

DeCodiFi™ Long&Complex

A Validated Polymerase for PacBio's Ampli-Fi Protocol

DeCodiFi™ Long&Complex is validated for long-read sequencing applications and is officially included as a recommended polymerase in the PacBio Ampli-Fi protocol.



Key Performance Characteristics

Based on PacBio's evaluation, DeCodiFi™ Long&Complex demonstrates the following operational parameters:

- ✓ **Amplification Yield:** Reaches the 500 ng benchmark required for downstream sequencing from 1 ng of input DNA.
- ✓ **Low Amplification Bias:** Provides uniform genome coverage during amplification, minimizing representational bias even across complex genomic regions.
- ✓ **Fragment Maintenance:** Amplifies library fragments up to 20 kb; for the Ampli-Fi workflow, PacBio recommends shearing DNA to 7–11 kb.
- ✓ **Assembly Contiguity:** Supports contiguous de novo genome assemblies by providing the required read length and quality to resolve complex genomic regions.

RECOMMENDED SETUP

Libraries of 7–11 kb, 50 µL reaction with 20 ng of library DNA

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	1 min	1
Denaturation	98°C	10 sec	10 cycles*
Annealing	58.8°C	15 sec	
Extension	68°C	12 min (30 s/kb)	
Final Extension	68°C	12 min	1
Hold	4°C	Hold	-

*For <5 ng input DNA, 14 PCR cycles are recommended.

Note: For the comprehensive library preparation and sequencing workflow, please refer to the official PacBio Procedure & Checklist: Amplifying genomic DNA for SMRTbell library preparation and HiFi sequencing (Document 103-648-000).

Official PacBio Evaluation Results

The following performance metrics were generated by the PacBio team during the validation of DeCodiFi™ Long&Complex on a human reference genome (HG002), using the Ampli-Fi protocol.

1. Sequencing Statistics

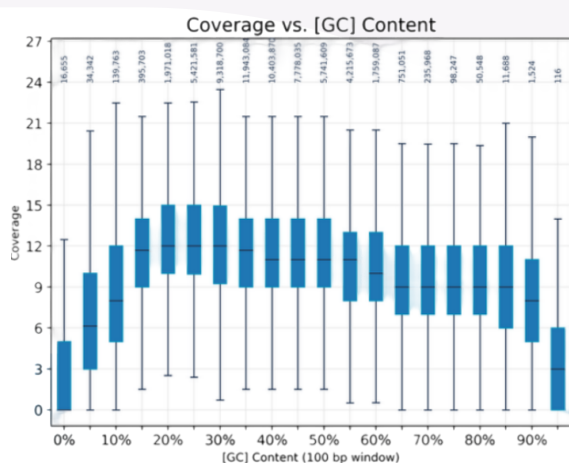
Overview of the initial sequencing output, demonstrating raw yield, read length, and accuracy.

HiFi Reads	HiFi Yield	HiFi Read Length (Mean)	HiFi Read Quality (Median)
7.68 M	78.0 Gb	10.15 kb	Q41

DeCodiFi™ Long&Complex successfully maintains long fragment lengths (10.15 kb mean) during amplification without compromising sequencing quality fidelity, achieving a median quality score of Q41

3. Alignment to Human Genome and GC bias (HG002 T2T v1.0.1)

Evaluation of deduplicated HiFi read coverage across the telomere-to-telomere human reference genome.



Mean Coverage of GC windows	11.7
Bases with 0 Coverage	0.15%

DeCodiFi™ Long&Complex provides uniform genome coverage, with 0.15% of the genome without coverage.

2. Demultiplexing and Deduplication

Post-sequencing metrics detailing the recovery of usable reads after demultiplexing and the removal of PCR duplicates.

SMRTbell Demux Rate	Twist Demux Rate	Duplicate Rate	Dedup HiFi Reads	Dedup HiFi Yield	Dedup HiFi Read Length	Dedup HiFi Quality
99.8%	89.7%	0.59%	6.83 M	69.1 Gb	10.12 kb	Q41

Deduplicated HiFi reads were used in all subsequent analyses. Coverage depth was 10.8X.

DeCodiFi™ Long&Complex recorded a low PCR duplicate rate of 0.59%, resulting in 69.1 Gb of usable yield post-deduplication.

4. De Novo Assembly Metrics

Genome assembly contiguity and completeness statistics across both haplotypes.

Haplotype	Size	N Contigs	N50	L50	auN
Hap 1	3.03 Gb	14,577	405 kb	2,013	588 kb
Hap 2	2.82 Gb	12,843	351 kb	2,290	525 kb

auN: Area under the curve (kb)

The amplified reads generated from low-pass sequencing (10.8X coverage) provide the necessary structural information to assemble and phase both haplotypes, yielding complete genome representations (~3 Gb) with contig N50 values of 405 kb and 351 kb.

5. Variant Calling Accuracy (GRCh38 vs. GIAB v4.2.1)

Precision and recall metrics for identifying Single Nucleotide Variants (SNVs) against the Genome in a Bottle (GIAB) benchmark.

Variant Type	Truth Total	Query TP	Query FP	Truth FN	Phred (Recall)	Phred (Precision)	Phred (F1)
SNV	3,367,450	3,357,639	3,374	9,811	Q25	Q30	Q27

In SNV detection, the output achieved Q30 Precision and Q25 Recall, registering over 3.35 million true positives against the GIAB benchmark.

Test it yourself!

If you would like to test DeCodiFi™ Long&Complex in your PacBio Ampli-Fi Protocol, contact us at sales@kurabiotech.com