LockSeq: Sensitive and Scalable On- and Off-Target Validation Using a Padlock Probe-Based NGS Library Prep Workflow

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Introduction

Genomic safety assessment is central to gene editing product development, and recent FDA guidance[1] emphasizes the need for comprehensive, unbiased off-target evaluation. Current targeted approaches, including multiplex PCR and hybrid capture, often require trade-offs between sensitivity, panel size, and cost, increasing the risk of incomplete off-target detection. LockSeq addresses this limitation through a gap-fill based target capture chemistry designed to reduce dropouts and enable scalable, quantitative gene editing characterization.

1-to-1 target capture: The 'molecular lock' chemistry for high-fidelity multiplexing

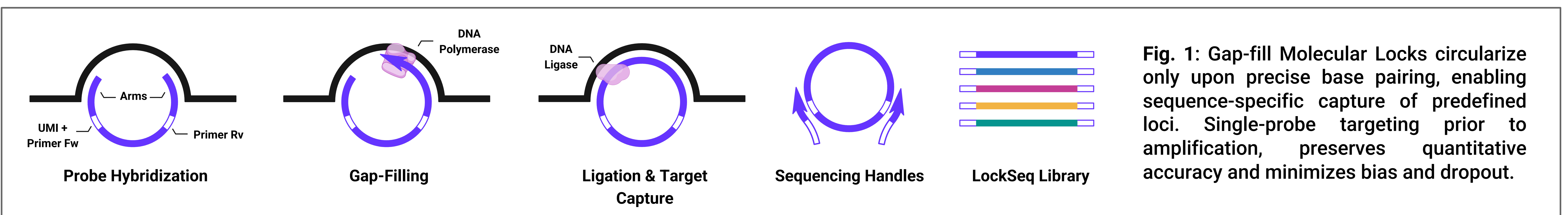

Fig. 1: Gap-fill Molecular Locks circularize only upon precise base pairing, enabling sequence-specific capture of predefined loci. Single-probe targeting prior to amplification, preserves quantitative accuracy and minimizes bias and dropout.

Encoded UMIs[2]: High-accuracy quantification

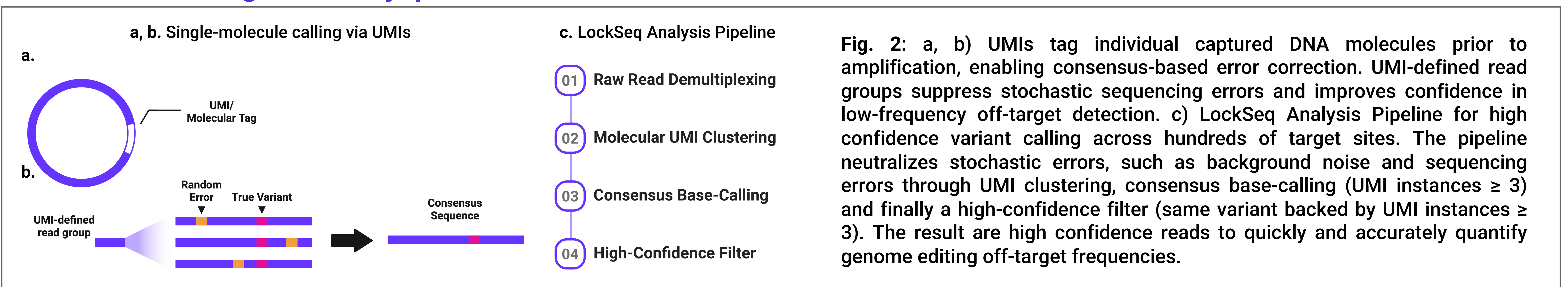

Fig. 2: a, b) UMIs tag individual captured DNA molecules prior to amplification, enabling consensus-based error correction. UMI-defined read groups suppress stochastic sequencing errors and improves confidence in low-frequency off-target detection. c) LockSeq Analysis Pipeline for high confidence variant calling across hundreds of target sites. The pipeline neutralizes stochastic errors, such as background noise and sequencing errors through UMI clustering, consensus base-calling (UMI instances ≥ 3) and finally a high-confidence filter (same variant backed by UMI instances ≥ 3). The result are high confidence reads to quickly and accurately quantify genome editing off-target frequencies.

LockSeq chemistry minimizes target dropouts and non-specific amplification

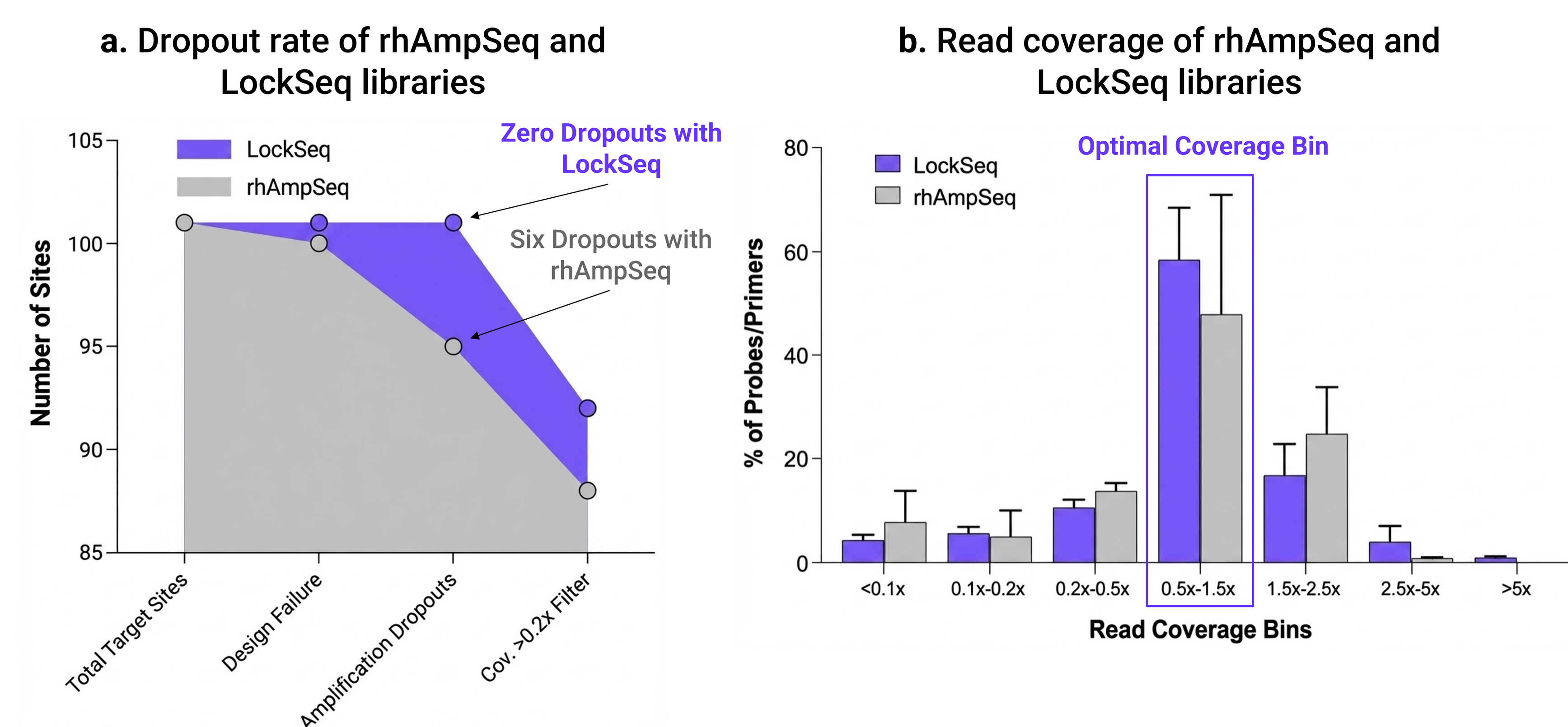

Fig. 3: Dropout rate and read coverage of LockSeq and rhAmpSeq™ (IDT). LockSeq covered all target sites while rhAmpSeq missed 6 target sites due to their design constraints and amplicon dropouts (Fig. 3a). For this, EMX1 canonical gRNA off-target sites were adapted from Dobbs *et al.*[3]

LockSeq enables accurate and sensitive variant quantification

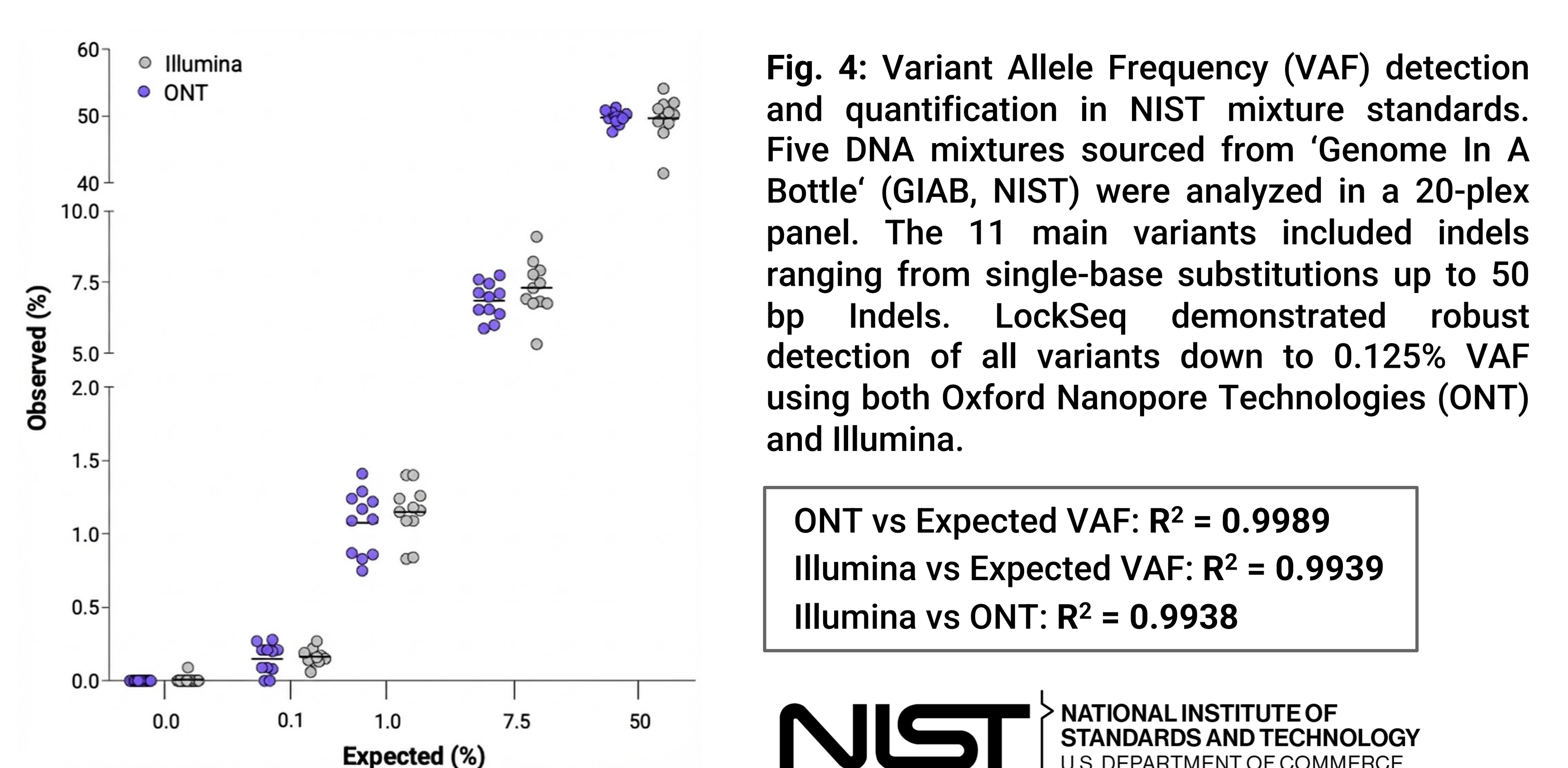

Fig. 4: Variant Allele Frequency (VAF) detection and quantification in NIST mixture standards. Five DNA mixtures sourced from 'Genome In A Bottle' (GIAB, NIST) were analyzed in a 20-plex panel. The 11 main variants included indels ranging from single-base substitutions up to 50 bp Indels. LockSeq demonstrated robust detection of all variants down to 0.125% VAF using both Oxford Nanopore Technologies (ONT) and Illumina.

ONT vs Expected VAF: R^2 = 0.9989
Illumina vs Expected VAF: R^2 = 0.9939
Illumina vs ONT: R^2 = 0.9938

LockSeq chemistry offers scalable accuracy and panel flexibility

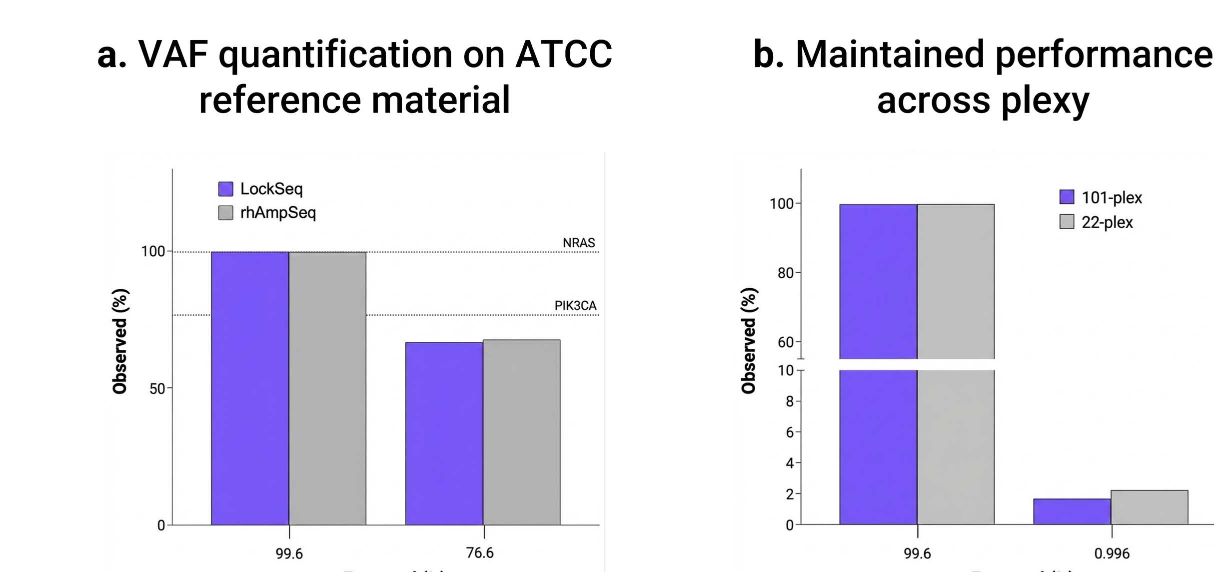

Fig. 5: Accuracy for VAF quantification was confirmed on ATCC reference material. a) LockSeq accurately quantifies both mutant variants - MDA-MB-453 (*PIK3CA* mutant) and SW 1271 (*NRAS* mutant) - when compared to rhAmpSeq. b) LockSeq maintains accuracy across scales. Additionally, we successfully pooled up to six panels (reaching 396-plex) while maintaining performance levels like the individual panels (data not shown).

LockSeq makes it easy to analyse & assess edit quality with confidence across hundreds of sites

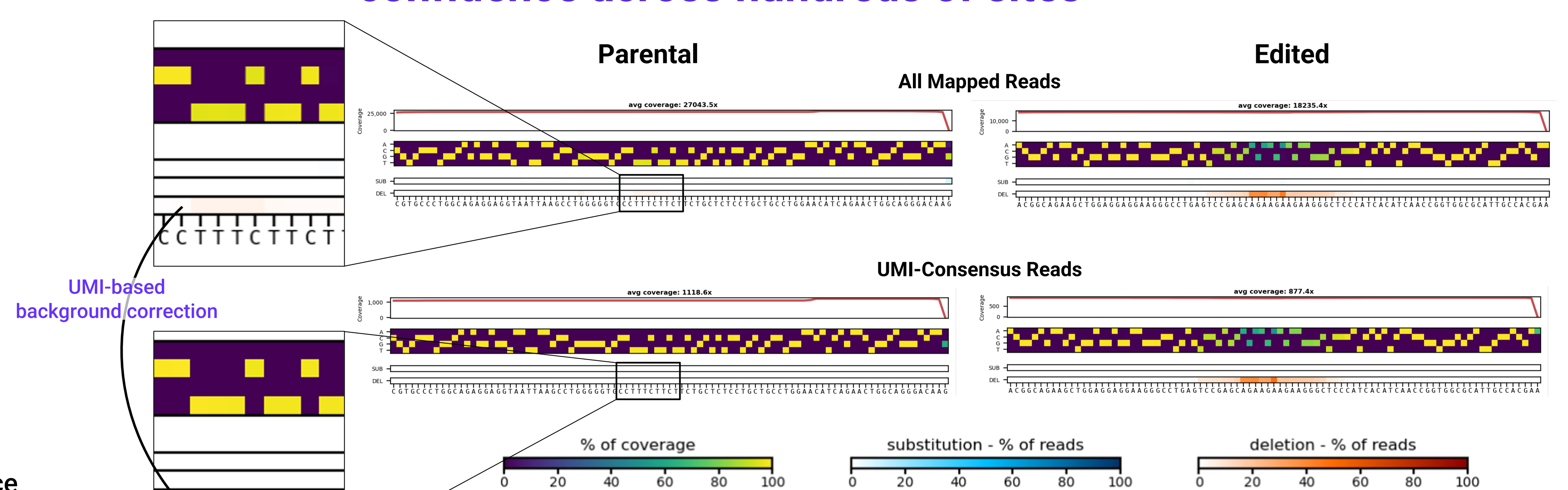

Fig. 6: Exemplary figures from the LockSeq Analysis Pipeline illustrating the read-confidence enhancement via our UMI-consensus engine. The pipeline automatically analyses multiplex validation library data and neutralizes background noise to offer an extremely low False Positive Rate (FPR) (see inset 'UMI-based background reduction'). Additionally, the pipeline assigns high confidence variants and reports results in an intuitive format for actionable decision-making.

LockSeq enables high-fidelity NGS-based on- and off-target confirmation

- Enables quick and accurate on- and off-target confirmation at scale
- Minimizes dropouts and delivers uniform target coverage
- Supports a standardized workflow with panel flexibility across development phases
- Available as an end-to-end service, with a reagent licensing model under development

References

1. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-products-incorporating-human-genome-editing
2. Hiatt *et al.* Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. *Genome Res.* 2013. 23: 843-854. Doi: 10.1101/gr.147686.112
3. Dobbs, van Eijk, Fellows *et al*. Precision digital mapping of endogenous and induced genomic DNA breaks by INDUCE-seq. *Nat Commun* 13, 3989 (2022). Doi: 10.1038/s41467-022-31702-9