# Development and Validation of a High-Throughput CAP/CLIA-Compliant Laboratory-Developed Test for Comprehensive Genomic Profiling with Integrated RNA Fusion Detection Across Multiple Specimen Types

## Development update and interim performance: FFPE solid-tumor workflow

Rwik Sen, Jonathan H. Schultz, Bo Wang, Yan Ding, Michelle T. Carlson, Caroline H. Mitchell, Shareef A. Nahas

Abstract
#2466485

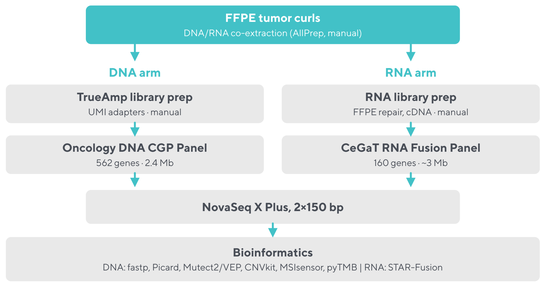
Meet our team at
Booth #1039

## Introduction

Comprehensive genomic profiling (CGP) combines SNV, indel, CNV, TMB and MSI testing in one assay and conserves scarce tissue. A targeted RNA arm adds fusion detection, which DNA capture alone misses.

Sampled built this *de novo* LDT from research-use-only Twist chemistry, starting with FFPE tumor material.

## Materials and methods




Targets: DNA ≥500× mean coverage; RNA 40–50M read pairs. Study design follows AMP/CAP guidance (Jennings et al., 2017).

## How Sampled builds *de novo* LDTs

- RUO kits become a locked, document-controlled test: SOPs, batch records, every reagent lot tracked in our QMS.
- Development runs were manual, by highly trained technicians with documented competency, setting the baseline for automation.
- Built for scale: one FFPE co-extraction for DNA and RNA, 96-reaction formats, NovaSeq X Plus, and transfer to automated extraction and Sciclone NGSx library prep.

## Samples and reagents

Extracted Sept–Oct 2026. Sequencing so far covers reference materials only.

**16 DNA extracts**
- **8** internal FFPE specimens
- **7** FFPE reference materials
- **1** extracted DNA mix

**18 RNA extracts**
- **10** internal FFPE specimens
- **6** FFPE reference materials
- **2** extracted RNA/TNA mixes

### FFPE reference materials

| | |
|---|---|
| **0496** | FFPE Tumor Fusion RNA v4 (18 fusions) |
| **1710** | Compromised FFPE WT (DIN 5.5, RIN 2.5) |
| **3634** | Solid Tumor FFPE DNA |
| **2236** | FFPE MSI-High |
| **1307** | FFPE TMB score 26 (WES 24.05) |
| **1310** | FFPE TMB score 7 (WES 6.9) |
| **2865** | FFPE Solid Tumor CNV |
| **HD200** | Horizon Mimix Quantitative Multiplex FFPE |

Seraseq (LGC Clinical Diagnostics) except HD200 (Horizon).
Extracted mixes 0097, 0497 and the TNA wild-type mix supplement the FFPE set.

### Reagents and lot control

23 reagent lots recorded with expiry dates; earliest expiry April 2027. All Twist chemistry: TrueAmp and RNA library prep, UMI/UDI adapters, hybridization and wash, purification beads.

Twist Alliance CeGaT RNA Fusion Panel: 160 fusion-associated genes, 66 with breakpoint tiling, 7,394 probes; ISO 13485 certified.

## Results: DNA arm

16 libraries from 8 reference materials (4 in triplicate), 30–83M read pairs each.

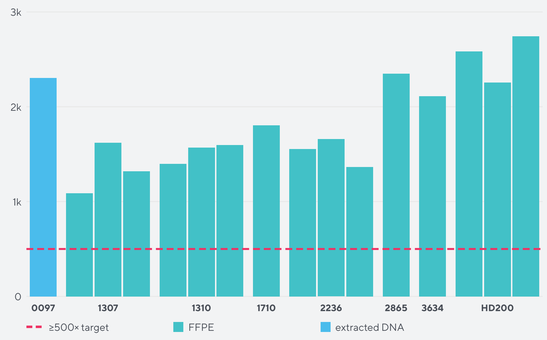
**1,639×**
median mean target coverage (1,089–2,743×)

**≥95.5%**
of target bases at ≥500×~ in every library

**1.44**
median fold-80 base penalty (1.30–1.68)

**>99.99%**
of reads aligned in every library

### Mean target coverage per library (×)




≥95.5% of target bases at ≥500× in every library; ≤0.22% zero-coverage targets; duplicate rate 25–39%.

### Replicate concordance

9 replicate pairs agreed 97.9–99.3% (1,165–1,352 variants each); a between-material pair agreed 55.9%, confirming sample identity.

## Results: RNA fusion arm

24 libraries from 8 reference materials; 0496 and 1710 titrated at 100, 50 and 25 ng in triplicate.

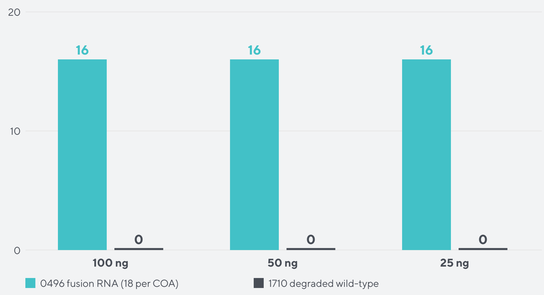
**100%**
of 48 FASTQ files pass per-base quality

**39.0**
mean Phred quality (38.7–39.4)

**32.7M**
median read pairs per library

**21/24**
libraries ≥25M read pairs (provisional minimum)

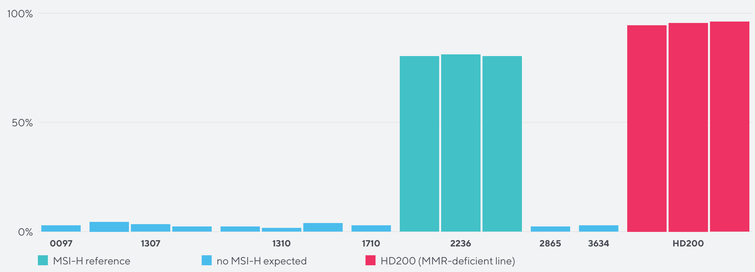
### Fusion calls across a 4-fold input range




STAR-Fusion calls per pooled triplicate. Matching the 16 calls to the 18 expected fusions is under review.

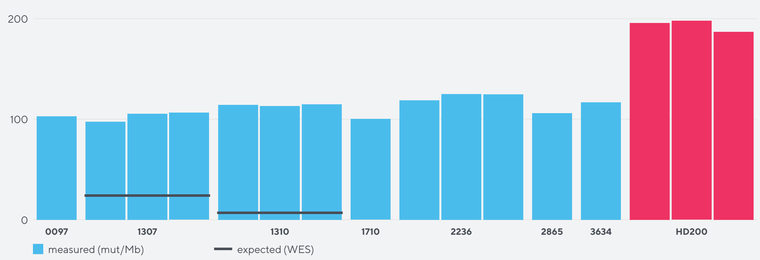
## Results: genomic signatures (DNA arm)

### MSI: MSI-High separates cleanly from MSS




MSI-H reference 2236: 80–81% unstable sites; other materials 1.7–4.5% (18-fold gap).

### TMB: reproducible, not yet calibrated




Replicate CV ≤4.7%, but a ~100 mut/Mb background (likely germline and FFPE artifacts) masks clinical-range TMB.

### CNV: 9 of 12 COA amplifications detected

| Gene | COA (+copies) | log2 | Call | Gene | COA (+copies) | log2 | Call |
|---|---|---|---|---|---|---|---|
| **AKT2** | +2.5 / +2.0 | +1.56 | Gain | **KRAS** | +1.0 / +0.9 | -2.11 | Not detected |
| **BRAF** | +3.2 / +2.3 | +0.34 | Borderline | **MET** | +2.9 / +2.5 | +0.99 | Gain |
| **EGFR** | +3.9 / +4.1 | +1.97 | Gain | **MYC** | +2.5 / +2.9 | +1.79 | Gain |
| **ERBB2** | +3.2 / +3.6 | +1.47 | Gain | **MYCN** | +2.4 / +2.4 | +1.37 | Gain |
| **FGFR3** | +4.9 / +5.6 | +1.91 | Gain | **NTRK1** | +2.4 / +4.5 | +1.39 | Gain |
| **KIT** | +2.8 / +1.8 | +0.85 | Gain | **PIK3CA** | +2.6 / +1.0 | -0.82 | Not detected |

2865, CNVkit gene-level (bins); gain = log2 ≥0.4. 4 of 368 non-COA genes reached 0.4 (3 probes each).

## Next steps to a clinical LDT

**1. Complete analytical validation**
- SNV/indel PPA/PPV vs COA (target ≥95%); reconcile 16 vs 18 expected fusions.
- Calibrate TMB vs WES; set the MSI-H cutoff; confirm CNV calls in the pipeline.
- LoD, repeatability and reproducibility; sequence internal FFPE specimens.

**2. Automate the workflow**
- Move extraction to Revvity and Qiagen automated chemistries.
- Move library prep and capture to Sciclone NGSx workstations.
- Bridge automated vs manual on the same reference materials.

**3. Clinical LDT readiness**
- Lock pipeline versions, filters and QC acceptance criteria.
- Per-batch NTC and positive control; confirm CLIA/CAP, NJ, NYS.
- Validation report with Laboratory Director sign-off.

## Conclusion

This interim development update shows that the core of Sampled's high-throughput comprehensive genomic profiling LDT, which pairs a DNA panel with an integrated RNA fusion arm, performs as designed on FFPE tumor material. On the DNA arm, every library exceeded the ≥500× coverage target at least 2-fold, with uniform capture (fold-80 ≤1.68). On the RNA arm, fusion calls held steady across a 4-fold input range (100 to 25 ng), with no false calls in degraded wild-type FFPE. MSI calling cleanly separated MSI-High from MSS references, and gene-level CNV analysis detected 9 of 12 certified amplifications. TMB still needs background removal and calibration before it can be reported. Together, these results establish the analytical foundation to build a single, scalable workflow that captures SNVs, CNVs, MSI, TMB and fusions from one specimen. The remaining work, namely SNV/indel accuracy, limit-of-detection and precision studies, automation of the workflow, and extension beyond FFPE to additional specimen types, defines the path from this foundational work to a fully validated, CAP/CLIA-compliant clinical test.