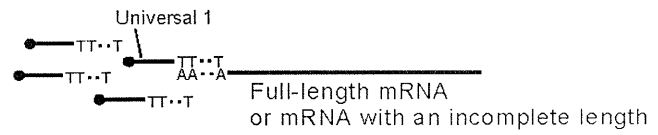


Liquid-phase nonTS/TAS-Seq using a reverse transcriptase having terminal transferase activity

Step 1:

Target RNA capture

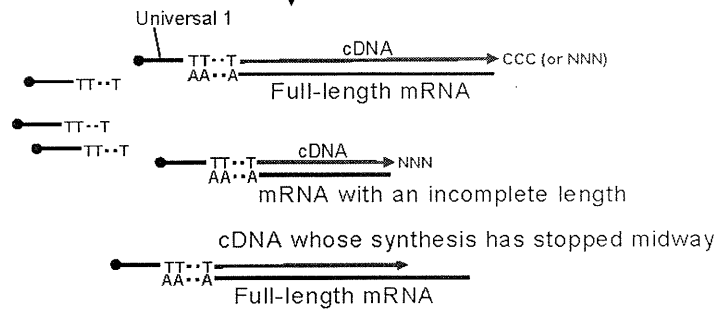
mRNA is captured using a poly(A) RNA-capture oligo.



Step 2:

Reverse transcription

Reverse transcription reaction is carried out using a reverse transcriptase having terminal transferase activity (synthesis of cDNA, addition of an additional sequence such as CCC to the 3' -end).

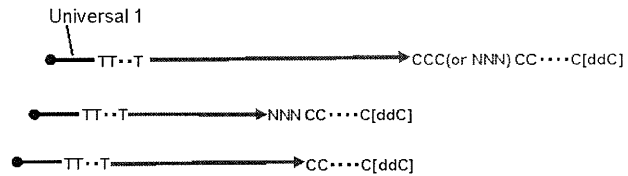


Step 3:

Removal of unreacted oligos by column purification etc.

Step 4:

RNA degradation and homopolymer tailing

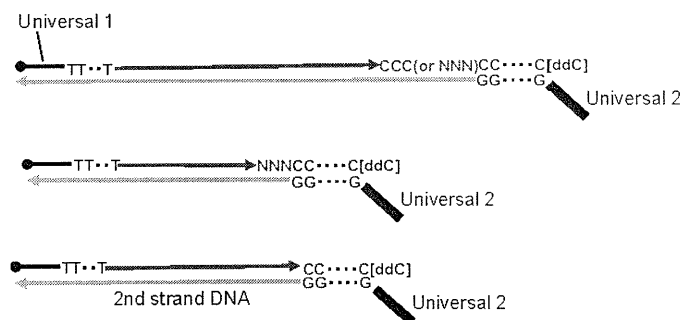


Step 5:

2nd strand synthesis

A primer

5'-[GG...G]-3' is added to carry out second-strand synthesis.



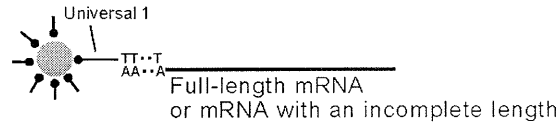
Step 6: PCR amplification

Fig.1

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

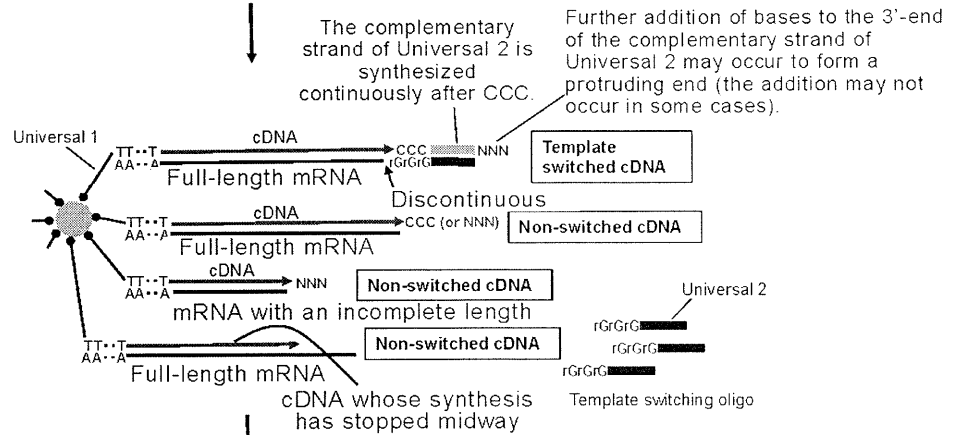
Step 1:

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



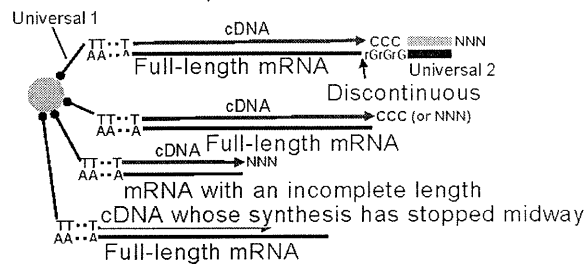
Step 2:

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



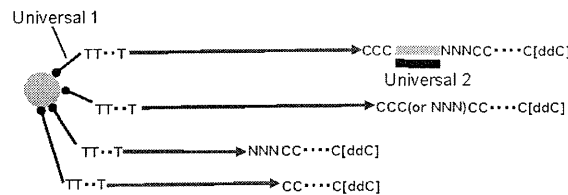
Step 3:

Removal of unreacted oligos by exonuclease treatment
Unreacted template switching oligos are removed by washing the solid phase, and then unreacted poly(A) RNA-capture oligos are removed by exonuclease treatment.



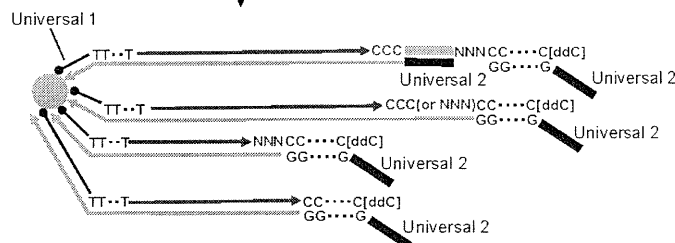
Step 4:

RNA degradation and homopolymer tailing



Step 5:

2nd strand synthesis
A long primer
5'-GG...G-3'
and a short primer
5'-...-3' are added to carry out second-strand synthesis.



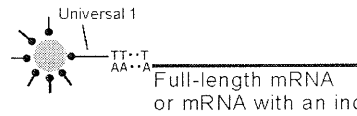
Step 6: PCR amplification

Fig.2

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

Step 1:

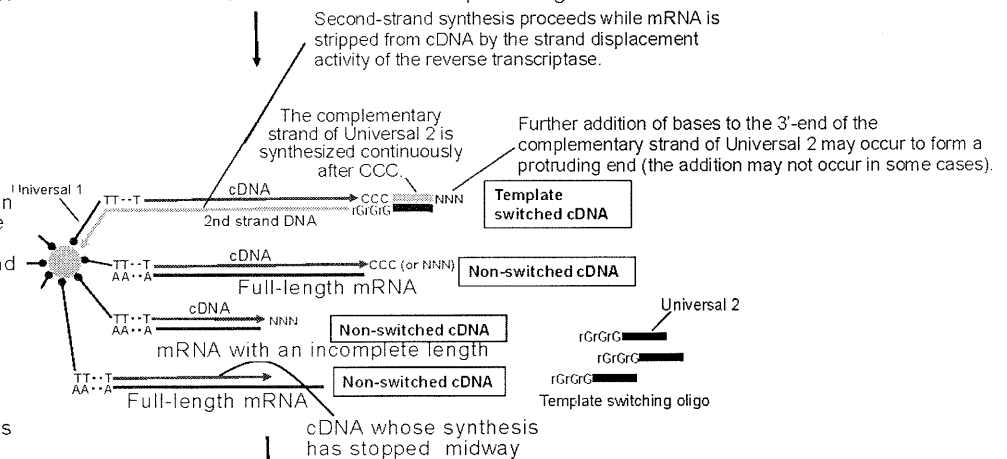
Target RNA capture
mRNA is captured using a poly(A) RNA-capture oligo.



Step 2:

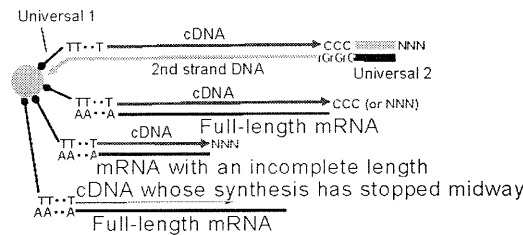
Reverse transcription and template switching

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).



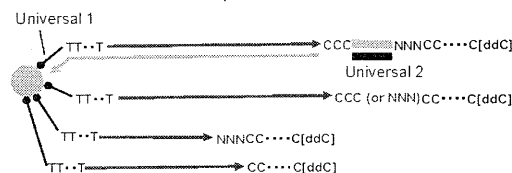
Step 3:

Removal of unreacted oligos by exonuclease treatment
Unreacted template switching oligos are removed by washing the solid phase, and then unreacted poly(A) RNA-capture oligos are removed by exonuclease treatment



Step 4:

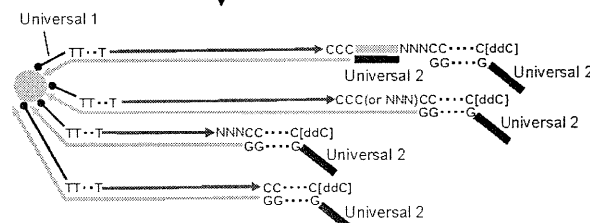
RNA degradation and homopolymer tailing



Step 5:

2nd strand synthesis

A long primer
5'-[GG...G]-3'
and a short primer
5'-[GG...G]-3' are added to carry out second-strand synthesis.



Step 6: PCR amplification

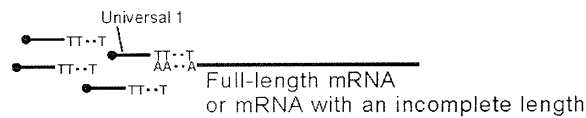
Fig.3

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

Step 1:

Target RNA capture

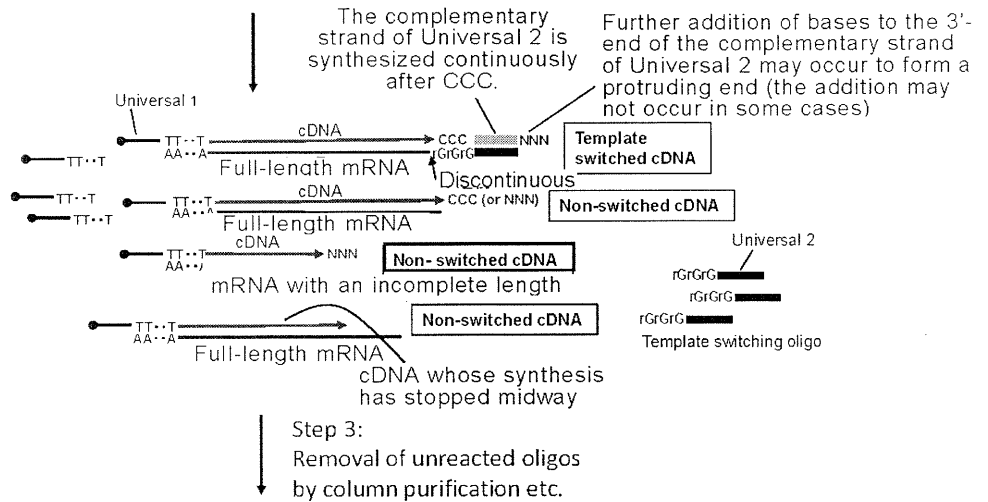
mRNA is captured using a poly(A) RNA-capture oligo.



Step 2:

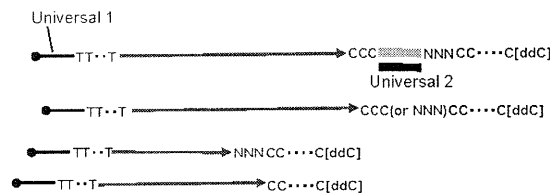
Reverse transcription and template switching

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



Step 4:

RNA degradation and homopolymer tailing



Step 5:

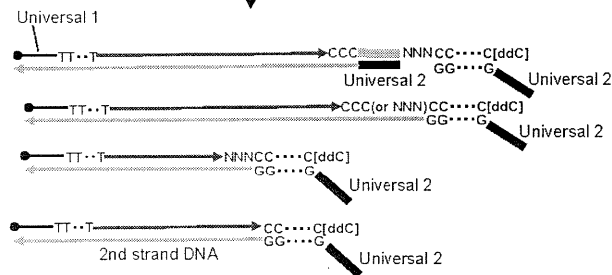
2nd strand synthesis

A long primer

5'-GG...G-3'

and a short primer

5'-...-3' are added to carry out second-strand synthesis.



Step 6: PCR amplification

Fig.4

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

Step 1:

Target RNA capture

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

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

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).

Addition of poly(C) to the 3'-non-protruding-end may occur although its efficiency is low.

Step 4:

RNA degradation and homopolymer tailing

Step 5:

2nd strand synthesis

A long primer 5'-[NNNN]GG...G-3' and a short primer 5'-[NNNN]-3' are added to carry out second-strand synthesis.

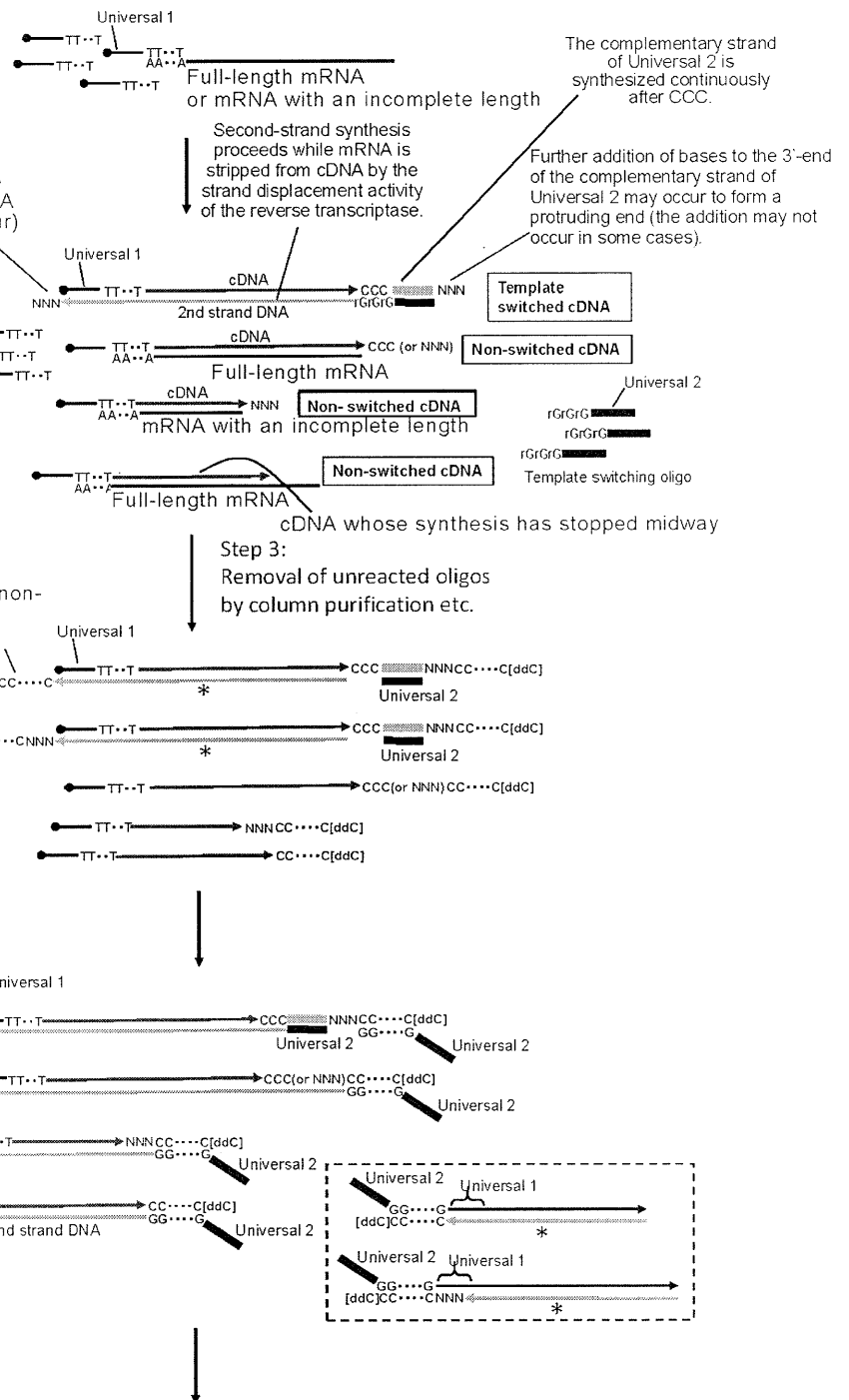


Fig.5

6/12

Human peripheral blood
mononuclear cells

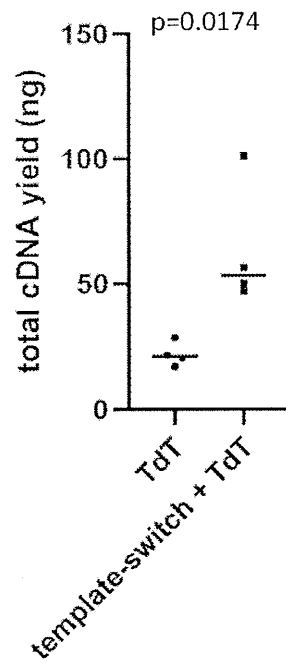
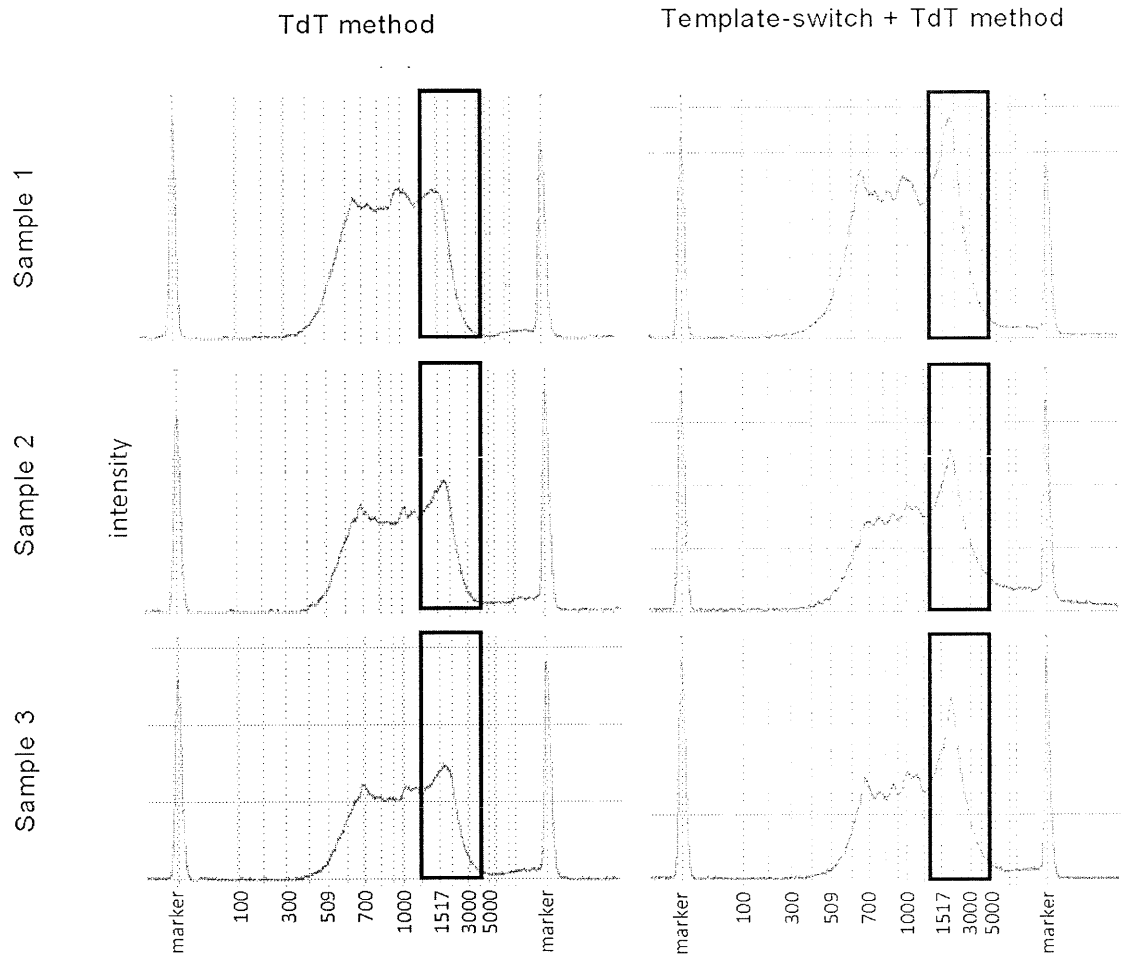
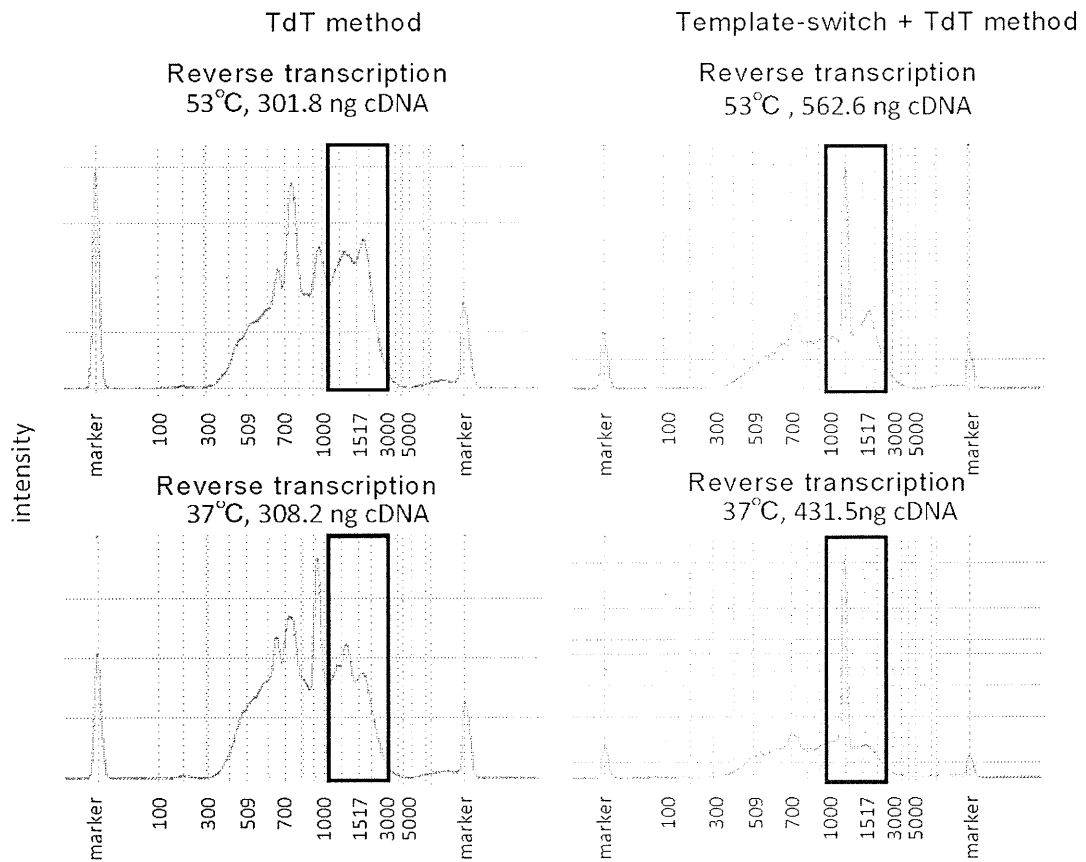


Fig.6

Human peripheral blood mononuclear cells

**Fig.7**

Human peripheral blood mononuclear cells

**Fig.8**

Total RNA derived from NIH3T3 cells

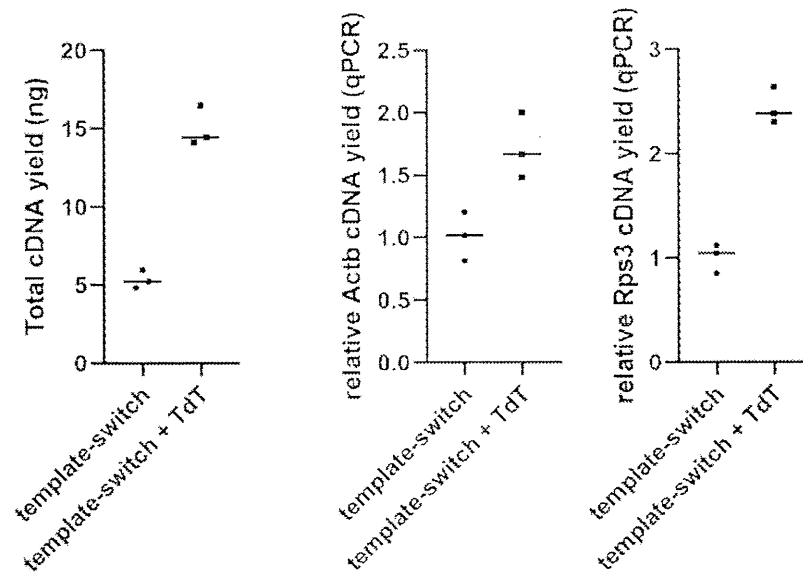


Fig.9

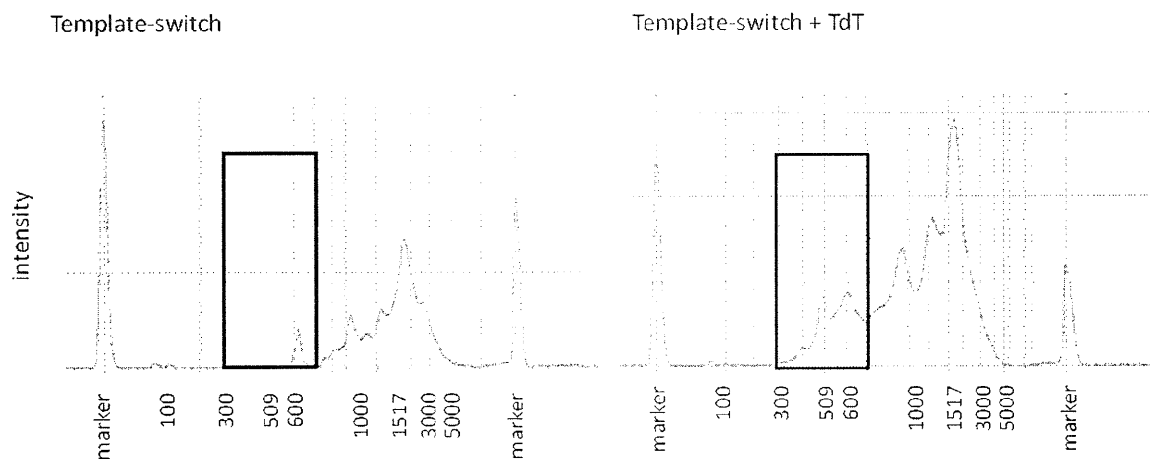
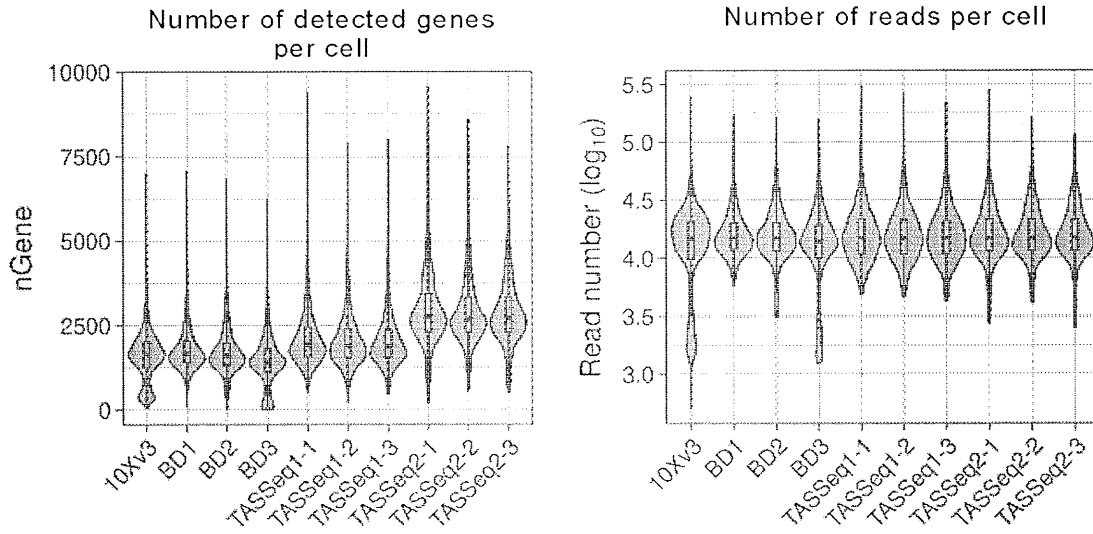


Fig.10

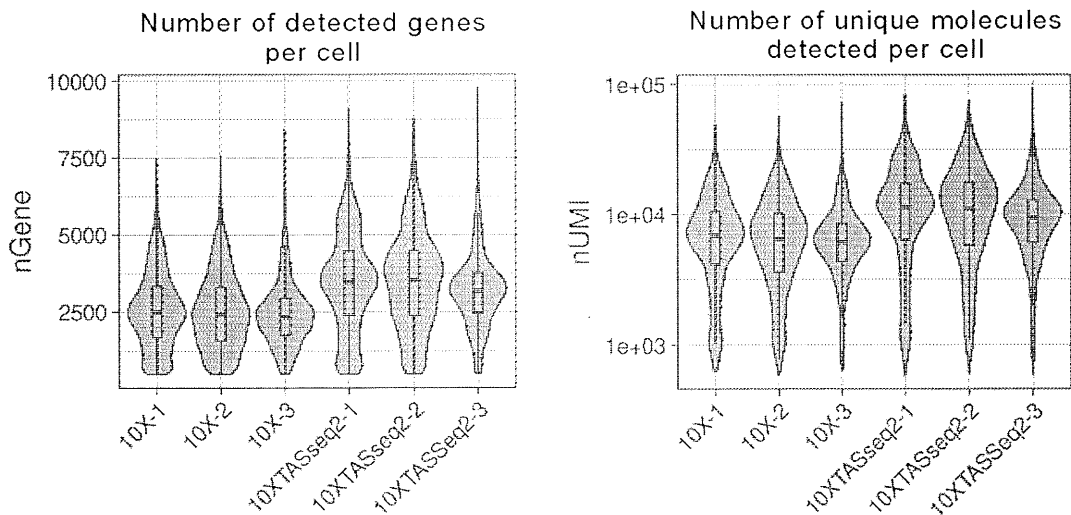
Mouse spleen cells



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

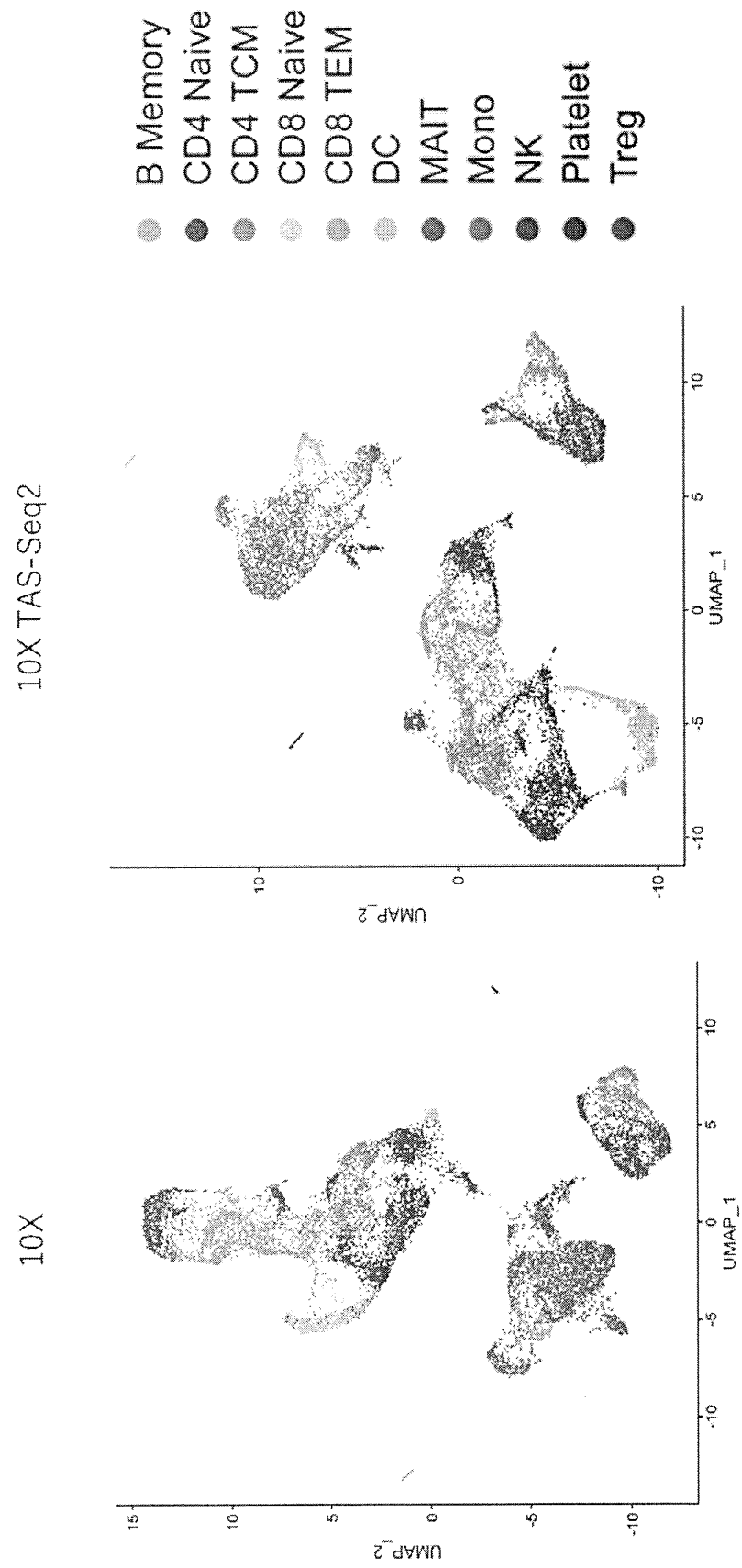


Fig.13

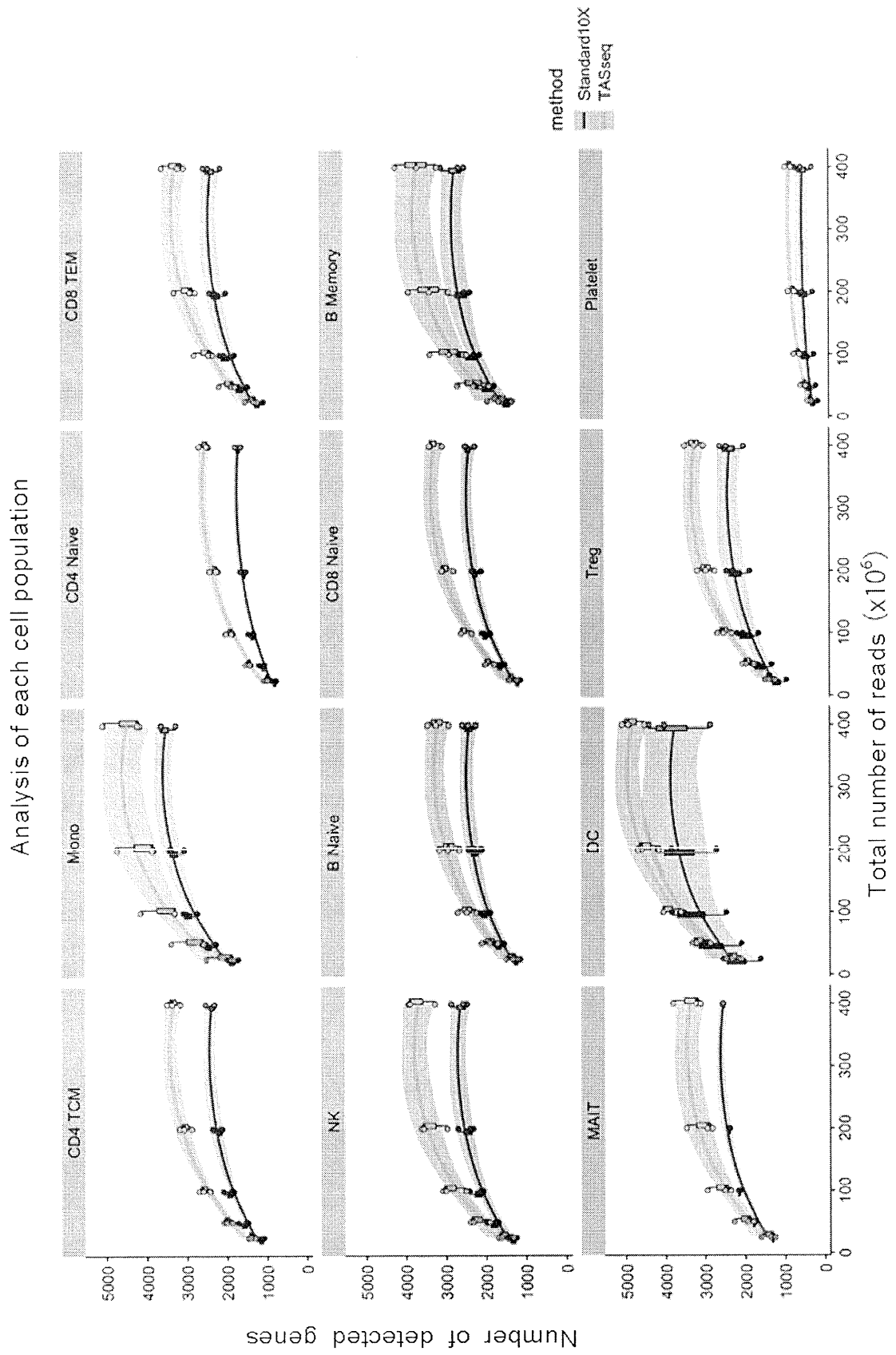


Fig.14