IGT® UMI Adapter & UDI Primer (for Illumina) ——Molecular tags for Illumina, facilitating low-frequency mutation detection in ctDNA

Circulating tumor DNA (ctDNA) refers to extra cellular DNA present in body fluids such as plasma, urine, and cerebrospinal fluid, measuring approximately 70~200 bp in length, it primarily originates from necrotic or apoptotic tumor cells, exosomes, and circulating tumor cells. While ctDNA analysis effectively addresses challenges in frequent tissue sampling and tumor heterogeneity, most ctDNA samples exhibit low detection rates, with approximately 10%~50% showing matching endogenous tags.

To address these technical challenges, based on self-developed TargetSeq One® hybridization capture system, by integrating paired-ended Universal Molecular Identifiers(UMI), iGeneTech can realize PCR and random sequencing error correction, significantly enhancing detection sensitivity and specificity in liquid biopsy applications. This innovative solution demonstrates broad applicability across multiple clinical fields, including early cancer diagnosis and staging, therapeutic efficacy evaluation, recurrence monitoring, and prognosis prediction in oncology.

Product Overview

The IGT® UMI Adapter & UDI Primer (for Illumina) comprises a paired-ended UMI adapter mix and UDI primers, specifically designed for TruSeq library prep on Illumina. The paired-ended UMI adapter mix contains 64 fixed UMI adapters of 6 bp length, generating 4, 096 unique combinations that ensure sufficient diversity to distinguish individual DNA molecules. With an edit distance of ⩾ 3 between UMI sequences, this design enhances accuracy in correcting errors during library prep, target region capture and sequencing processes of DNA molecules. In addition, the product line offers 384 UDI sequences for customers, meeting the polling requirements for ultra-high data yields per lane in high-throughput sequencing platforms.

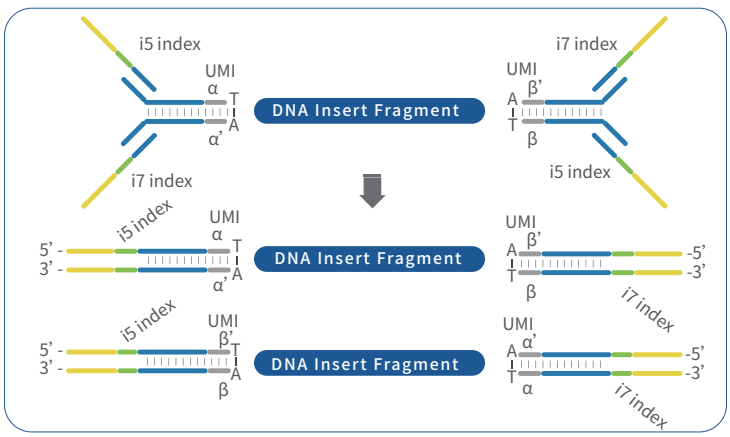


Figure 1. IGT® UMI Adapter & UDI Primer (for Illumina) Library Prep Process

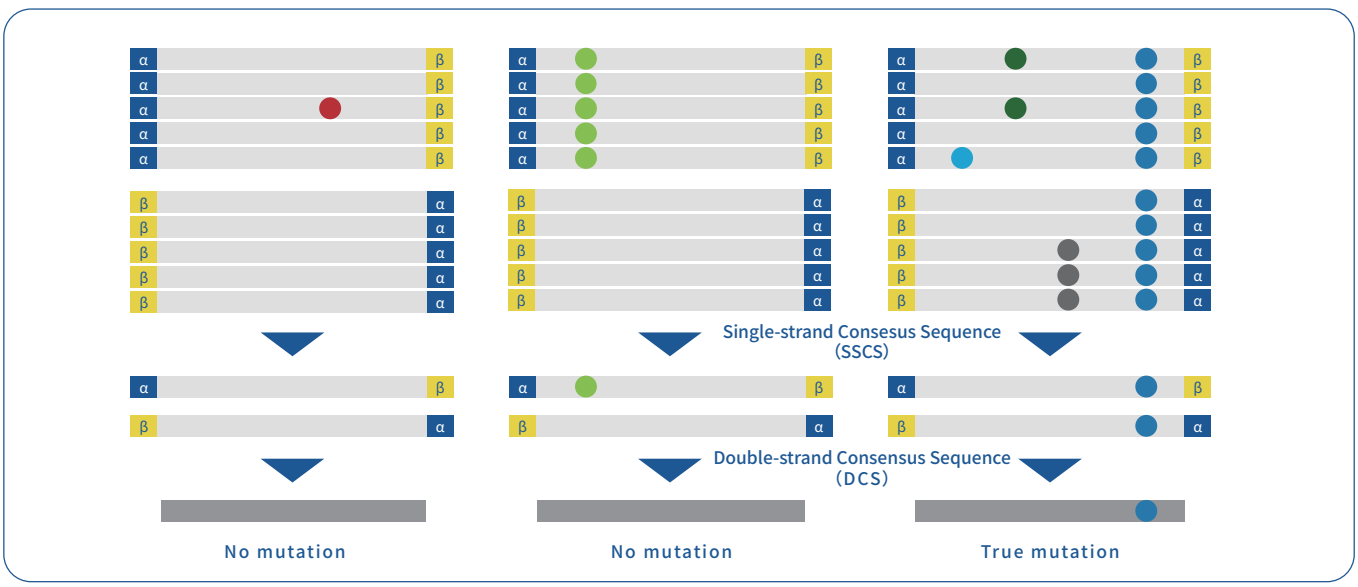


Figure 2. Principle of correcting data errors of Paired-ended UMI

Product Advantages

1 High efficiency of adapter connection

Optimize the adapter design and systhesis to ensure high library conversion efficiency and high sensitivity detection of low frequency variation

2 Assist to achieve accurate detection of low-frequency variants

Stable detection of abundance as low as 0.02% of ctDNA. 99.9% of 2 background noise can be filtered out by paired-ended UMI correction.

3 Unique UMI sequence design

The design of UMI sequences was not completely random (edit distance ⩾ 3) to avoid false positive variation caused by UMI sequence error.

4 Contains UDI amplification primers

It contains a paired-ended Unique Dual Index (UDI) to avoid cross-contamination to the greatest extent.

Case

- Input 25 ng of 0.1% Horizon cfDNA standard (Horizon Multiplex I cfDNA, catalog # HD780) for library prep, capture with a 52 kb panel, followed by sequencing on the NovaSeq 6000 platform using PE150. Under conditions of 20,000× average sequencing depth in the target region before redundancy removal, all 8 known mutation sites were detected with 100% sensitivity (Table1).

Table 1. Sequencing data of Horizon 0.1% cfDNA Reference Samples

EGFR L858R	*EGFR* T790M	*EGFR* delE746-A750	*EGFR* V769 -D770insASV	*KRAS* G12D	*NRAS* Q61K	*NRAS* Q61K	*NRAS* A59T	*PIK3CA* E545K	灵敏度
0.08%	0.10%	0.09%	0.06%	0.10%	0.10%	0.12%	0.16%	0.21%	100%

- The implementation of molecular labeling technology in low-frequency ctDNA detection significantly reduces false positive site identification number. After standard deduplication, 526 mutations with VAF below 3% were detected in the Horizon standard panel. When combining UMI with genome coordinate alignment for single-stranded consensus sequence (SSCS deduplication & correction), 163 mutations with VAF < 3% were identified. In contrast, using UMI for cfDNA molecular positive/negative strand error correction(DCS)combination, only 15 low-frequency mutations with VAF < 3 % were detected, including 8 known mutation sites. The paired-ended UMI technology demonstrated a 97% reduction in low-frequency alleledetection rates compared to standard deduplication methods (Figure 3).

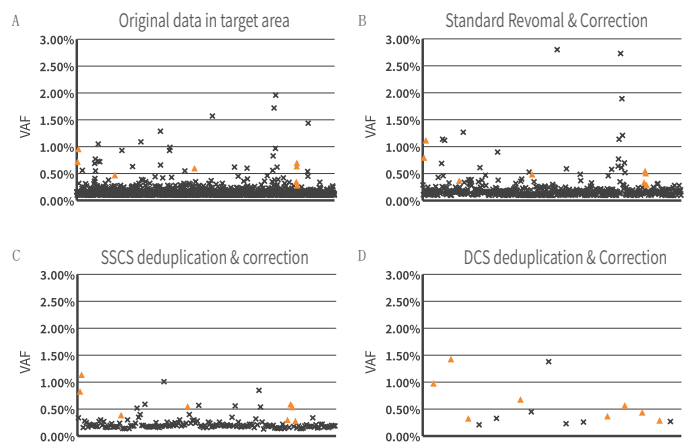


Figure 3. IGT™ UMI Adapter & UDI Primer (for Illumina) can significantly reduce background noise

Product Information

Product name	Spec.	Cat.
IGT™ UMI Adapter & UDI Primer 1-16 (for Illumina, tube)	16*1 rxn	C10131
IGT™ UMI Adapter & UDI Primer 1-96 (for Illumina, plate)	96*1 rxn	C10092
IGT™ UMI Adapter & UDI Primer 97-192 (for Illumina, plate)	96*1 rxn	C10102
IGT™ UMI Adapter & UDI Primer 193-288 (for Illumina, plate)	96*1 rxn	C10112
IGT™ UMI Adapter & UDI Primer 289-384 (for Illumina, plate)	96*1 rxn	C10122

*For more adapter reagents, please consult iGeneTech customer manager.

Official Wechat

Website：www.igenetech.com
Email：sales@igenetech.com
Tel：010-89146623

Headquarter: Building 13, No.1A Chaoqian Road, Science and Technology Park, Changping District, Beijing, 102200 China

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