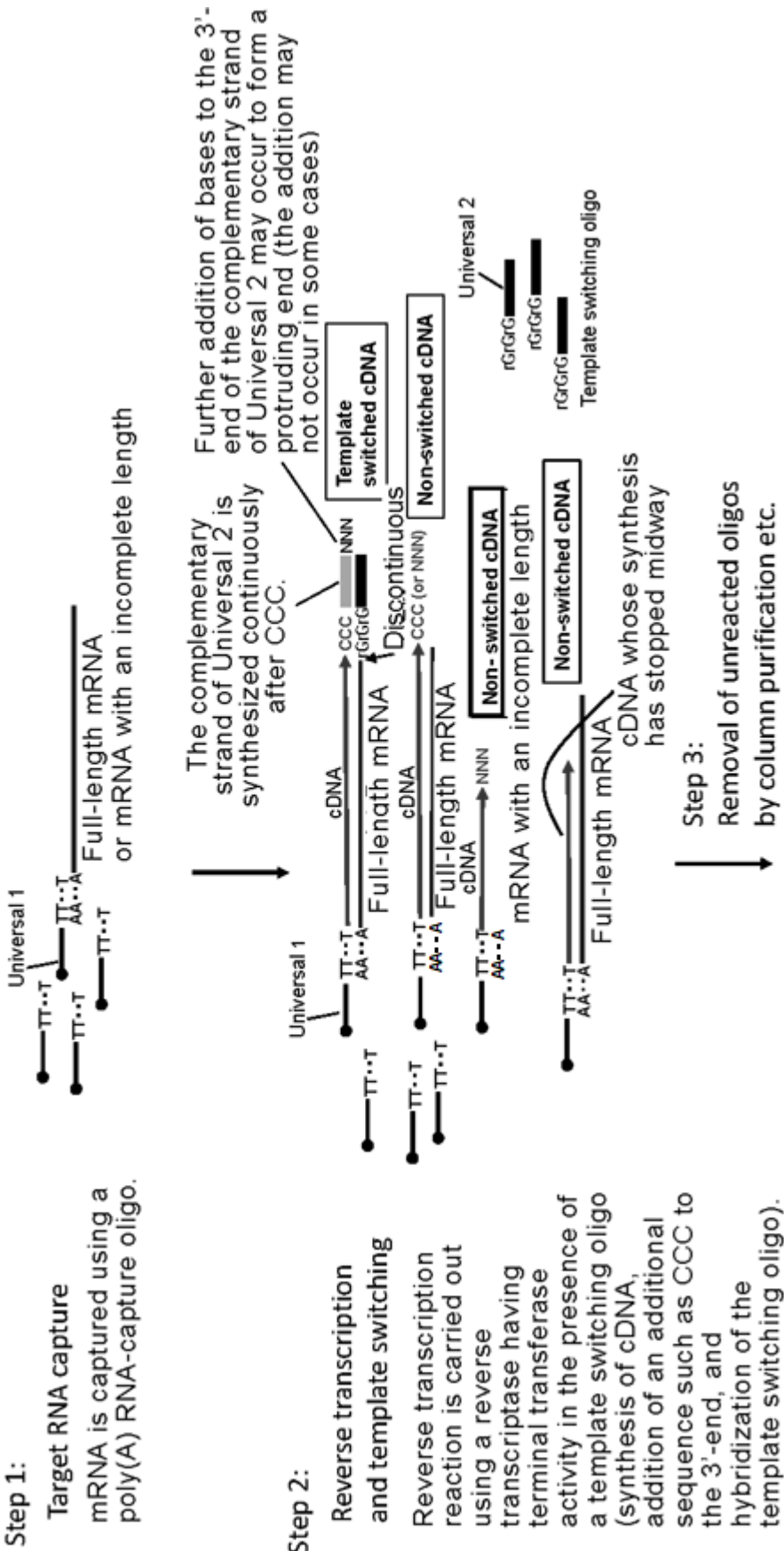


Fig.4-1

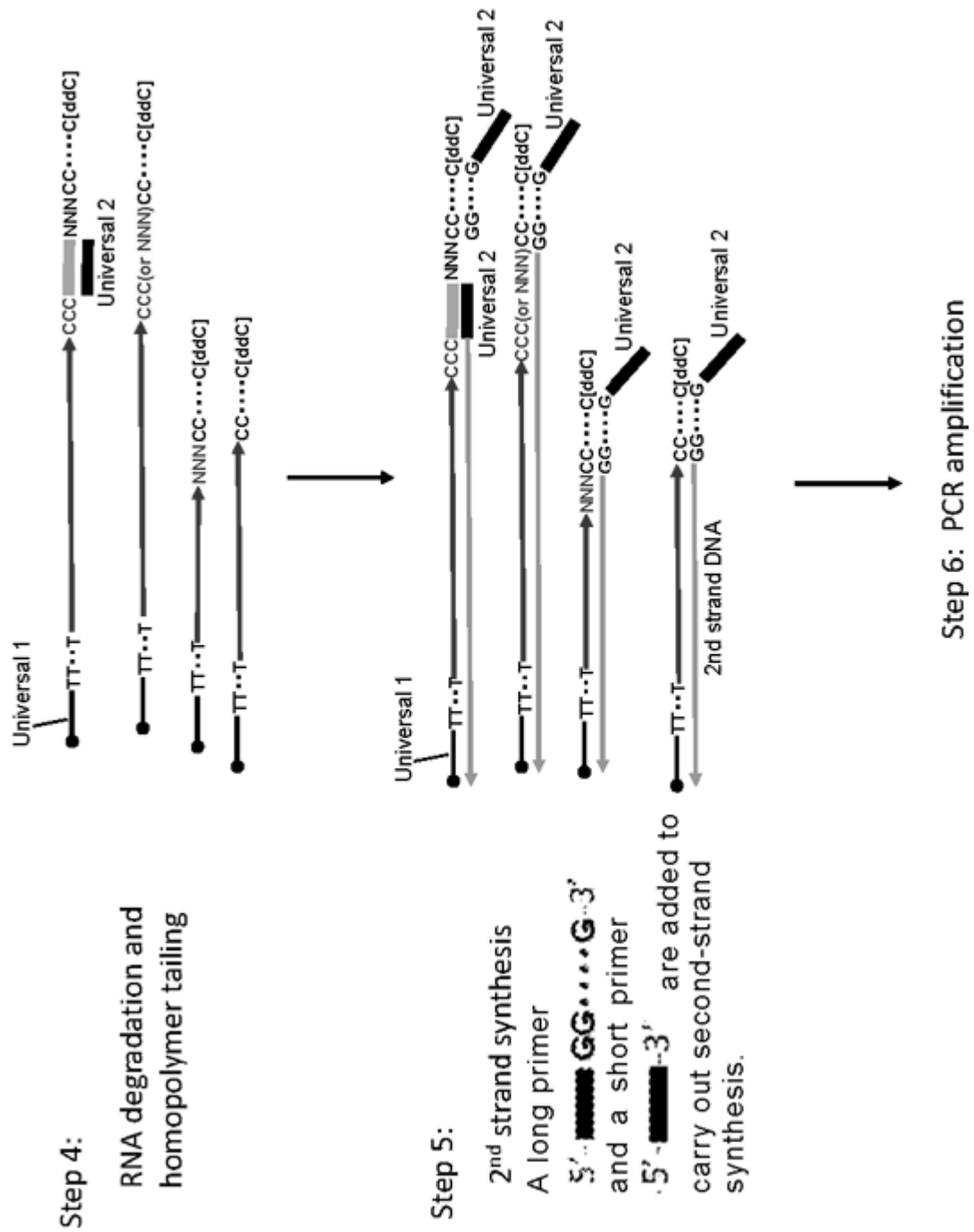


Fig.4-2

Step 1:

Target RNA capture

mRNA is captured using a poly(A) RNA-capture oligo.

The complementary strand of Universal 2 is synthesized continuously after CCC.

Full-length mRNA or mRNA with an incomplete length

Second-strand synthesis proceeds while mRNA is stripped from cDNA by the strand displacement activity of the reverse transcriptase.

Further addition of bases (NNN) to the 3'-end of the complementary strand synthesized 2nd strand DNA may occur (or may not occur).

Further addition of bases to the 3'-end of the complementary strand of Universal 2 may occur to form a protruding end (the addition may not occur in some cases).

Step 2:

Reverse transcription and template switching

Reverse transcription reaction is carried out using a reverse transcriptase having terminal transferase activity and strand displacement activity in the presence of a template switching oligo (synthesis of cDNA, addition of an additional sequence such as CCC to the 3'-end, hybridization of the template switching oligo, and synthesis of the second strand for the switched cDNA).

Universal 1

cDNA

2nd strand DNA

ccc (or NNN)

Full-length mRNA

Non-switched cDNA

Template switched cDNA

Universal 2

rGrGg

Template switching oligo

Non-switched cDNA

Full-length mRNA

Full-length mRNA whose synthesis has stopped midway

Step 3:

Removal of unreacted oligos by column purification etc.

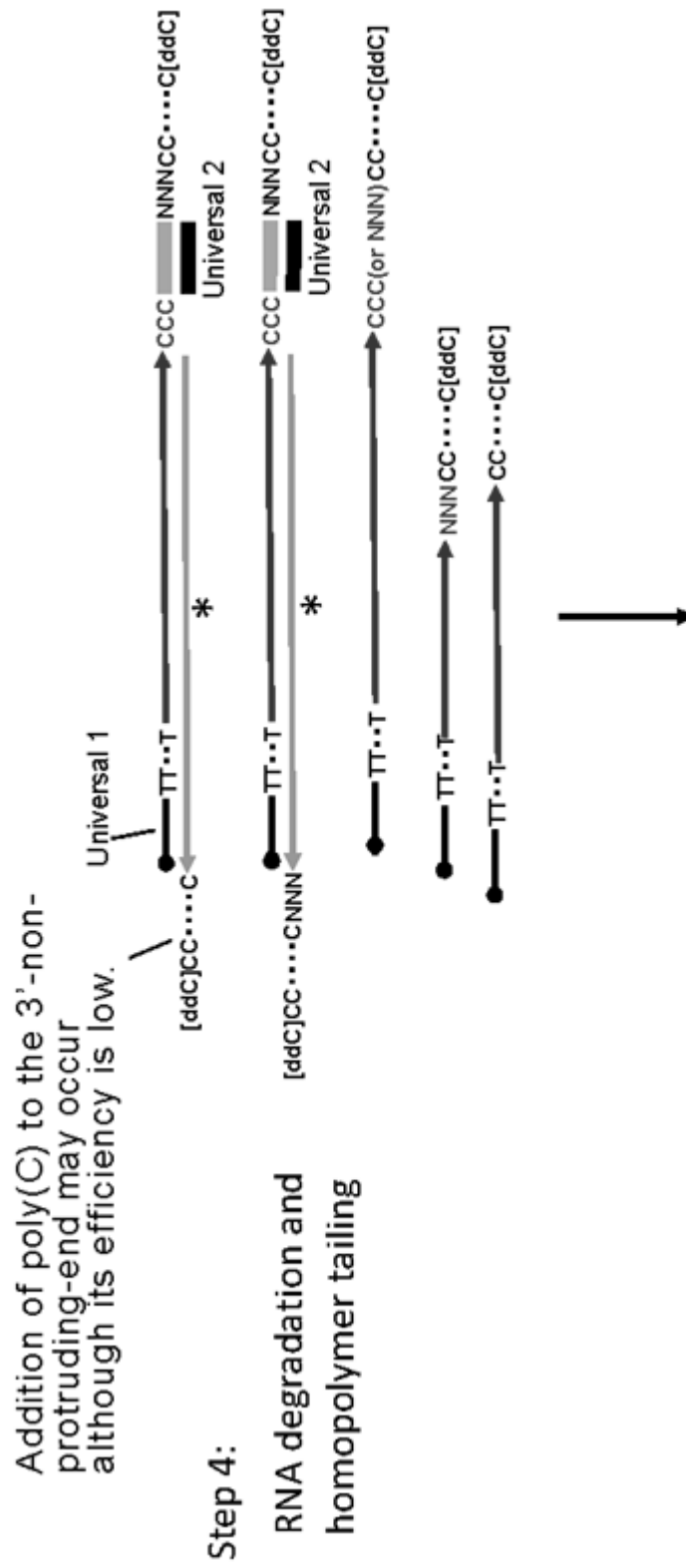


Fig.5-2

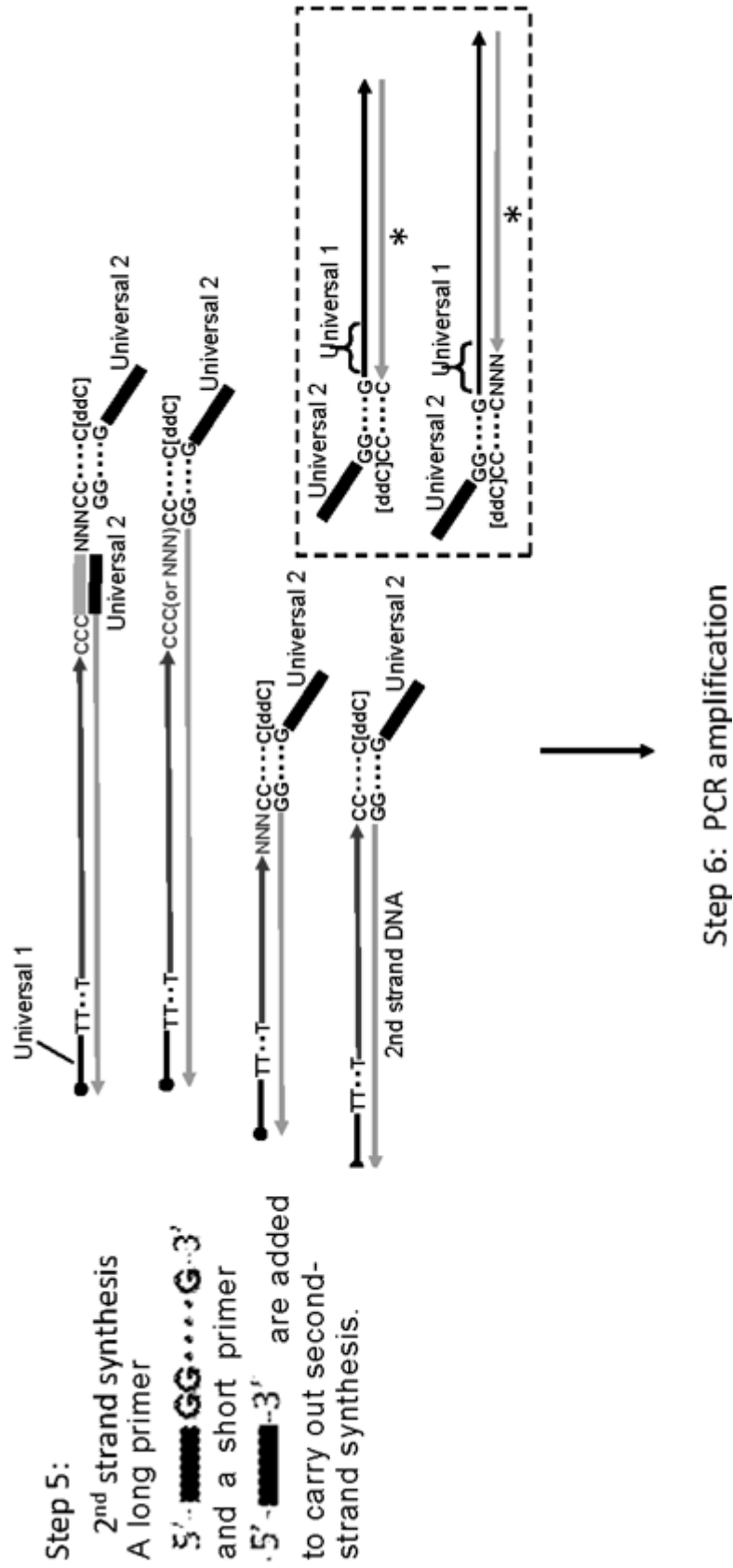


Fig.5-3

14/20

Human peripheral blood
mononuclear cells

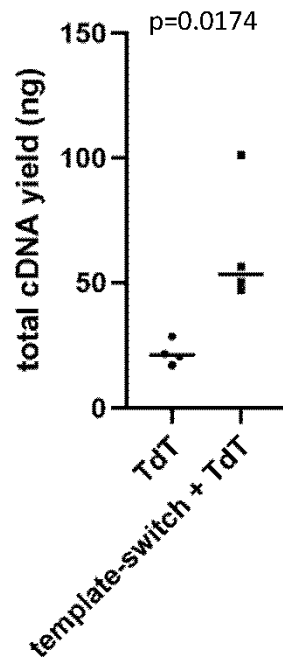


Fig.6

Human peripheral blood mononuclear cells

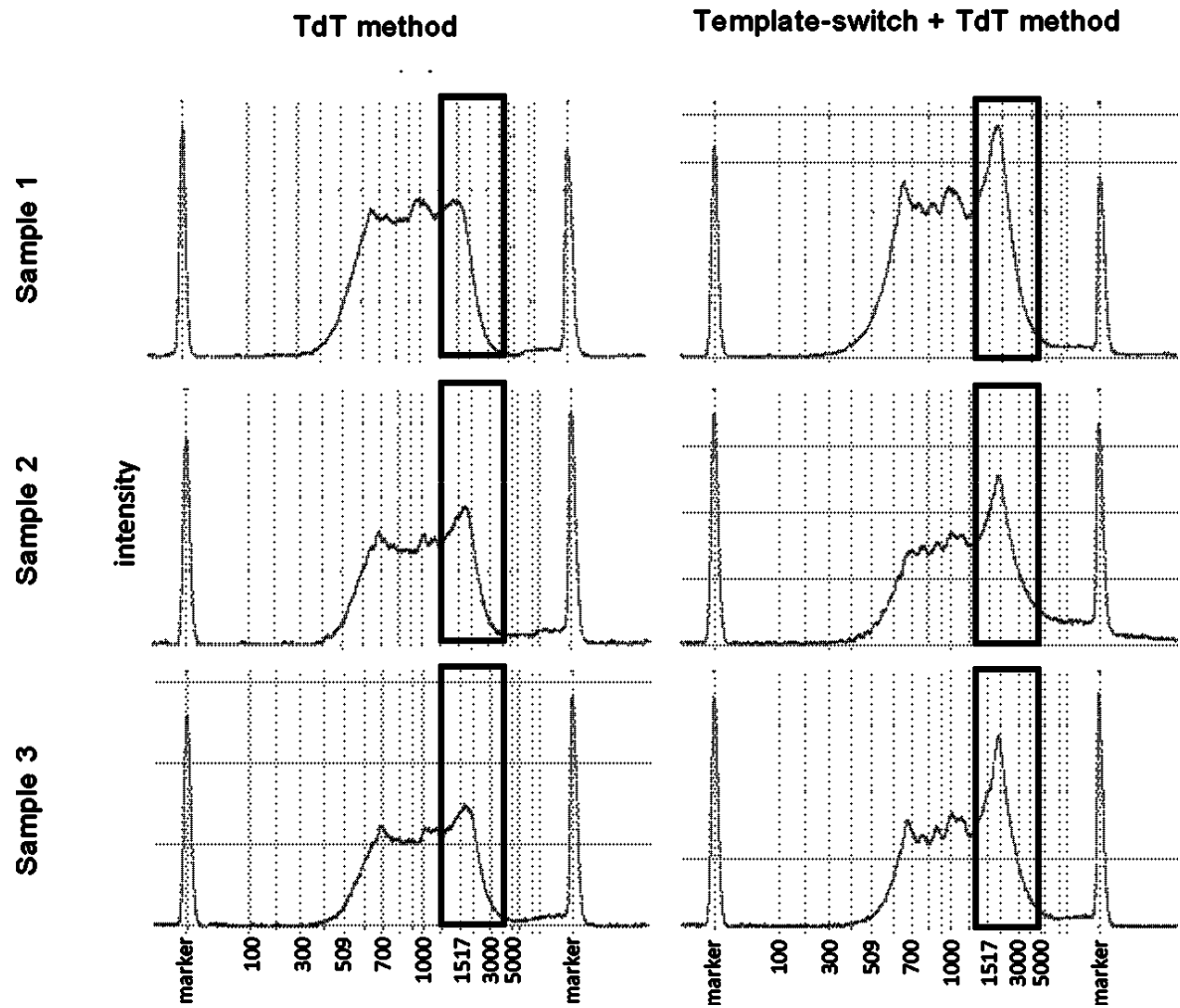


Fig.7

Human peripheral blood mononuclear cells

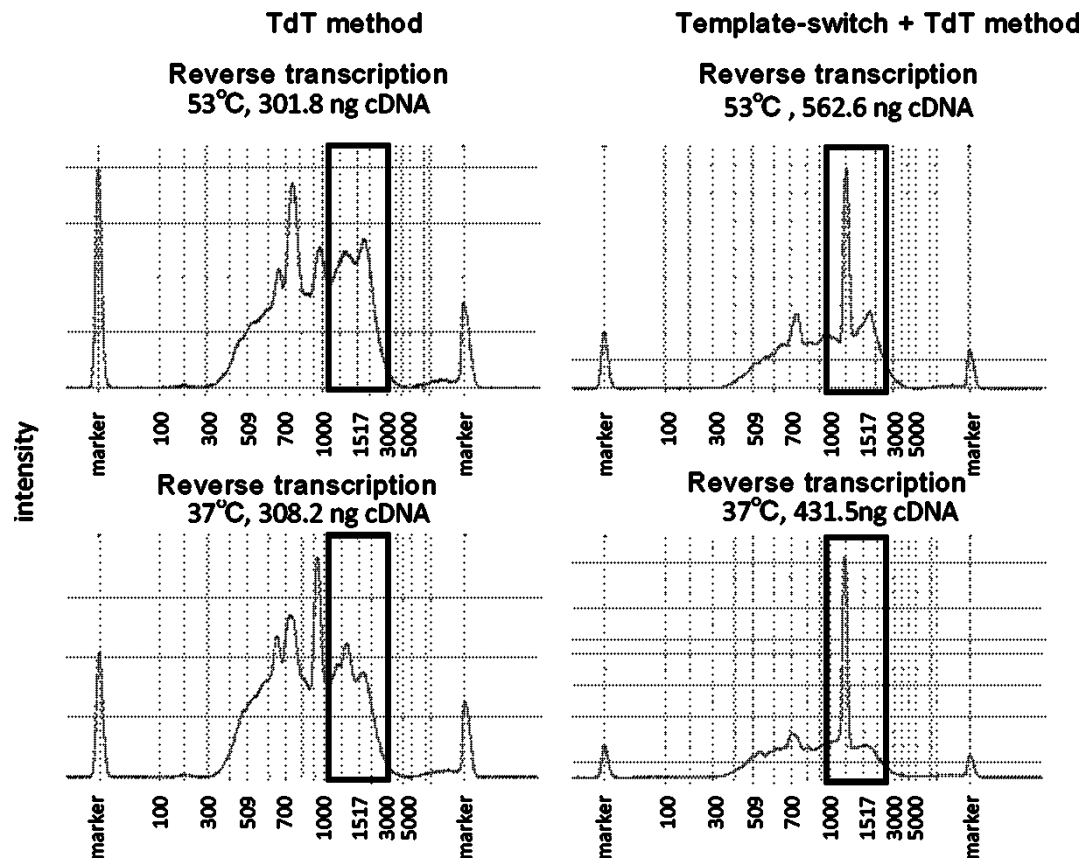


Fig.8

Total RNA derived from NIH3T3 cells

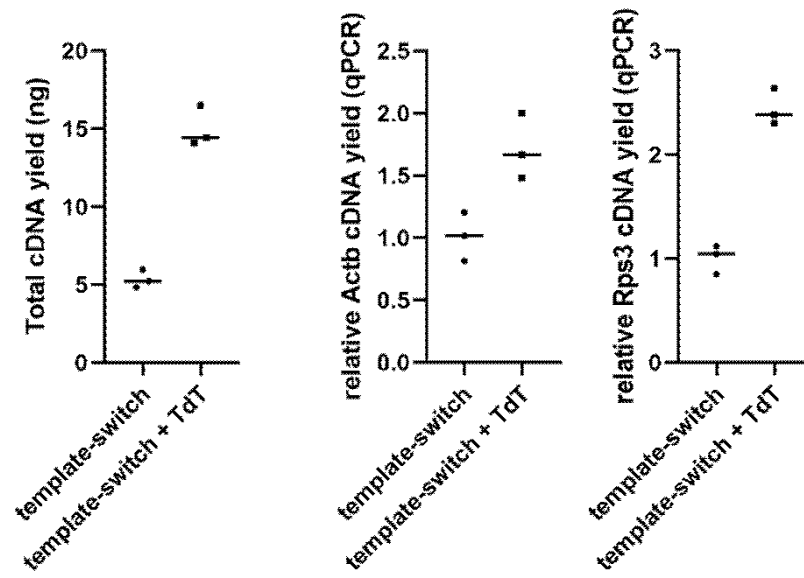


Fig.9

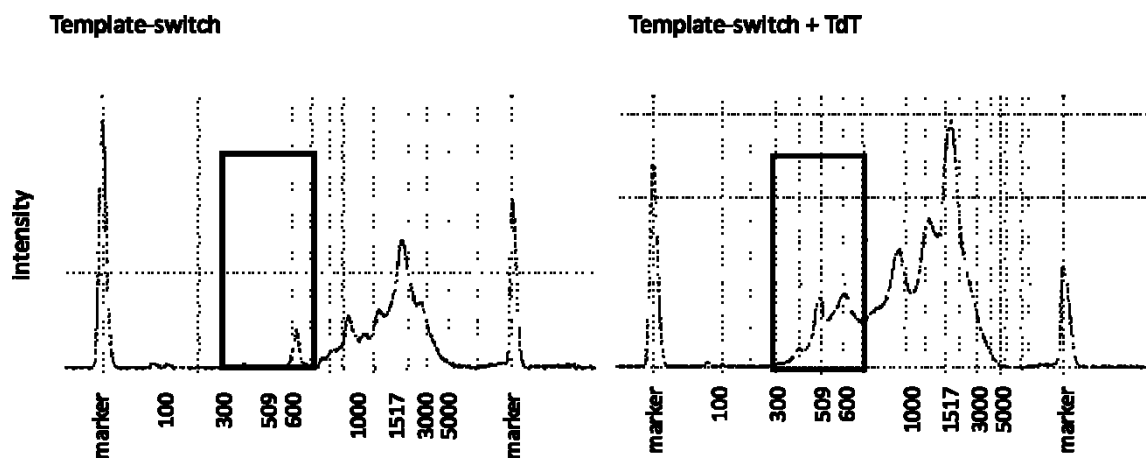
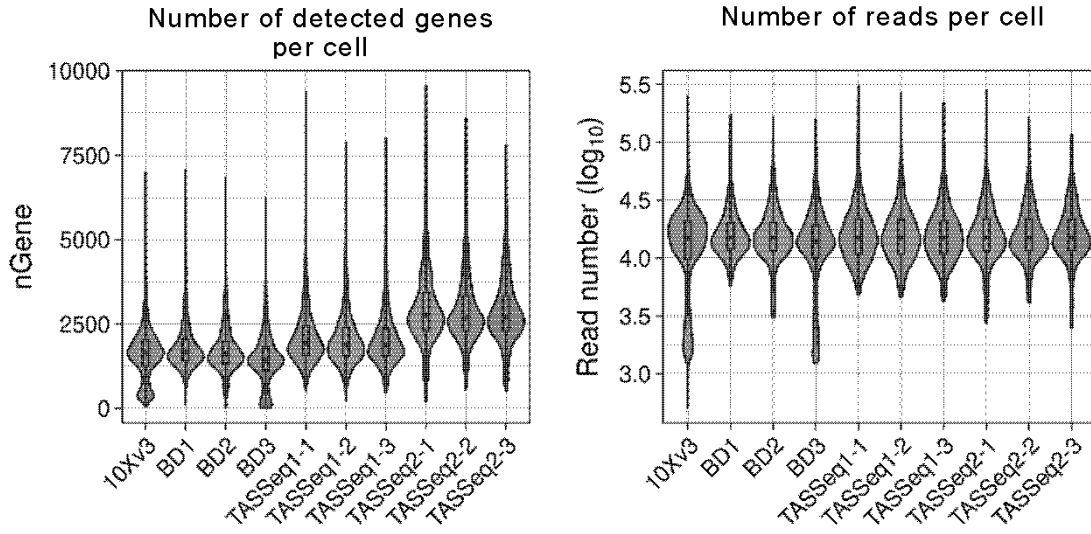


Fig.10

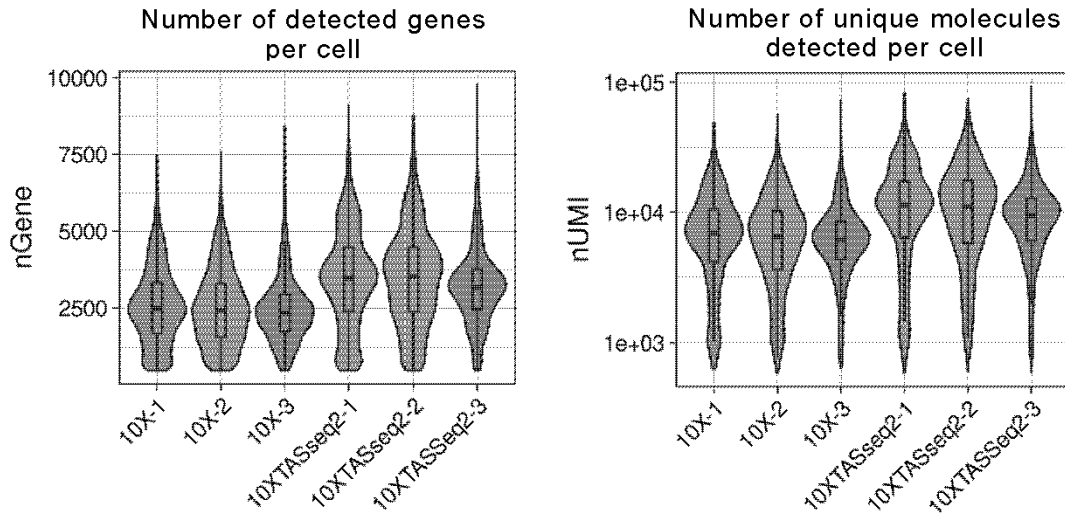
Mouse spleen cells



10X v3: 10X Chromium v3 (GSE192930) include-intron mode (template switching method, liquid phase)
 BD1-BD3 : BD Rhapsody WTA kit (random priming method, solid-phase beads)
 TAS-Seq1-1~1-3 : TAS-Seq (TdT method alone, solid-phase beads)
 TAS-Seq2-1~2-3 : TAS-Seq2(template-switch + TdT method, solid-phase beads)

Fig.11

Human peripheral blood mononuclear cells



10X: 10X Chromium v3(template switching method, liquid phase)
 10XTASSeq2: 10X Chromium v3 + TAS-Seq2(template switching + TdT method, liquid-phase)
 Each number at the end indicates the sample type.

Fig.12

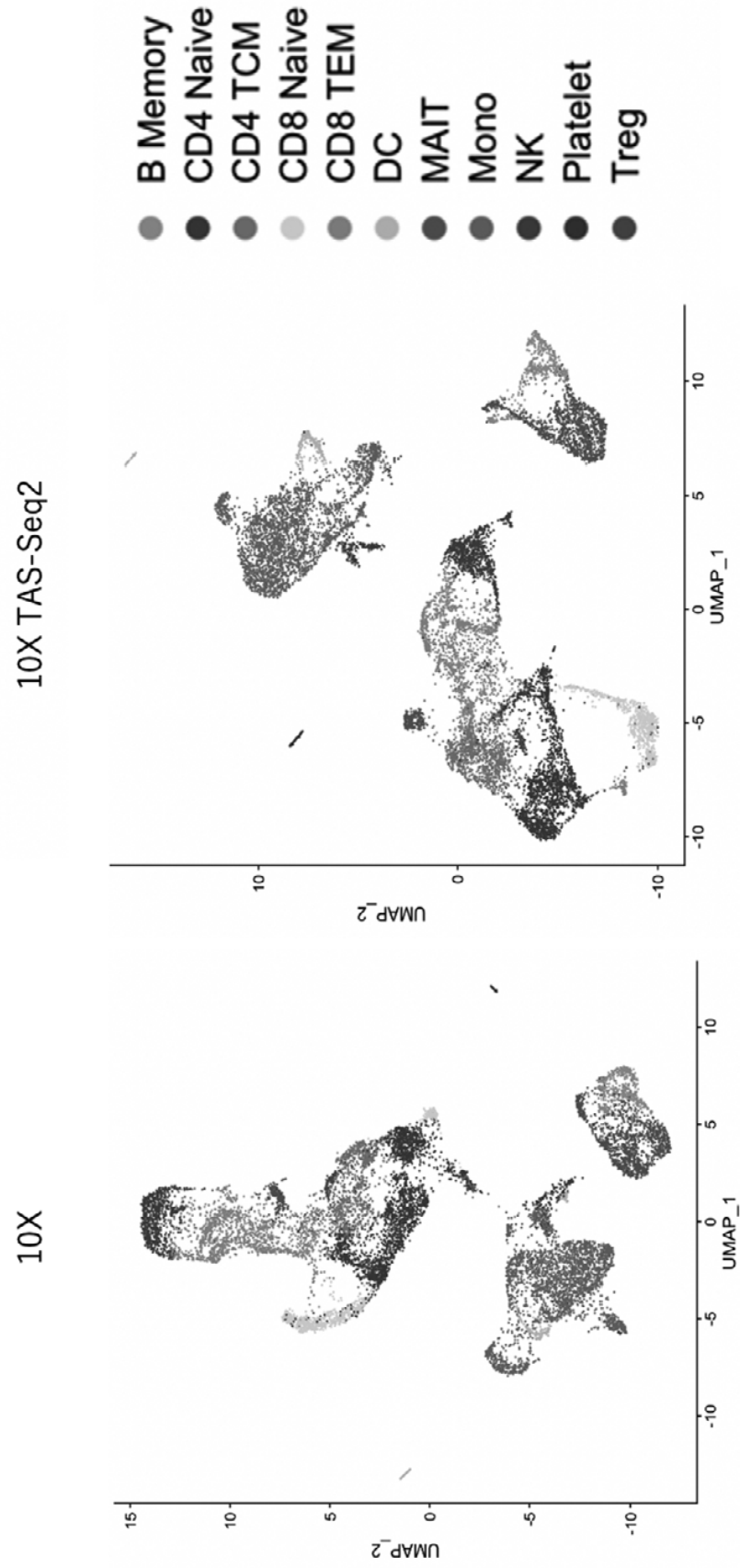


Fig.13

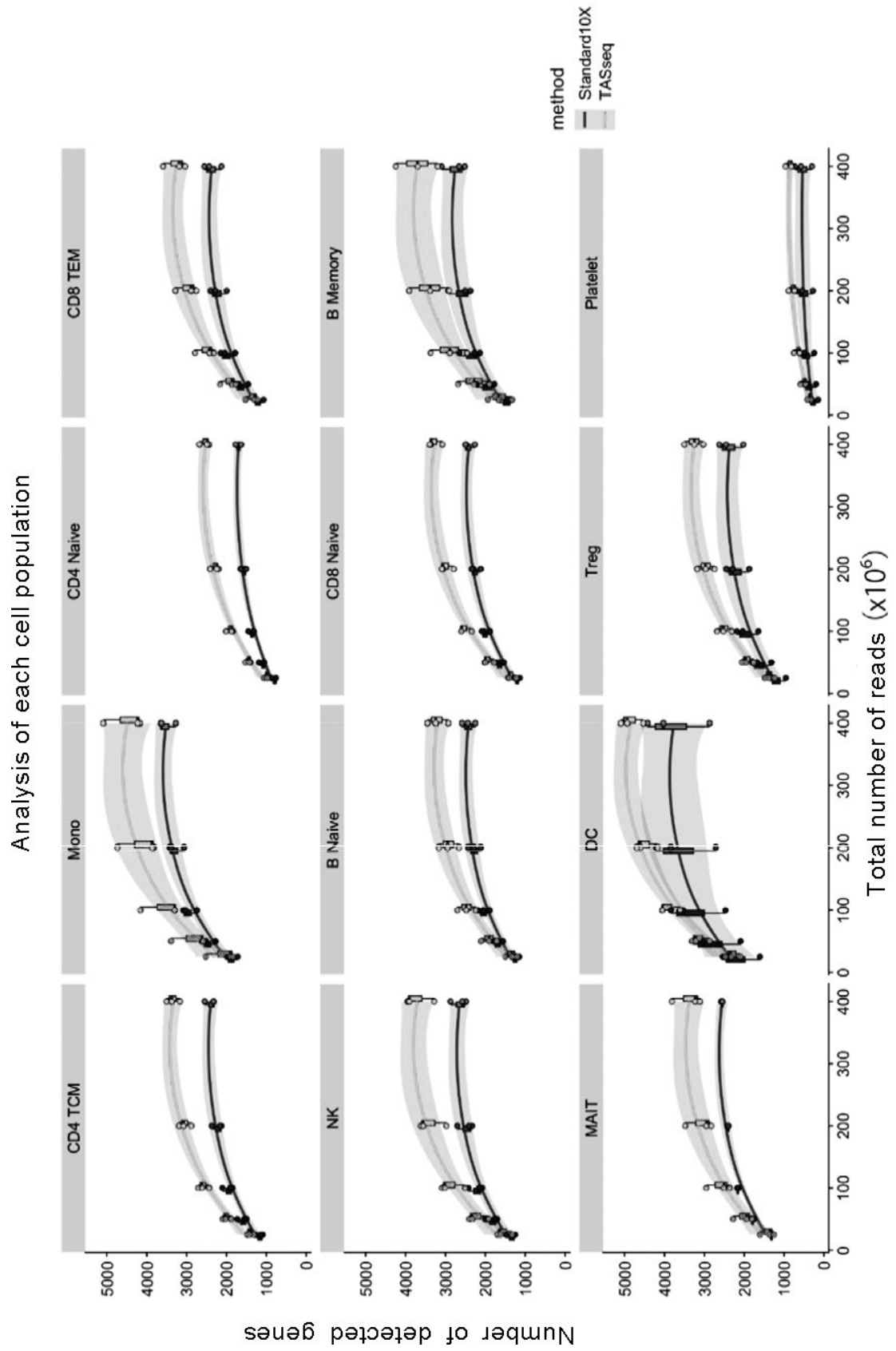


Fig.14