Comprehensive Genomic Profiling of FFPE Samples Using the Twist Oncology – DNA CGP Panel on the MGI T1+ Platform

INTRODUCTION

Comprehensive genomic profiling (CGP) workflows require highly uniform target enrichment, sensitive low-frequency variant detection, and robust compatibility with challenging FFPE-derived samples. The Twist Oncology – DNA CGP Panel from Twist Bioscience is designed to support CGP of solid tumors through hybrid-capture enrichment.

This application note demonstrates the performance of the Twist Oncology – DNA CGP Panel on MGI's T1+ platform with downstream analysis using QIAGEN CLC Genomics Workbench. The T1+ platform enables direct loading of non-phosphorylated double-stranded DNA (dsDNA) libraries without requiring additional manual library conversion, circularization or DNB making, supporting straightforward implementation of established hybrid-capture enrichment workflows while maintaining familiar operational procedures.

FFPE-compromised Horizon reference materials representing mild, moderate, and severe degradation states were processed to evaluate workflow robustness, sequencing quality, and low-frequency somatic variant detection sensitivity under challenging sample conditions.

HIGHLIGHTS

- **Streamlined hybrid-capture workflow compatibility**
  Direct loading of Twist libraries enables straightforward implementation of established hybrid-capture sequencing workflows on the T1+ platform.
- **Robust FFPE sequencing performance**
  The Twist Oncology – DNA CGP Panel maintains strong target enrichment performance and high coverage uniformity across FFPE-compromised samples.
- **Sensitive low-frequency somatic variant detection**
  The workflow enables reproducible detection of low-frequency somatic variants, including variants near 1% variant allele frequency (VAF).
- **Integrated downstream analysis workflow**
  QIAGEN CLC Genomics Workbench supports streamlined somatic variant detection and visualization for hybrid-capture NGS data.

TECHNOLOGY

Twist Oncology – DNA CGP Panel

The Twist Oncology – DNA CGP Panel is a hybrid-capture NGS panel targeting approximately 2.4 Mb across 560 clinically relevant genes for comprehensive genomic profiling (CGP). The panel supports detection of SNVs, indels, CNVs, MSI, TMB, and selected fusion-associated rearrangements.

MGI T1+ Sequencing Platform

The MGI T1+ platform utilizes DNBSEQ™ technology, which combines DNA nanoball (DNB) generation, patterned array flow cells, and combinatorial Probe Anchor Synthesis (cPAS) sequencing chemistry to support high sequencing accuracy and strong coverage uniformity. The T1+ automates phosphorylation, circularization and DNB generation internally, allowing non-phosphorylated dsDNA libraries to be loaded directly.

The platform supports multiple library input formats, including phosphorylated and non-phosphorylated dsDNA, single-stranded circular DNA (ssCircDNA), and DNB libraries. This flexibility enables straightforward sequencing of libraries generated using established hybrid-capture workflows, without requiring additional manual conversion steps.

Flexible sequencing configurations are supported through independently addressable Flow Cell Large (FCL) and Flow Cell Small (FCS) formats, enabling efficient scaling across targeted sequencing, exome, and transcriptomics applications while maintaining high sequencing quality and operational flexibility.

METHODS AND MATERIALS

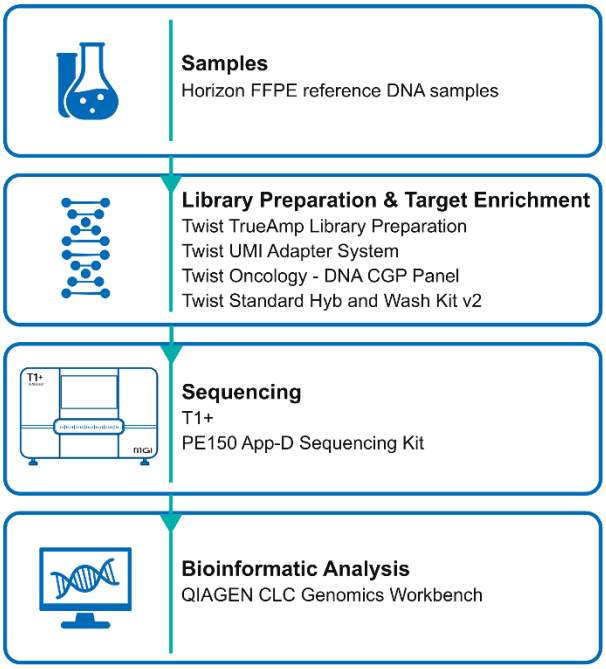

Figure 1. Workflow.

Sample Preparation

Formalin-compromised reference DNA samples representing mild (HD798), moderate (HD799), and severe (HD803) degradation conditions were used to evaluate workflow robustness and low-frequency somatic variant detection performance (Table 1). The Horizon Mimix™ Quantitative Multiplex fcDNA reference standards contain 11 verified variants across BRAF, cKIT, EGFR, KRAS, NRAS, and PIK3CA with expected VAFs ranging from 1.0% to 24.5%, enabling assessment of hybrid-capture sequencing performance across degraded FFPE-like sample conditions.

Name	Vendor Cat. No.	Purpose
Formalin-Compromised DNA (Mild) Reference Standard	Horizon Discovery HD798	Evaluate low-frequency somatic variant detection and enrichment performance under mild FFPE degradation conditions
Formalin-Compromised DNA (Moderate) Reference Standard	Horizon Discovery HD799	Evaluate workflow robustness and variant detection sensitivity under moderate FFPE degradation conditions
Formalin-Compromised DNA (Severe) Reference Standard	Horizon Discovery HD803	Evaluate sequencing quality and low-frequency variant detection under severe FFPE degradation conditions

Table 1. Details of benchmarking control samples, product information and intended function.

Library Preparation and Target Enrichment

Libraries were prepared from 50 ng DNA input material using Twist TrueAmp Library Preparation workflows with TruSeq-compatible adapters. Individual pre-capture libraries of 300 ng each were pooled into an 8-plex enrichment workflow using the Twist Oncology – DNA CGP Panel and Twist Standard Hybridization v2 chemistry for overnight hybridization. Post-capture libraries had a mean library size of approximately 426 bp prior to sequencing. Detailed operating procedures are described in the corresponding Twist Bioscience library preparation and target enrichment user manuals.

Sequencing

Sequencing was performed on the MGI T1+ platform using a paired-end 150 bp (PE150) sequencing workflow with App-D sequencing primers. The Flow Cell Large (FCL) PE150 AppD sequencing kit supports four sequencing lanes per flow cell. Each sequencing workflow utilized a single T1+ lane containing an 8-plex pool of CGP libraries derived from mild, moderate, and severe FFPE-compromised reference samples. Recommended loading input for phosphorylated and non-phosphorylated dsDNA libraries is 200 fmol per lane.

For the phosphorylated dsDNA library workflow, the library pool underwent conversion or 5′ phosphorylation using the MGIEasy Universal Library Conversion Kit (App-A), including a 10-cycle PCR. Per lane, a total of 200 fmol of the dsDNA library pool was loaded into the T1+ DNB Make and Load Module for circularization, DNB making and flow cell loading prior to sequencing.

For the non-phosphorylated dsDNA library workflow, pooled CGP panel enriched dsDNA libraries were directly loaded into the T1+ DNB Make and Load Module without additional library conversion or phosphorylation steps. Due to concentration limitations, a total of 113 fmol of the non-phosphorylated dsDNA library was used as input, with the resulting DNBs loaded onto a single flow cell lane.

Sequencing performance metrics from both workflows demonstrated high sequencing quality and efficient data generation across the CGP workflow.

Bioinformatics Analysis

Sequencing data generated on the MGI T1+ platform were analyzed using QIAGEN CLC Genomics Workbench with the "Twist Oncology – DNA CGP hybrid capture analysis" workflow.

The workflow supports detection of SNPs, indels, copy number variants, and selected fusion-associated rearrangements from hybrid-capture sequencing data and is available as either a QIAGEN-hosted secondary analysis workflow or an on-site CLC workflow deployment.

RESULTS

Sequencing & Enrichment Performance

Sequencing performance was evaluated for both phosphorylated and non-phosphorylated dsDNA CGP library workflows on the MGI T1+ platform. High sequencing quality metrics were observed across both approaches, including Q30 scores above 96%, Q40 scores above 91%, and barcode split rates above 94%, demonstrating robust sequencing performance for hybrid-capture CGP libraries. (Table 2)

	Phosphorylated Workflow	Non-Phosphorylated Workflow
Total Reads per Lane	632.81 M	565.04 M
Q30 (%)	98.33	96.56
Q40 (%)	95.79	91.16
Barcode Split Rate (%)	95.35	94.05
Mean Target Coverage	522×	525×
Duplication Rate (%)	30.08	29.4
Fold-80 Base Penalty	1.37	1.33
Target Bases ≥20X (%)	99.60	99.61
Target Bases ≥30X (%)	99.57	99.59

Table 2. Sequencing performance metrics for CGP libraries sequenced on the MGI T1+ platform via phosphorylated or non-phosphorylated workflows.

Relevant hybrid-capture QC metrics were averaged across mild, moderate and severe samples downsampled to 32M PE150 reads (~2000x raw coverage).

The Twist Oncology – DNA CGP workflow maintained strong target enrichment performance, high target coverage uniformity, and consistent on-target sequencing metrics across mild, moderate, and severe FFPE-compromised sample conditions (Figure 2).

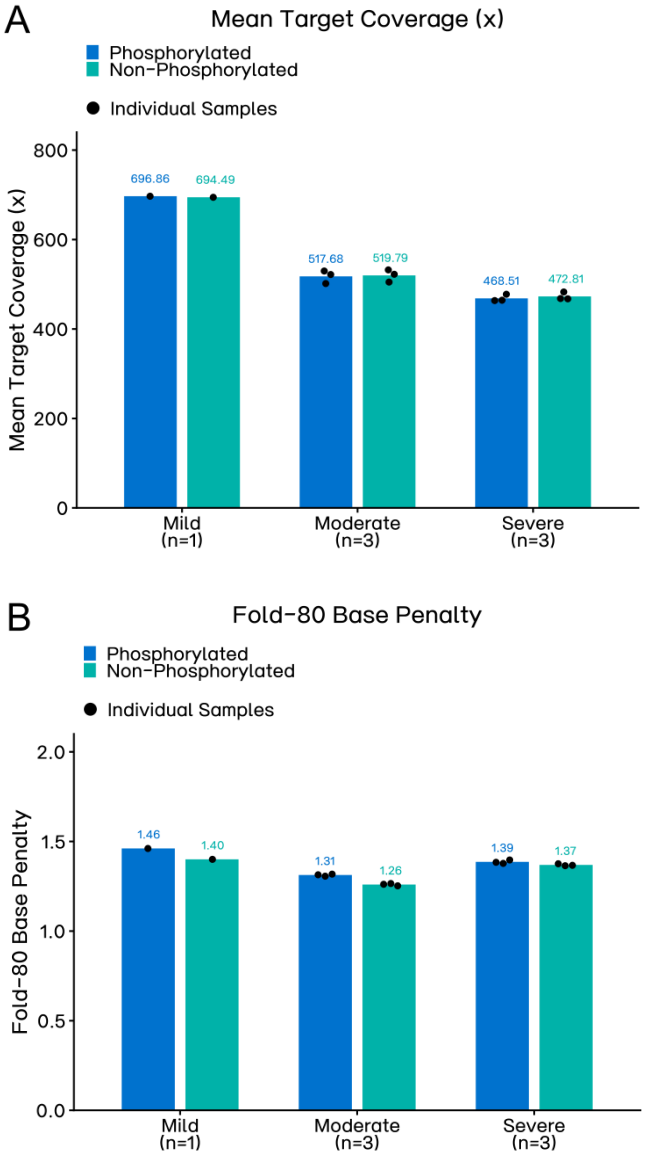

Figure 2. Consistent target coverage and low fold-80 base penalty values were maintained across all FFPE degradation states, demonstrating robust target enrichment performance and consistent coverage uniformity across FFPE degradation conditions. **(A)** Mean target coverage and **(B)** Fold-80 base penalty across mild, moderate, and severe FFPE conditions for both workflows.

Bars represent average values and dots represent individual replicates.

Comparable sequencing and enrichment results observed between phosphorylated and non-phosphorylated dsDNA workflows further demonstrate compatibility of standard hybrid-capture CGP libraries with direct loading on the MGI T1+ platform.

Somatic Variant Detection Performance

To further evaluate somatic variant detection performance, expected and observed VAFs from FFPE-compromised Horizon reference standards were compared across mild, moderate, and severe degradation conditions (Table 3). The Twist Oncology – DNA CGP workflow combined with QIAGEN CLC Genomics Workbench demonstrated robust detection of expected SNPs and indels across all FFPE sample conditions, including low-frequency variants near 1% VAF.

The Horizon FFPE DNA reference standards contain verified variants across clinically relevant oncology genes including BRAF, cKIT, EGFR, KRAS, NRAS, and PIK3CA, with expected VAFs ranging from 1.0% to 24.5%. Consistent variant detection performance was observed across mild (HD798), moderate (HD799), and severe (HD803) FFPE-compromised samples.

Gene	Protein	Expected VAF (%)	Mutation	Mild (HD798) (%)	Moderate (HD799) (%)	Severe (HD803) (%)
NRAS	Q61K	12.5	G>T	10.31–10.78	8.96–10.26	8.95–12.35
PIK3CA	E545K	9	G>A	7.83–8.81	7.14–8.99	6.21–7.72
PIK3CA	H1047R	17.5	A>G	18.09–18.33	17.37–21.58	16.11–17.74
cKIT	D816V	10	A>T	7.82–8.35	7.74–9.86	7.20–10.32
EGFR	G719S	24.5	G>A	21.60–22.98	21.95–23.08	17.17–20.42
EGFR	ΔE746-A750	2	Deletion	0.74–1.01	1.05–1.54	1.00–1.83
EGFR	T790M	1	C>T	0.80–0.87	0.64–1.48	1.00–1.35
EGFR	L858R	3	T>G	4.39–4.53	2.37–3.75	3.56–4.12
BRAF	V600E	10.5	A>T	12.10–12.36	11.11–13.18	10.84–12.13
KRAS	G13D	15	C>T	13.64–14.34	13.26–15.97	10.29–13.91
KRAS	G12D	6	C>T	5.52–5.58	4.26–5.42	7.06–9.11

Table 3. Representative expected and observed somatic VAFs across FFPE-compromised reference samples. Values represent combined results from phosphorylated and non-phosphorylated workflows.

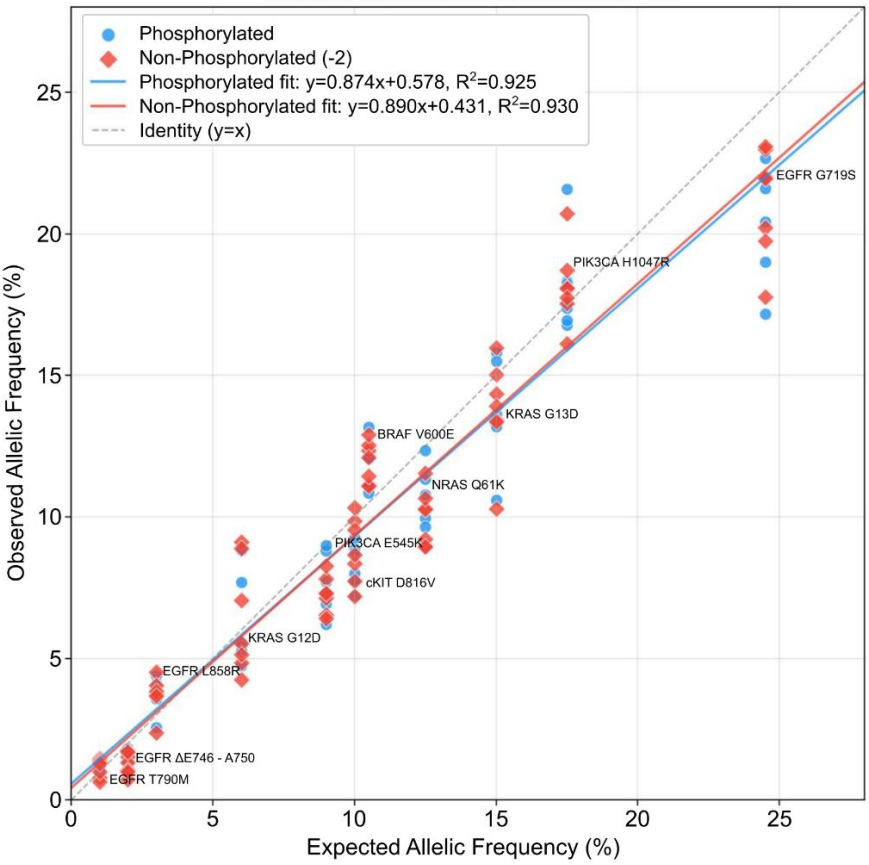

Figure 3. Correlation between observed and expected somatic VAFs across FFPE-compromised reference samples. Phosphorylated and non-phosphorylated dsDNA workflows are shown separately, with linear regression fits for each workflow. The dashed line represents the expected 1:1 relationship.

Strong concordance between expected and observed VAFs was observed for both phosphorylated and non-phosphorylated dsDNA workflows across a broad range of allele frequencies. Combined analysis demonstrated high correlation between expected and observed VAF values ($r = 0.963$, $R^2 = 0.927$), including low-frequency variants near 1% VAF (Figure 3). These results demonstrate consistent somatic variant detection across FFPE-compromised sample conditions.

CONCLUSION

The Twist Oncology – DNA CGP workflow on the MGI T1+ platform demonstrated robust sequencing performance, consistent target enrichment, and accurate low-frequency somatic variant detection across FFPE-compromised sample conditions. Direct loading of the non-phosphorylated CGP dsDNA libraries supports straightforward sequencing of established hybrid-capture workflows on the T1+ platform while maintaining high sequencing quality and sensitive variant detection performance.

ORDERING INFORMATION

Category	Product	Cat. No.
Instruments	MGI DNBSEQ-T1+ Sequencer	900-000991-00
Software	QIAGEN CLC Genomics Workbench	—
Library Preparation	Twist TrueAmp Library Preparation, 16 | 96 Samples	116480 | 116481
	Twist UMI Adapter System, 16 | 96 Samples	105040 | 105041
Target Enrichment	Twist Oncology – DNA CGP Panel, 2.4 Mb, 2 | 12 Reactions	116360 | 116361
	Twist Standard Hyb v2 and Wash Kit, 2 | 12 Reactions	116613 | 116614
	Twist Universal Blockers, 2 | 12 Reactions	100856 | 100578
	Twist Binding and Purification Beads, 2 | 12 Reactions	101262 | 100983
Sequencing Reagents	DNBSEQ-T1+ PE150 App-D Sequencing Kit (FCL)	940-002982-00
	DNBSEQ-T1+ PE150 App-D Sequencing Kit (FCS)	940-003021-00

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