DNAscope™: A novel chromogenic in-situ hybridization technology for high-resolution detection of DNA copy number and structural variations

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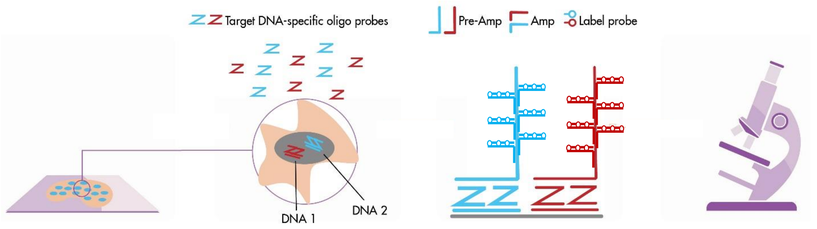
INTRODUCTION

Genomic DNA anomalies such as copy number variations (gene duplication, amplification, deletion) and gene rearrangements are important biomarkers and drug targets in many cancer types. DNA in-situ hybridization (ISH) is the gold standard method to directly visualize these molecular alterations in formalin-fixed paraffin-embedded (FFPE) tumor tissues at single-cell resolution within a histological section. However, currently available fluorescent ISH (FISH) assays provide limited morphological detail due to the use of fluorescent nuclear staining compared to chromogenic staining. Furthermore, FISH techniques rely on expensive fluorescence microscopes, risk loss of fluorescent signal over time and involve tedious imaging at high magnifications (100X). There is thus an unmet need for a sensitive and robust chromogenic DNA-ISH assay that can enable high-resolution detection of genomic DNA targets with the ease of bright-field microscopy.

We present here DNAscope - a novel chromogenic DNA-ISH assay - for detecting and visualizing genomic DNA targets under a standard light microscope. DNAscope is based on the widely used RNAscope® double-Z probe design and signal amplification technology and provides unparalleled sensitivity and specificity with large signal dots readily visualized at 40X magnification and with full morphological context. Furthermore, DNAscope ensures specific DNA detection without interference from RNA due to the use of a novel RNA removal method. Using a duplex chromogenic detection assay in red and blue, we demonstrate highly specific and efficient detection of gene rearrangements (ALK), gene amplification (ERBB2, EGFR, MET) and deletion (TP53 and CDKN2A). The DNAscope assay has been carefully optimized for probe signal size and color contrast to enable easy interpretation of signals under conventional light microscopy or digital pathology. Compared to conventional FISH assays, DNAscope probes are standard oligos that are designed *in silico* to be free of any repetitive sequences and can be rapidly synthesized for any DNA target. In conclusion, the DNAscope assay provides a powerful and convenient alternative to commonly used FISH assays in many cancer research applications.

ASSAY WORKFLOW



1: Tissue section	2: Hybridize to target DNA	3: Amplify signal	4: Image
Start with properly prepared tissue sections and pretreat to allow access to target DNA.	Hybridize two sets of gene-specific ZZ probe pairs to two target DNAs.	Use two orthogonal signal amplification systems to detect two target DNAs. Capture a cascade of signal amplification molecules onto each target via hybridization. Detect with two chromogenic substrates.	Visualize target DNA using a standard bright-field microscope.

Figure 1: Assay workflow describing duplex chromogenic DNA-ISH using DNAscope™ assay.

RESULTS

Detection of copy number variations (gene amplification and deletion) using DNAscope assay

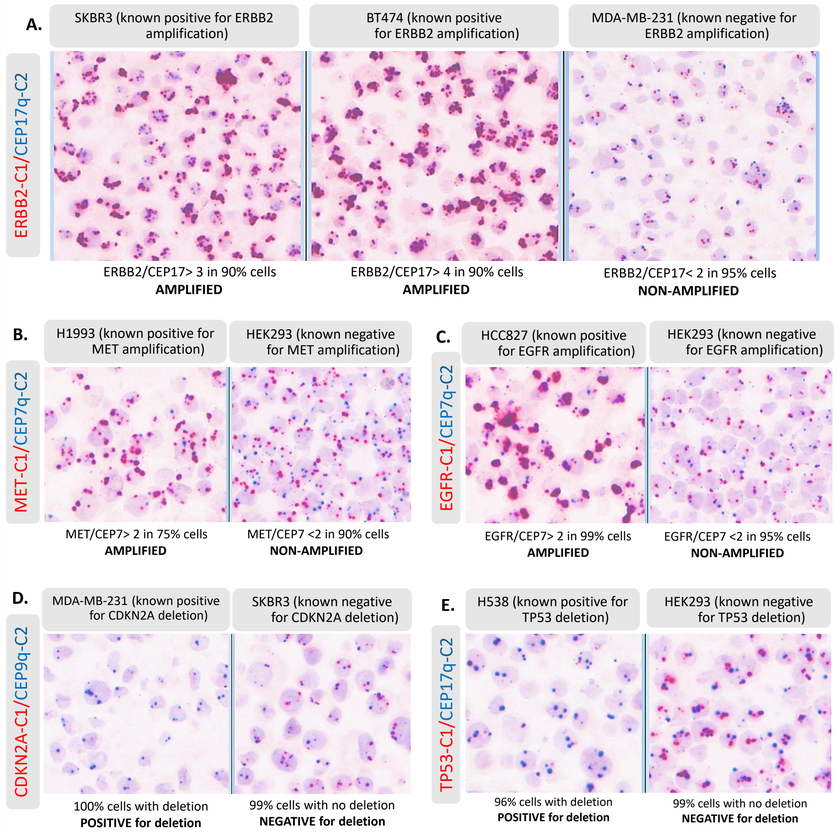


Figure 2: Detection of copy number variations with DNAscope probes and assay. (A). ERBB2 (red) amplification with respect to chromosome 17 probe (blue) demonstrated in known amplified cell lines SK-BR-3, BT474 and known negative cell line MDA-MB-231. **(B,C).** Positive and negative cell samples showing MET and EGFR gene amplification respectively in red with respect to chromosome 7 (blue). **(D,E).** Gene deletion demonstrated with CDKN2A and TP53 probes in red respectively, relative to chromosome probes.

Detection of gene fusion using DNAscope break-apart assay

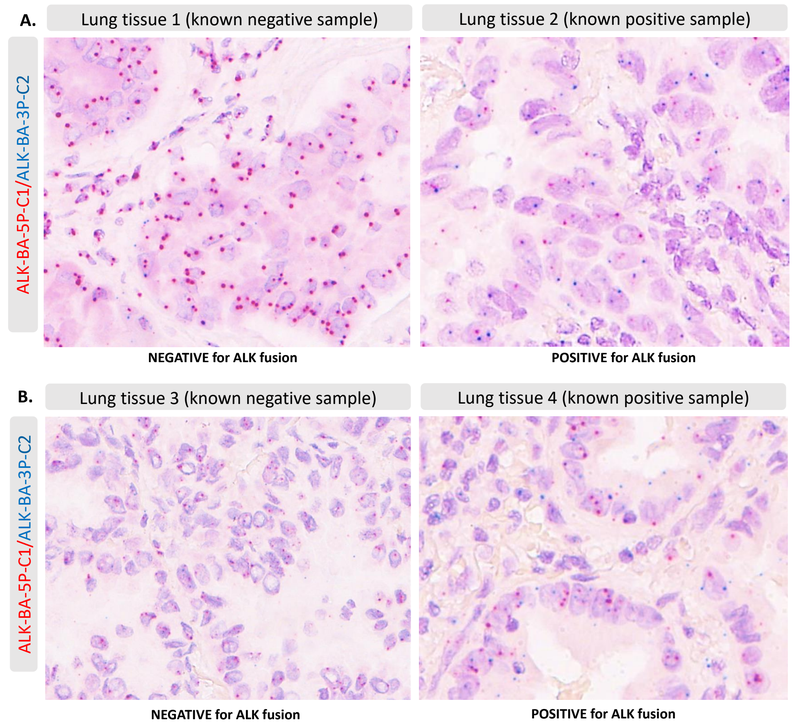


Figure 3: DNAscope detection of ALK gene fusion in FFPE lung tissue samples. Detection of ALK gene in validated positive and negative lung tissue samples showing break-apart positive samples identified by the appearance of pure blue dots.

CONCLUSION

- DNAscope™ assay is a novel chromogenic DNA-ISH technology that enables high-resolution chromogenic detection of genomic aberrations.
- High sensitivity and specificity of the assay and probes was demonstrated for copy number variations and gene translocations/fusions.
- Superior morphological context is facilitated by chromogenic detection, enabling easy interpretation without specialized equipment.

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