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TruSight™ Oncology UMI Reagents

Error correction with unique molecular identifiers (UMIs) for sequencing libraries.

Highlights

- **Reduced error rate**
  Enables the reduction of error rates down to 0.007% or lower
- **Integrated with TruSight Oncology DNA library prep and enrichment assay**
  Allows creation of UMI-containing sequencing libraries with the TruSight Tumor 170 DNA oligonucleotides (oligos) on cell-free DNA (cfDNA)
- **Accessible analysis software**
  Provides error correction software in BaseSpace™ Sequence Hub or for local installation

Introduction

Next-generation sequencing (NGS) has enabled cancer researchers to assess numerous genes in a single assay. Illumina technology provides highly accurate sequencing data, yet any synthesis-based method involves inherent errors. Although the error rate is low enough (less than 0.5%) to successfully accomplish many NGS-based applications, new approaches that use noninvasive methods for sample collection may require a lower error rate. For example, analysis of cfDNA can be used to detect somatic variants in blood without the need for biopsy; however, the low percentage of circulating tumor DNA (ctDNA) within total cfDNA causes variant allele frequencies to exist near the limit of detection of existing methods.

To address this challenge, Illumina offers the TruSight Oncology UMI Reagents, which implement UMIs and error correction software to lower the rate of inherent errors in NGS data. The UMI reagents integrate easily with the TruSight Oncology workflow[1] by simply replacing standard Y-shaped adapters with UMI-containing adapters to barcode each individual DNA strand. Reducing the background noise leads to a lower limit of detection (Figure 1). With the TruSight Tumor 170 DNA assay,[2] the TruSight Oncology UMI Reagents reduced the error rate to ≤ 0.007% or lower. Lower error rates increase analytical specificity, and the resulting higher confidence in variant calls help put ctDNA analysis into the hands of translational cancer researchers.

Simple integration into the TruSight Oncology workflow

The TruSight Oncology UMI Reagents provide UMI-adapters and indexes, plus TruSight Oncology DNA library prep and enrichment reagents, that can be paired with the TruSight Tumor 170 DNA oligos to create UMI-containing sequencing libraries. By replacing the standard adapters with the UMI-containing adapters during the ligation step, integration of UMIs does not create any extra steps in the library prep workflow (Figure 2).

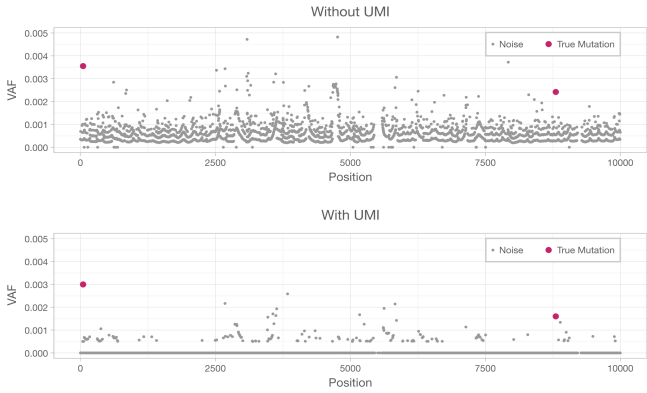

Figure 1: Background reduction facilitates accurate variant calling—To enable accurate detection of rare variants, UMIs are integrated with error correction software enabling true mutations to be distinguished from background noise. The bottom panel illustrates how, by removing inherent errors that result in false positives, the reduced error rate allows true mutations (red dots) to be better distinguished from background noise (grey dots)

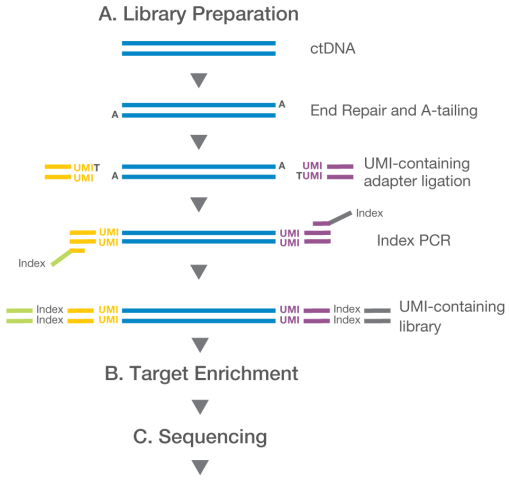

Figure 2: Target capture library prep workflow—UMIs can be integrated into TruSight Oncology Y-adapter ligation without interrupting the standard workflow. During library preparation, the adapters from the original library prep kit are simply replaced by the UMI adapters.

Intuitive software for error correction

After UMI-containing libraries are sequenced, the UMI Error Correction App aligns reads, then collapses the sequences with shared UMIs down to unique reads (Figure 3). This process enables the filtering of false positives, which are excluded from the collapsed reads, reducing the error rate for variant calling (Table 1). The UMI Error Correction App is available in the cloud-based BaseSpace Sequence Hub, or for installation on a local workstation.

Table 1: Reduction of error rates with UMIs

Sequencing Run	Mean Error Rate (Uncollapsed Reads)	Mean Error Rate (Collapsed Reads)
1	0.038%	0.0023%
2	0.043%	0.0024%
3	0.035%	0.0024%
4	0.084%	0.0019%

Library preparation was performed using the TruSight Oncology UMI reagents paired with DNA content from the TruSight Tumor 170 DNA assay, and 31 samples were distributed among four independent sequencing runs on the HiSeq™4000 System. Mean error rates are shown with and without collapsed reads using the UMI Error Correction App.

Summary

The TruSight Oncology UMI Reagents enable the reduction of sequencing error rate to 0.007% or lower. With a significant reduction of background noise, the detection of variant frequencies lower than 1% is possible. This addresses the need for higher accuracy in methods that assess low-frequency variants, such as ctDNA analysis.

Ordering information

Product	No. of Samples	Catalog No.
TruSight Oncology UMI Reagents	48	20024586

Learn more

For more information about the Oncology UMI Reagents, visit www.illumina.com/UMI-Reagents

For more information about TruSight Tumor 170, contact your account manager, or visit www.illumina.com/TruSightTumor170

References

1. TruSight Oncology. www.illumina.com/products/by-type/clinical-research-products/trusight-oncology.html. Accessed January 31, 2018.
2. TruSight Tumor 170. www.illumina.com/products/by-type/clinical-research-products/trusight-tumor-170.html. Accessed January 31, 2018.

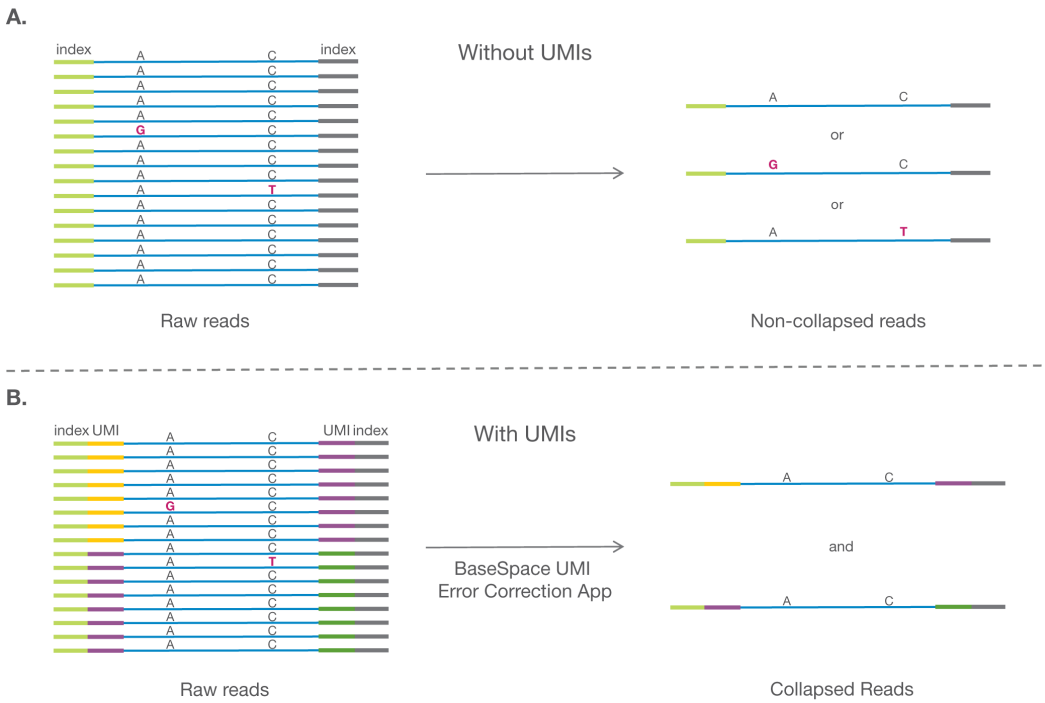

Figure 3: Error correction—(A) Without the use of UMIs, inherent errors persist during variant calling. Shown are 16 reads with two variants that could either be true rare variants or inherent errors. Without error correction, it is impossible to distinguish between true variants and false positives. (B) Integration of UMIs into the sequencing library enables the UMI Error Correction App to recognize multiple reads from the same target molecule and collapse them into a single read, reducing errors in final variant calls. Shown are two UMIs capturing the same DNA target. Each set of reads contains one error. After error correction, only one correct sequence remains.

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