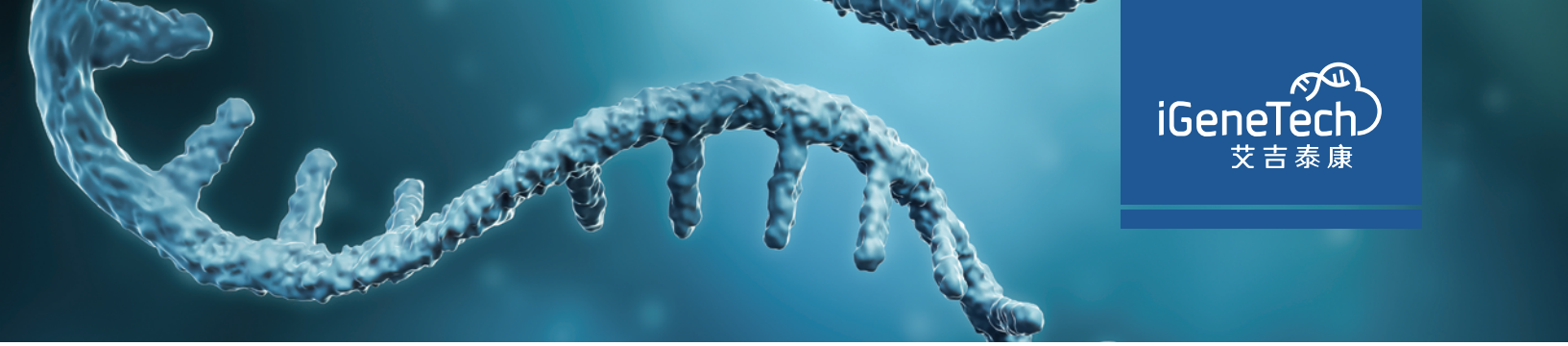

Single Stranded DNA Library Prep Kit

Suitable for low initial sample size of methylation library & genomic library prep, facilitating early tumor screening

IGT® ssDNA Library Prep Kit is a universal single-stranded DNA library prep kit, applicable to methylation library & genomic library prep starting from 1 ng ~ 50 ng DNA. The constructed libraries can be further used for target region hybridization capture sequencing or direct sequencing. During the construction of methylation libraries, bisulfite treatment can lead to severe DNA damage, fragmentation, and cleavage. Additionally, low-quality or severely degraded DNA also contains a large number of single-stranded of DNA. IGT® ssDNA Library Prep Kit can perform adapter ligation on single-stranded DNA, significantly improving the utilization rate of original DNA molecules and the library complexity. It has significant advantages in the construction of methylation libraries and genomic libraries with low initial sample sizes.

Application Background

ctDNA is a DNA fragment derived from tumor cells, carrying genetic info such as mutation, insertion, deletion, rearrangement, copy number variation & methylation[1]. There're significant differences in DNA methylation status between normal cells and tumor cells. Methylation changes can often be detected in early stage of tumor occurance, and DNA methylation has significant tissue specificity[2]. Detecting status of DNA methylation can realize tissue traceability. Therefore, DNA methylation has potential to serve as a biomarker for early tumor screening. However, in early stage of cancer, the content of ctDNA in body fluids is relatively low and bisulfite method can cause significant damage to DNA. This requires a library prep method targeting low initial amounts & broken DNA to increase molecular recovery rates and improve the accuracy of early screening data.

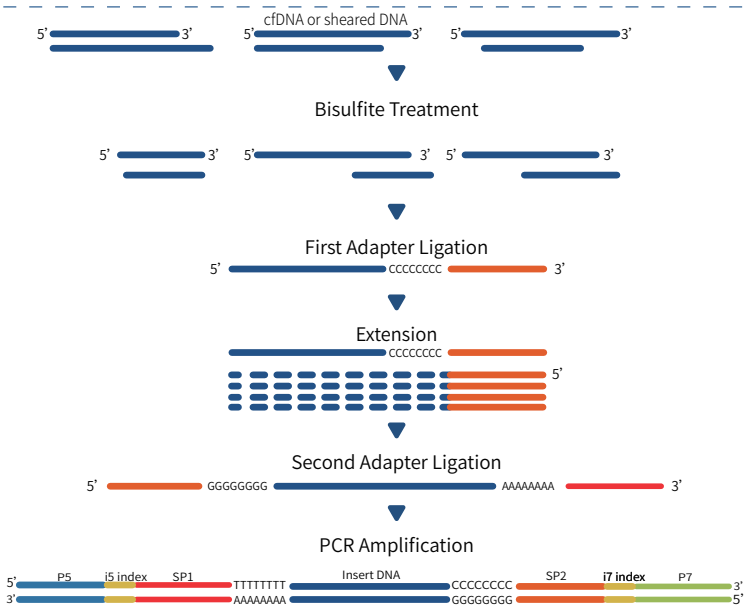

Figure 1. Flowchart of methylation library prep of IGT®ssDNA Library Prep Kit

Product advantages

1. Suitable for samples with low initial amounts such as cfDNA and FFPE DNA etc., the initial amount for library prep can be as low as 1 ng.

2. In the process of methylation library prep, the Post-BS library prep strategy is adopted, which significantly improved the utilization rate of DNA templates.

3. Can enhance the library complexity effectively and increase the effective sequencing data.

4. Compatible with whole-genome methylation sequencing, target region methylation sequencing, whole-genome sequencing and target region hybrid capture sequencing.

Data of Methylation library sequencing

1. Sample performance of different initial quantities

IGT® ssDNA Library Prep Kit, in combination with the BisCap® methylation hybrid capture system, is suitable for samples of different starting amounts and the sequencing results are stable.

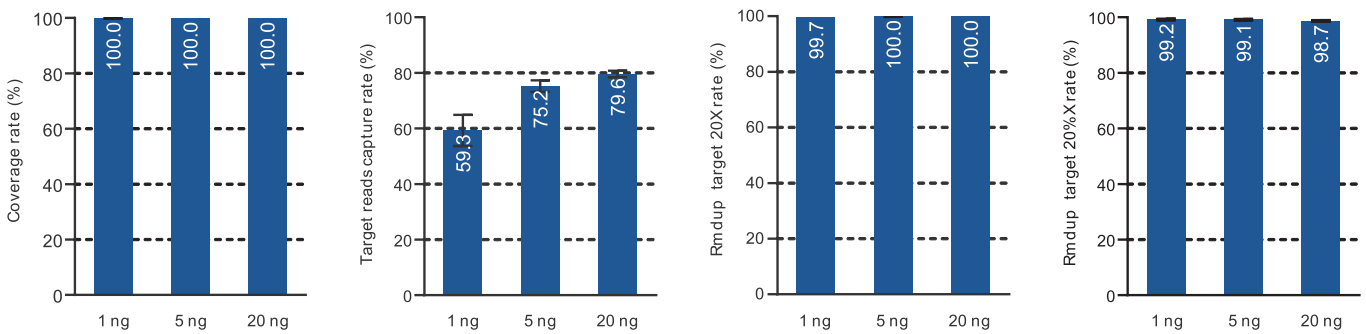

Figure 2. Capture data of IGT® ssDNA Library Prep Kit with different input amount. Seperately input 1 ng、5 ng、20 ng cfDNA (sample is NA12878 cell line gDNA, ultrasound interruption of simulated cfDNA) for single stranded library prep, library prep adopts IGT® ssDNA Library Prep Kit and IGT® ssDNA Adapter & UDI Primer (for Illumina), capture with 123 kb Panel, sequence with PE150 on NovaSeq 6000, the average volume of sequencing data is 1.8 Gb.

2. Extremely high cytosine conversion rate

During the library prep of IGT® ssDNA Library Prep Kit, the Bisulfite method was adopted for transformation, and the cytosine conversion rate of non-CG sequences (including CHG and CHH) was above 99.2%.

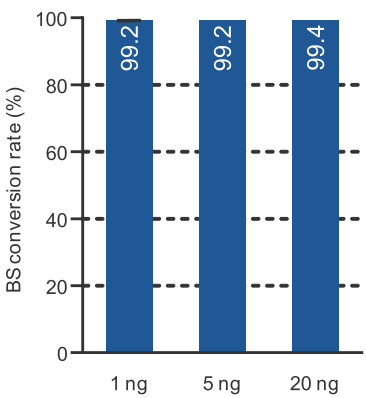

Figure 3. Conversion rate of IGT® ssDNA Library Prep Kit with different input of the sample Seperately input 1 ng、5 ng、20 ng cfDNA (sample is NA12878 cell line gDNA, ultrasound interruption of simulated cfDNA) for Bisulfite transformation and library prep, capture with 123 kb Panel, seq uence with PE150 on NovaSeq 6000, the average volume of sequencing data is 1.8 Gb.

3. High utilization rate of the original DNA molecule

Under the same sequencing data volume, the input for single-stranded library prep is only 1/10 of that for double-stranded library prep, and the effective depth after deduplication in single-stranded library prep is higher.

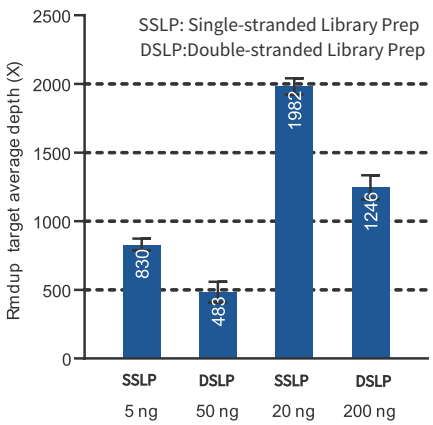

Figure 4. Effective depth of IGT® ssDNA Library Prep Kit and Double-stranded DNA Library Prep Kit. NA12878 cell line gDNA, seperately input 5 ng、20 ng、50 ng 、200 ng for library prep, among which 5 ng and 20 ng library prep adopt IGT® ssDNA Library Prep Kit and IGT® ssDNA Adapter & UDI Primer (for Illumina), 50 ng and 200 ng library prep adopt IGT® Methyl Fast Library Prep Kit v2.0 and IGT® methyl Adapter M& UDI Primer (for Illumina), capture with 123 kb panel, extract 1 Gb of data for effective in-depth analysis.

4. Excellent repeatability of methylation levels after library capture

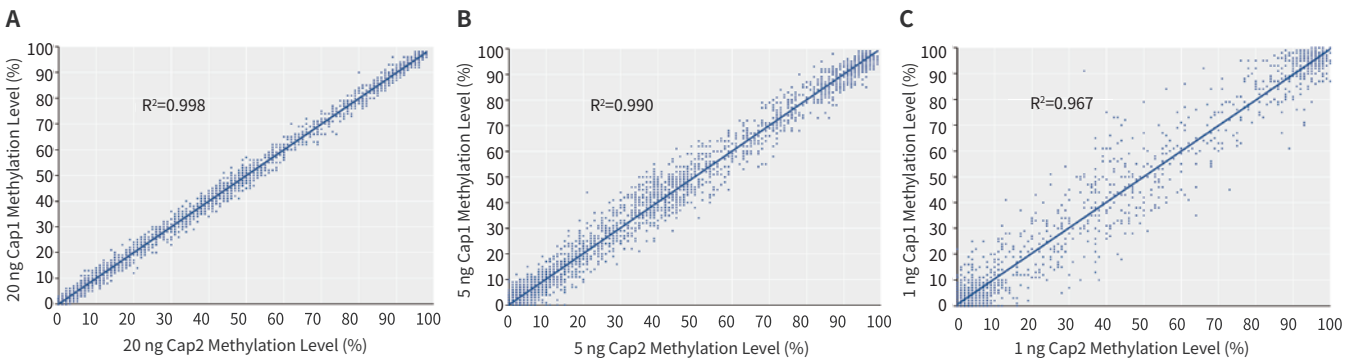

Figure 5. Repeatability of methylation levels at different DNA inputs in single-stranded DNA library prep kits

Input A: 20 ng, B: 5 ng, and C: 1 ng of cfDNA respectively (the sample was gDNA of NA12878 cell line, ultrasonally disruption to simulate cfDNA) for Single-stranded library prep. Library prep adopts IGT® ssDNA Library Prep Kit and IGT® ss DNA Adapter&UDI Primer (for Illumina). Capture with 123 kb Panel and sequence with PE150 on NovaSeq6000. Analyze the repeatability of methylation levels at CpG sites of the same sample in different batch of capture experiments.

5. Highly consistent methylation levels of single-stranded libraries and double-stranded libraries

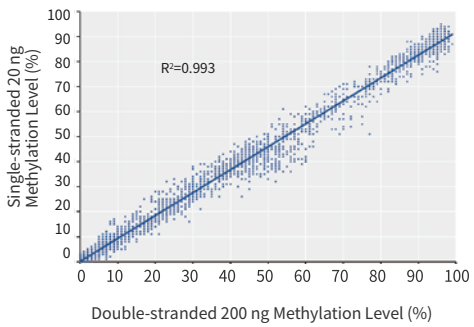

Figure 6. Correlation of methylation levels between single-stranded & double-stranded library prep kit

Input 20 ng of cfDNA (sample: gDNA of NA12878 cell line, ultrasonally broken to simulate cfDNA) and 200 ng of gDNA (sample: gDNA of the NA12878 cell line) for ultrasonally broken library prep. The library prep of 20 ng input samples adopts IGT® ssDNA Library Prep Kit and IGT® ssDNA Adapter & UDI Primer (for Illumina). The library prep of 200 ng input sample adopts IGT ® Methyl Fast Library Prep Kit v2.0 and IGT® Methyl Adapter & UDI Primer (for Illumina), capture with 123 kb Panel, sequence with PE150 on NovaSeq6000. The correlation of methylation levels between single-stranded library prep kits and double-stranded library prep kits was analyzed at the same sequencing depth (1000 ×).

* Applications

The size of BisCap® Human CpG Island Panel is 21.2 Mb, covering 27,000 CpG island, use 5 ng and 20 ng simulated cfDNA for single-stranded library prep and capture, sequence with PE150 on NovaSeq 6000, the data volume is about 8.6 Gb. The average capture efficiency is above 59%, the coverage rate is above 98%, 20x coverage is above 90%, and the 20x uniformity is above 95%. The data indicators perform exceptionally well.

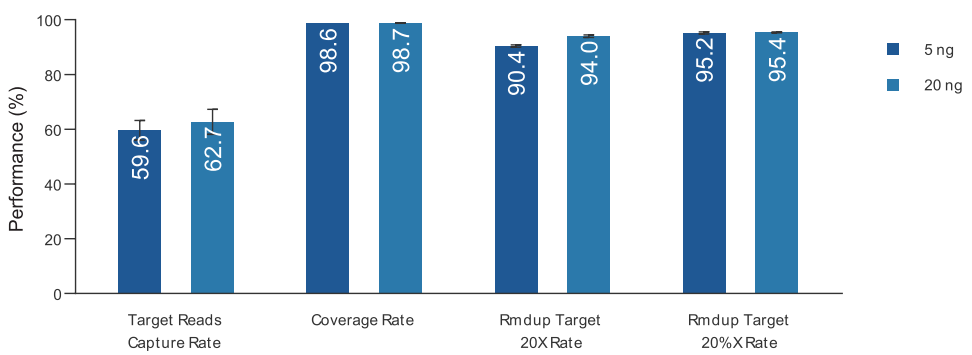

Figure 7. Data performance of CGI Panel combined with the single-stranded library prep kit.

Product Information

Kit Name	Spec.	Catalog #
IGT® ssDNA Library Prep Kit	16 rxn	C10911
IGT® ssDNA Library Prep Kit	96 rxn	C10912
IGT® ssDNA Adapter & UDI Primer 1-16 (for Illumina, tube)	16*1 rxn	C10861
IGT® ssDNA Adapter & UDI Primer 1-16 (for Illumina, tube)	16*6 rxn	C10862
IGT® ssDNA Adapter & UDI Primer 1-96 (for Illumina, plate)	96*1 rxn	C10872
IGT® ssDNA Adapter & UDI Primer 97-192 (for Illumina, plate)	96*1 rxn	C10882
IGT® ssDNA Adapter & UDI Primer 193-288 (for Illumina, plate)	96*1 rxn	C10892
IGT® ssDNA Adapter & UDI Primer 289-384 (for Illumina, plate)	96*1 rxn	C10902
TargetSeq® Eco Universal Blocking Oligo (for Illumina ssDNA Library)	16 rxn	C80791
TargetSeq® Eco Universal Blocking Oligo (for Illumina ssDNA Library)	96 rxn	C80792

References:

1. Jonathan C. M. Wan, Charles Massie. Liquid biopsies come of age: towards implementation of circulating tumour DNA[J]. Nature Reviews Cancer volume, 2017 Apr;17(4):223-238.

2. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges[J]. Nature Reviews Genetics, 2012, 13(10): 679-692.

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