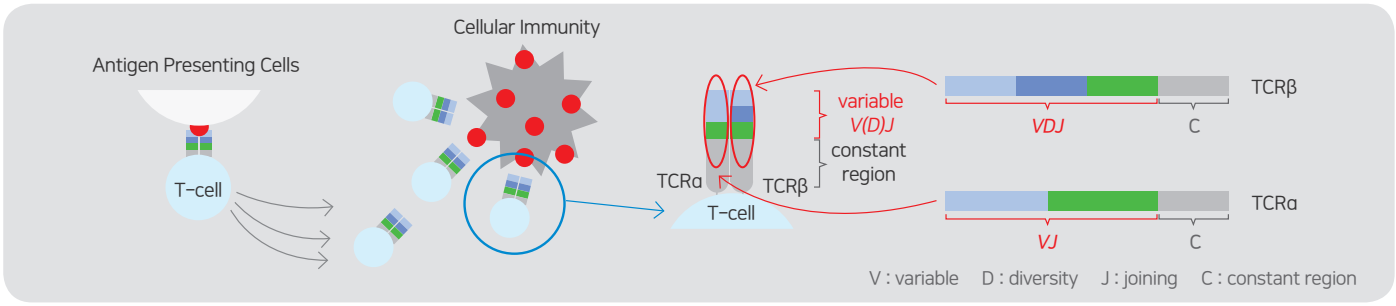

# SMARTer® Human TCR a/b Profiling Kit

## TCR repertoire 분석을 위한 SMARTer NGS solution

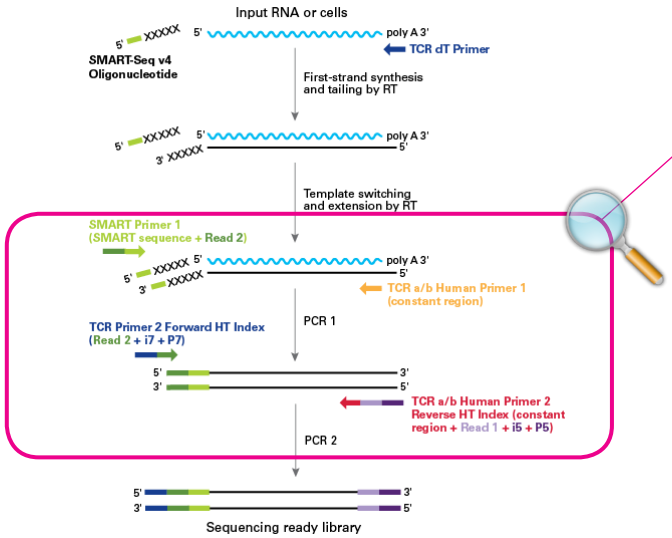
- TCR α, β chain을 동시에 또는 각각 분석 가능
- V(D)J segments의 full length 서열 분석
- 소량의 샘플 사용 : 10 ng ~ 3 µg total RNA, 50 ~ 10,000 purified T cells
- Illumina® ready NGS library 제작 (MiSeq® System 권장)

세포 매개성 면역의 핵심요소 중, T Cell 또는 T lymphocytes의 수용체인 T Cell Receptor (TCR)는 V(D)J somatic recombination에 의하여 "다양성 (diversity)"을 가진다. SMARTer® Human TCR a/b Profiling Kit는 SMART-Seq과 RACE 기술의 조합으로 기존 분석방법인 multiplex PCR에 비하여 소량의 샘플로부터 편향성 없는 full length V(D)J segments를 분석할 수 있다.



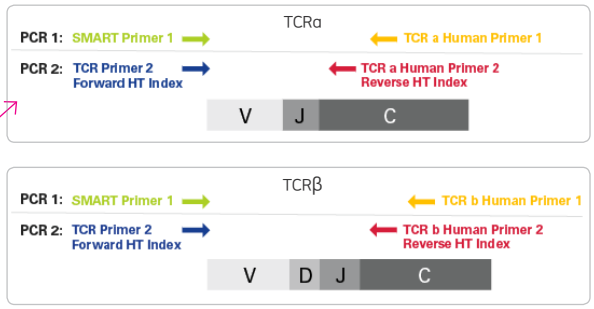

## SMART-Seq Technology

SMART = Switching Mechanism At 5'end of RNA Transcript




## RACE Technology

RACE = Rapid Amplification of cDNA Ends




V(D)J segment mRNA로부터 full length cDNA를 얻기 위하여 TCR mRNA 3' 말단의 constant region, 5' 말단의 SMART-Seq v4 oligo를 이용하여 semi-nested PCR을 수행한다.

that's GOOD science!

- 소량 (ng)의 total RNA로부터 NGS library 제작
- LNA™ oligo를 이용하여 더욱 개선된 민감도

SMART-Seq

+

- RACE : Rapid Amplification of cDNA Ends
- 알고 있는 서열 (constant)을 이용해 미지의 서열 (V(D)J) 분석

RACE PCR

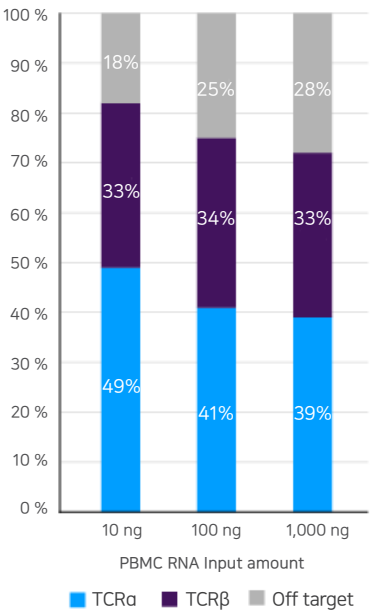
=

**SMARTer® Human TCR a/b Profiling Kit**

**소량의 샘플로부터 높은 민감도로 TCR 분석**

## ▪ 다양한 샘플 input 양에 따른 NGS 분석 결과 비교

A




B

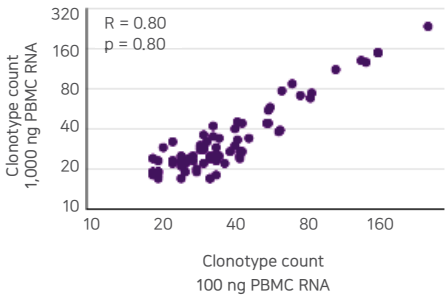
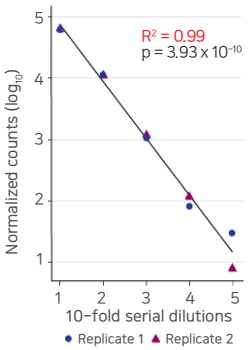



그림 1. Sequencing reads on target and correlation of clonotype count data for varying sample input amounts.
A. Percentages of sequencing reads that map to CDR3 regions in either TCRα (blue) or TCRβ (purple) or that represent off target reads (gray). The protocol was performed on peripheral blood RNA: 10 ng, 100 ng, and 1,000 ng. For each RNA sample amount analyzed, ≥ 70% of sequencing reads mapped to a CDR3 region in either TCRα or TCRβ, with the 10 ng sample amount yielding the highest percentage of on target reads.
B. Correlation of clonotype count data for varying sample input amounts. Another form of analysis involved plotting counts of the 100 most highly represented clonotypes for varying sample input amounts. Comparison of clonotype count data for the 100 ng and 1,000 ng sample amounts yielded a Pearson correlation coefficient of 0.80 and a Spearman coefficient of 0.80.

## ▪ SMARTer TCR 분석법의 재현성과 민감도

A




B

| Amount of Jurkat RNA | % spike-in (% Jurkat RNA in 10 ng PBMC RNA) | #TRBV12-3-TRBJ1-2 clonotypes identified | | Signal: Noise | Two-tailed, Student's t-test (p value) | p < 0.005? |
|---|---|---|---|---|---|---|
| | | Replicate 1 | Replicate 2 | Ratio (x̄ spike-in / x̄ control (0%)) | | |
| 0 | 0.000% | 619 | 480 | 1.0000 | – | – |
| 100 fg | 0.001% | 649 | 488 | 1.0346 | 0.87498 | False |
| 1pg | 0.010% | 702 | 597 | 1.1820 | 0.36972 | False |
| 10pg | 0.100% | 1,695 | 1,673 | 3.0646 | 0.00382 | True |
| 100 pg | 1.000% | 12,074 | 11,206 | 21.1829 | 0.00157 | True |
| 1 ng | 10.000% | 62,395 | 63,006 | 114.1592 | 0.00003 | True |

그림 2. Assessing the reproducibility and sensitivity of the SMARTer approach.
A. The protocol was performed in replicate on PBMC RNA samples spiked at varying concentrations (10%, 1%, 0.1%, 0.01%, and 0.001%) with RNA obtained from a homogenous population of Leukemic Jurkat T cells (TRBV12-3-TRBJ1-2 clonotype). The number of TRBV12-3-TRBJ1-2 specific sequence reads obtained for each spiked sample was normalized by subtracting the number of corresponding reads obtained for negative control samples consisting of unspiked PBMC RNA. Following $Log_{10}$ transformation, plotting of the data (spike-in dilution vs. normalized read count) and linear regression analysis revealed a statistically significant correlation ($p = 3.93 \times 10^{-10}$, $R^2 = 0.99$) between the amount of spiked-in Jurkat RNA and the number of TRBV12-3TRBJ1-2 specific sequence reads.
B. Count data, signal to noise ratios, and statistical analysis for TRBV12-3-TRBJ1-2 specific sequence reads obtained from spiked RNA samples. This result demonstrates that differences in the relative abundance of transcripts for a particular TCR clonotype are faithfully and reproducibly represented in sequencing libraries generated. Comparison of the number of TRBV12-3-TRBJ1-2 specific sequence reads obtained for the control vs. spike-in samples suggests that added Jurkat RNA at a concentration of 0.1% is detectable above background in the sequencing output ($p < 0.005$) at a depth of ~ 275,000 reads, evidence of the sensitivity afforded by the SMARTer approach.

## ▪ 제품리스트

| 제품명 | Code | 용량 |
|---|---|---|
| SMARTer® Human TCR a/b Profiling Kit | 635014 | 12회 |
| | 635015 | 48회 |
| | 635016 | 96회 |



TaKaRa

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