WHITE PAPER

A NOVEL CHROMOGENIC *IN SITU* HYBRIDIZATION ASSAY FOR HIGH-RESOLUTION DETECTION OF DNA COPY NUMBER AND STRUCTURAL VARIATIONS

Anushka Dikshit, Vasudha Murlidhar, Li-Chong Wang, Bingqing Zhang and Xiao-Jun Ma
Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA 94560

INTRODUCTION

Detection of important biomarkers in the form of genomic anomalies such as copy number variations (gene duplication, amplification, deletion) and gene rearrangements provide vital information for diagnosis and treatment strategies especially for cancer. In recent years, genome-wide high throughput techniques such as next-generation sequencing (NGS) have been used to identify these genetic variations. Although such array-based techniques allow profiling of copy number variations and chromosomal translocations on a large scale, the results from these techniques lack tissue and morphological context and often require further validation. For example, the PCR amplification steps prior to sequencing cause exponential growth in the number of copies that are ultimately sequenced. If there are differences in the efficiency of target amplification it could negatively impact the accuracy of the copy number estimates. Direct detection techniques such as DNA *in situ* hybridization (ISH) allow visualization of genetic alterations within the tissue and support molecular pathology research and diagnostics.

The new DNAscope™ HD Duplex assay is a novel chromogenic DNA ISH assay based on the RNAscope™ double-Z probe design and signal amplification technology that provides high sensitivity and specificity with an optimized workflow (**FIGURE 1**). It is a duplex assay designed to optimally visualize gene amplifications, deletions and rearrangements with robust reproducibility.

In situ hybridization is the gold standard to localize nucleic acid targets within cells and tissues with spatial information. The RNAscope *in situ* hybridization technology has been a leader in the field of spatial genomics providing single molecule detection of RNA at single-cell and subcellular resolution. The technology has evolved to encompass detection of specialized RNA targets such as splice variants, highly homologous sequences and point mutations using the BaseScope™ assays, and miRNA, siRNAs and anti-sense oligo detection using the miRNAscope™ assays.

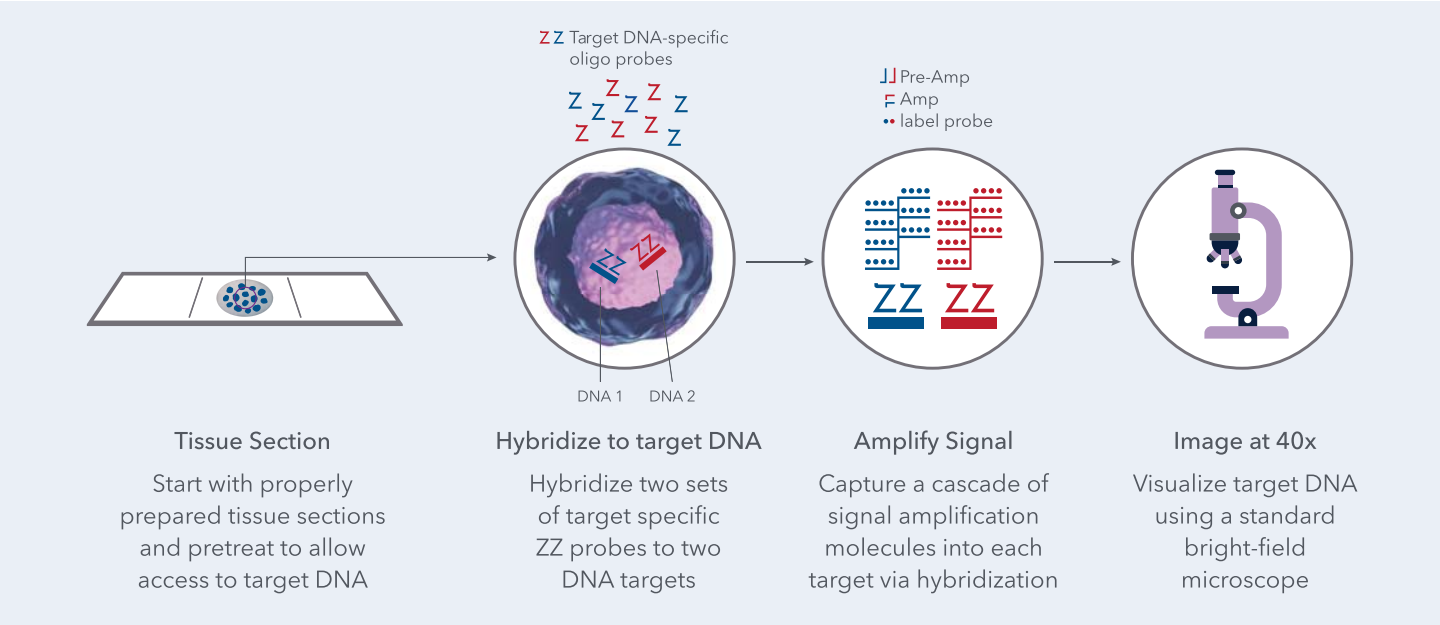


FIGURE 1. Schematic representing new DNAscope HD Duplex assay workflow.

CURRENT TOOLS FOR DNA *IN SITU* HYBRIDIZATION

DNA ISH is a spatial profiling tool based on hybridizing labeled complementary probes to the target DNA inside the cell nucleus to visualize any genetic alterations in tissues. FISH (fluorescent *in situ* hybridization) has emerged as the predominant DNA ISH tool for diagnostic analyses providing simultaneous detection of multiple targets on the same section. The advances in probe labeling and design strategies in the last six decades have contributed significantly to the adoption and utilization of DNA FISH assays for molecular diagnostics. However, there are still key limitations that hamper the routine use of a fluorescence-based ISH platform.

- FISH assays provide limited fields of view and morphological detail due to the use of fluorescent imaging at a high magnification making it challenging to distinguish tumor cells from non-tumor cells for interpretation and accurate scoring as well as overall assessment of tumor heterogeneity.
- Multicolor FISH assays require specialized high resolution fluorescent microscopes and highly trained personnel which are not always readily available in pathology labs.
- FISH assays mainly utilize Bacterial Artificial Chromosome (BAC) clone-based probes that are large and tend to contain multiple genes, thus lacking single gene specificity.
- BAC-based probes often contain numerous repetitive elements which can lead to unspecific signals despite optimized buffers and conditions.
- Preparing BAC-based FISH probes can be challenging and time consuming and subject to clone availability.

COMPARATIVE ANALYSIS OF DNAscope ASSAY WITH CURRENTLY AVAILABLE DNA ISH ASSAYS

Overexpression of ERBB2 was detected in breast cancer cell lines, BT474 and MCF7 using the DNAscope assay and compared to four other currently available manual and automated DNA ISH assays. Similarly, ROS1 rearrangement was studied in 'fusion-positive' HCC-78 lung cancer cell line and 'fusion negative' U87 glioblastoma cell line using the DNAscope assay and another chromogenic ISH assay (**FIGURE 2**). Compared to these traditional FISH and CISH (chromogenic *in situ* hybridization) assays, the new DNAscope assay demonstrated very high sensitivity and specificity with clear signal 'dots'. With the DNAscope assay, the clear and specific dots readily allow for the determination of changes in copy number or presence of gene fusion. However, the other assays showed either non-specific staining , very low signal intensity or indiscernible signal suggesting break apart due to poor chromogen contrast. These data indicate that the DNAscope assay has very high sensitivity and specificity over the standard DNA ISH assays that are currently available.

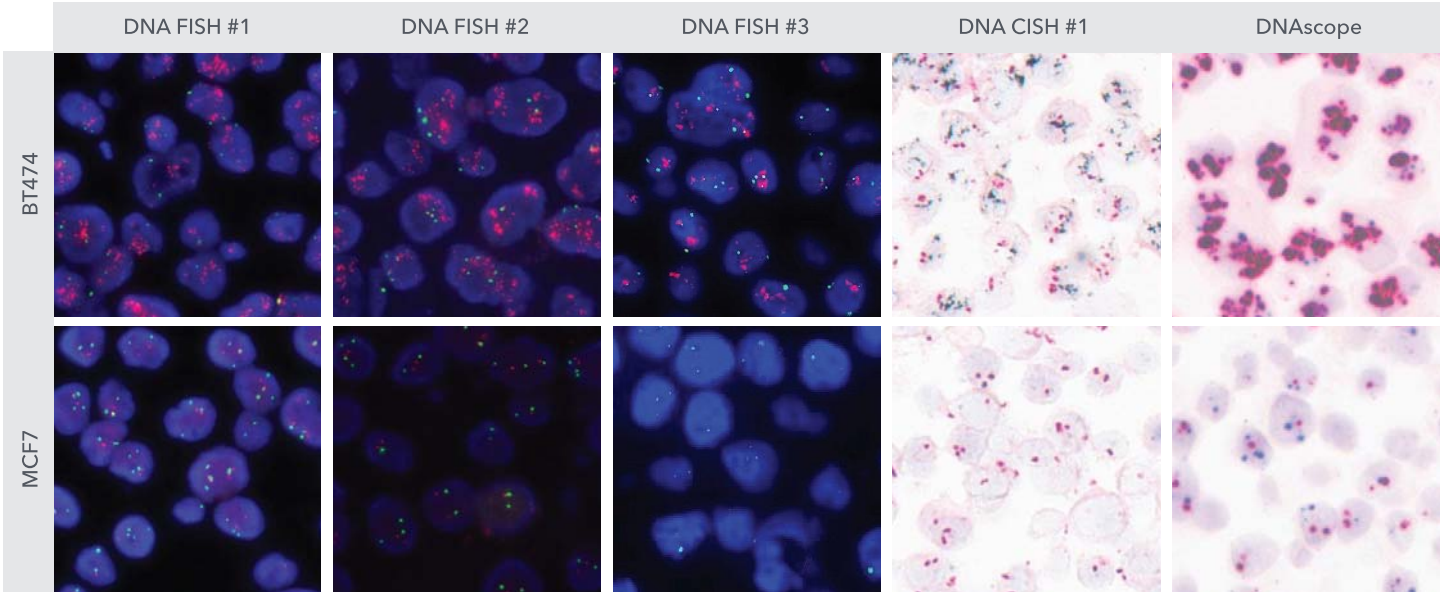


FIGURE 2A. Comparative analysis of ERBB2 gene amplification data using current market DNA *in situ* hybridization assays with the new DNAscope assay.

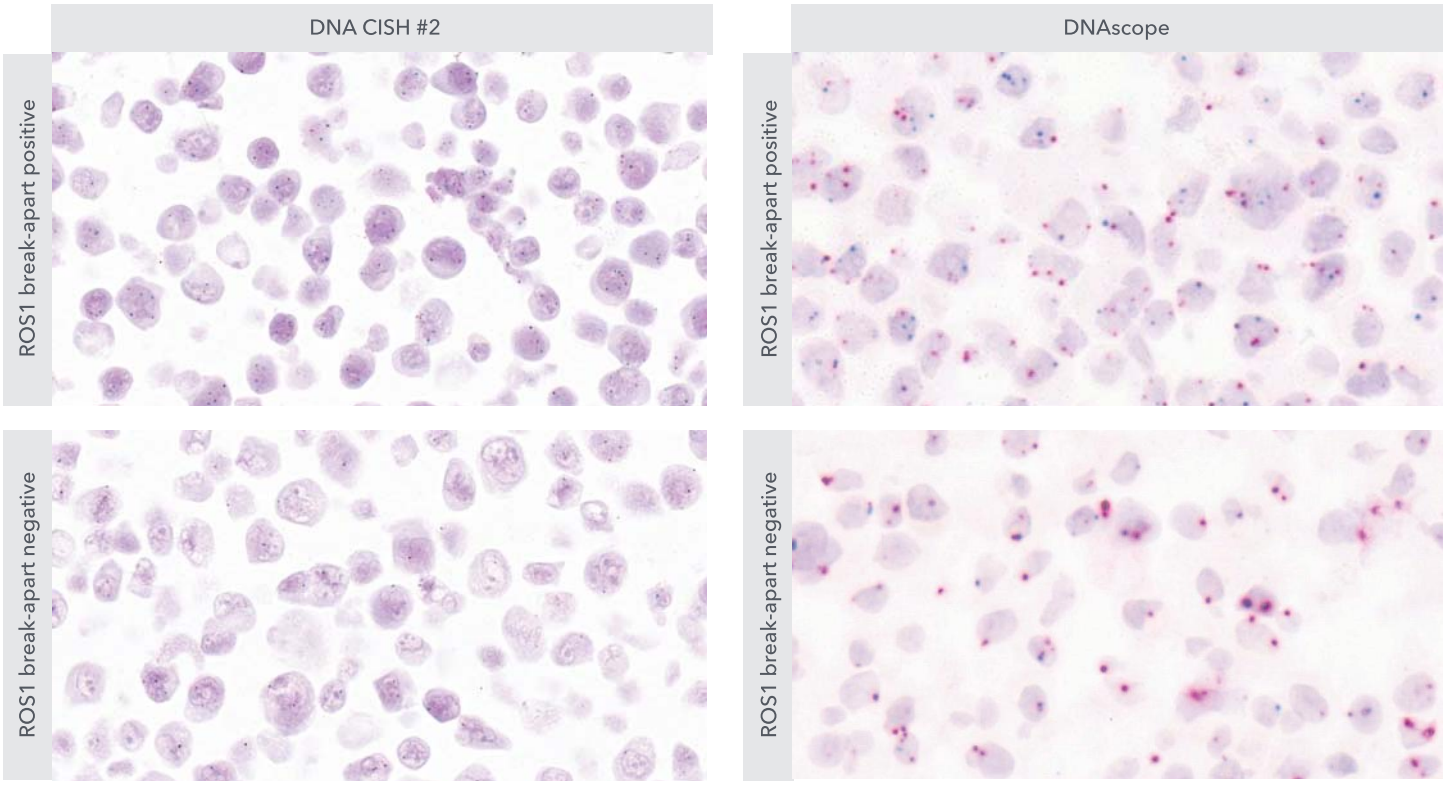


FIGURE 2B. Comparative analysis of ROS1 gene rearrangement detection using current market DNA *in situ* hybridization assay with the new DNAscope assay.

DNAscope HD DUPLEX ASSAY FOR THE DETECTION OF COPY NUMBER VARIATIONS, TRANSLOCATIONS AND GENE REARRAGEMENTS

The DNAscope assay was developed to overcome the limitations of conventional DNA ISH assays and provide high resolution detection of chromosomal aberrations with morphological and spatial context. This assay is designed as a duplex chromogenic detection platform using red and blue chromogens. This assay has some unique features that enable exceptional performance for detecting target DNA.

- The DNAscope chromogenic assay enables convenient signal detection using a brightfield microscope without the necessity for expensive image analysis equipment and specially-trained personnel.
- The DNAscope assay has been carefully optimized for probe signal size and color contrast allowing clear visualization of signal with easy data interpretation.
- 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.
- The signal amplification technology ensures high sensitivity and signal to noise ratio.
- This assay affords high resolution and precise detection of small genomic alterations.

DETECTION OF COPY NUMBER VARIATIONS

Copy number variations (CNVs) can occur due to spontaneous events or can be inherited and cause changes in the copy number of one or more genes due to deletions and duplications/amplifications. CNVs can be associated with a range of disease pathologies. CNVs of some genes can lead to initiation and/or progression of certain cancers. Therefore, identifying CNVs commonly found in multiple malignancies can provide information for understanding disease prognosis and development of treatment strategies. The DNAscope assay can be successfully used for detecting gene deletions and amplifications. This assay utilizes probes targeting centromeric regions as chromosome enumeration control probes (e.g., *CEP17/CEP9/CEP7*) which are visualized in blue and the target of interest is visualized in red. Detection of a pair of blue dots and a pair of red dots indicate no change in copy number. However, an increase in the number of red dots relative to blue dots indicates a copy number gain or amplification while a decrease in the number of red dots or no red dots indicates chromosomal loss or deletion (FIGURE 3A). Gene amplifications for *ERBB2, MET* and *EGFR* were successfully detected in breast cancer, lung cancer and H&N cancer cell lines, respectively, that are known to possess these gene amplifications (FIGURE 3 B). Similarly, gene deletions were detected for tumor suppressors such as *CDKN2A* and *TP53* in breast cancer and lung cancer cell lines, respectively (FIGURE 3C).

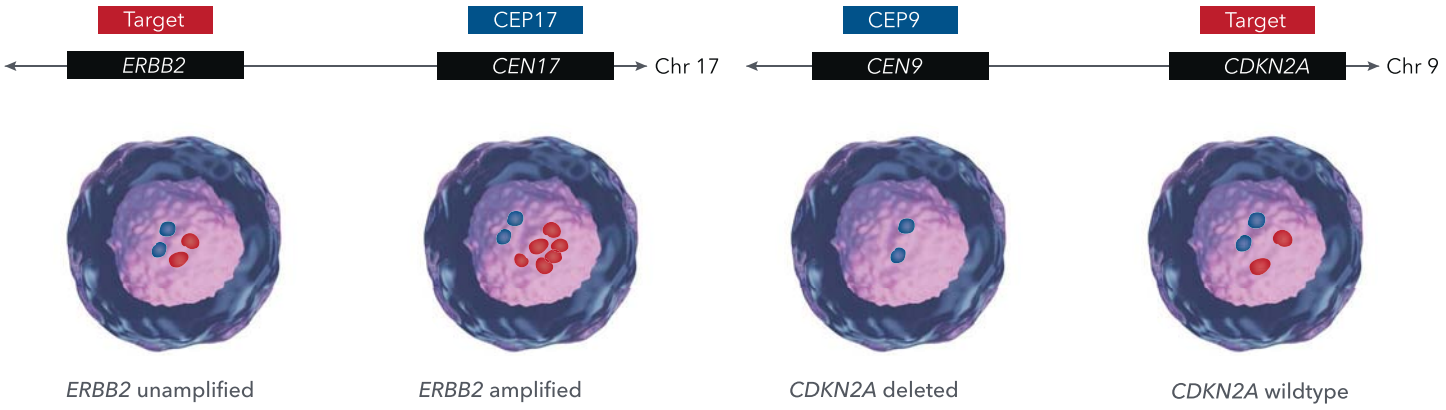


FIGURE 3A. Schematic depicting probe design strategy and data interpretation of DNAscope signal for detecting copy number variations.

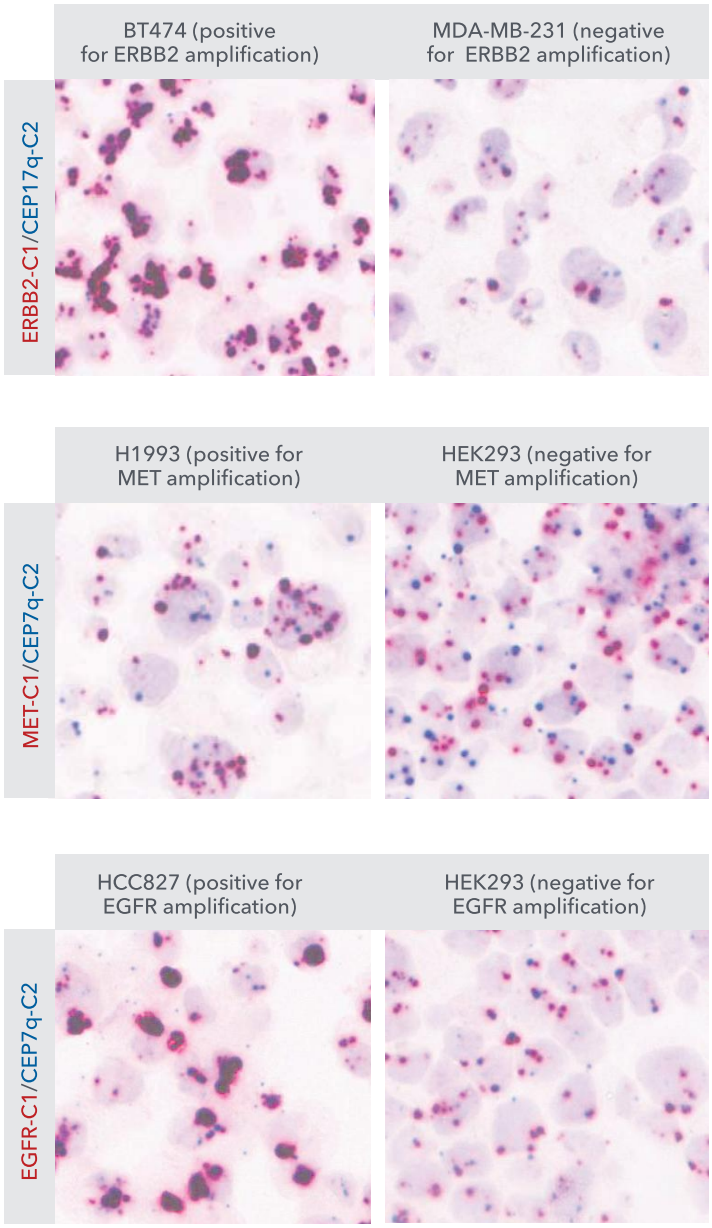


FIGURE 3B. Multiple red dots in the cell nuclei indicating amplification of ERBB2, MET and EGFR genes in BT474, H1993 and HCC827 cell lines, respectively.

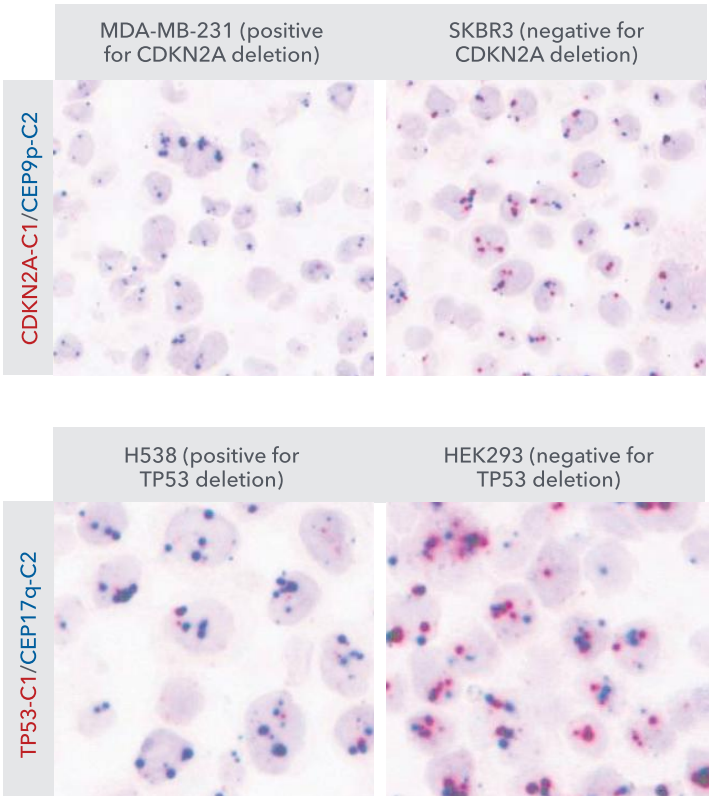
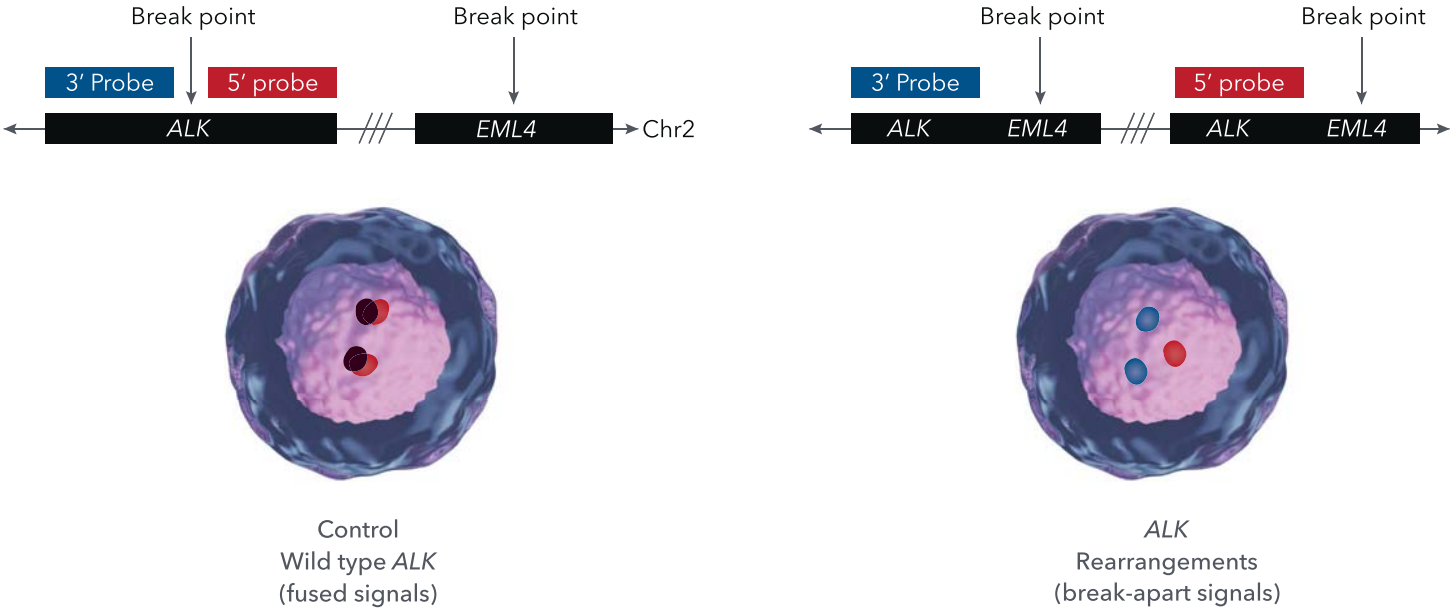


FIGURE 3C. Less than 2 red dots per cell nuclei indicating deletion of CDKN2A and TP53 target genes in MDA-MB-231 and H538 cell lines, respectively.

DETECTION OF GENE TRANSLOCATIONS AND REARRANGEMENTS

Gene translocations are chromosomal abnormalities that occur when a segment of one chromosome is transferred to a nonhomologous chromosome or to a different site on the same chromosome. Such translocations can impact the function of the genes present in the displaced segment of the chromosomal fragments. Some translocations can give rise to oncogenes that can induce malignant transformation of cells resulting in cancer. The DNAscope assay can be utilized to identify and visualize such rearrangements by detecting 'break-apart' events. The probes are designed such that in the absence of a break-apart event, the blue and red signals will coincide and appear as a big 'purple' or 'dark red' dot but when a break-apart event occurs, rearrangement of the target gene can be visualized as separated individual 'pure blue' and 'red' dots or only as 'pure blue' dots (**FIGURE 4A**). Rearrangement of ALK and ROS1 was successfully detected in lymphoma and lung cancer cell lines (**FIGURE 4B**), respectively, as well as in lung cancer tumor samples (**FIGURE 4C**).



BREAK-APART EVENTS ARE DETECTED THROUGH APPEARANCE OF PURE BLUE DOTS

FIGURE 4A. Schematic depicting probe design strategy and data interpretation of DNAscope signal for detecting gene rearrangement.

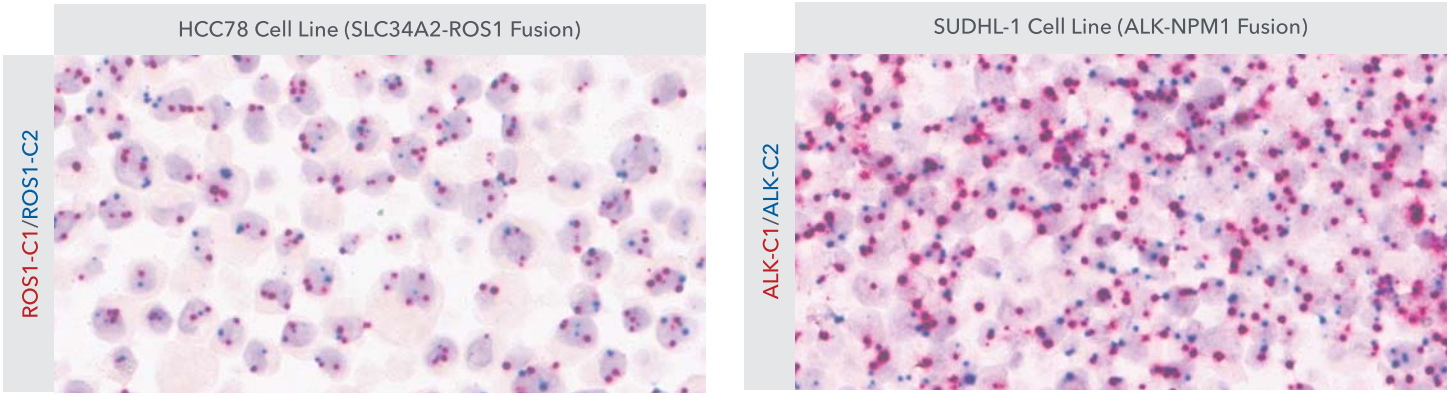


FIGURE 4B. Gene rearrangement of ROS1 and ALK targets detected by visualizing break-apart events in HCC78 and SUDHL-1cell line respectively.

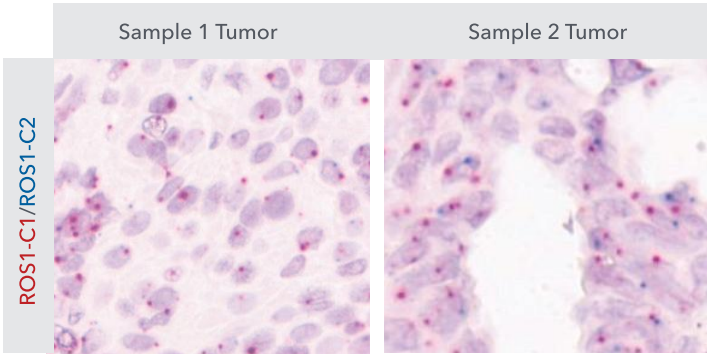


FIGURE 4C. Sample 2 tumor appeared to be positive for ROS1 gene rearrangement indicated by break-apart event visualized as bright blue dots.

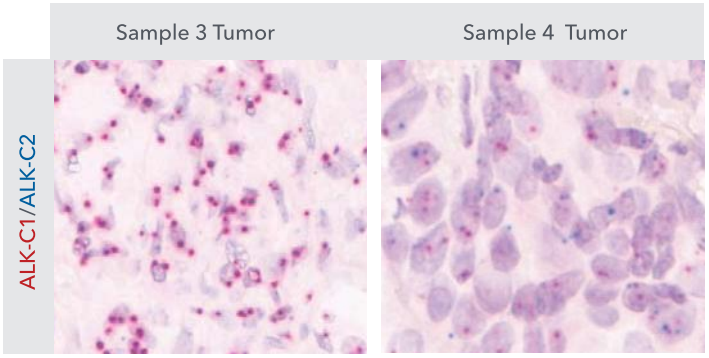


FIGURE 4D. Sample 4 tumor appeared to be positive for ALK gene rearrangement indicated by break-apart event visualized as bright blue dots.

SUMMARY

The DNAscope HD Duplex assay can reliably detect various chromosomal structural aberrations such as amplification, deletion and translocations/rearrangements at single-gene resolution. The new DNAscope assay provides very high sensitivity and specificity with large signal dots readily visualized at 40X magnification. The exceptionally high signal-to-noise ratio of this assay enables ease of visualization and interpretation. Compared to the use of large BAC clones to approximate specific chromosomal locations, the DNAscope assay allows precise targeting of genomic aberrations. The chromogenic detection system is a significant improvement over current FISH assays which require special equipment and highly trained specialists to use and interpret. Overall, the DNAscope assay is filling a long-standing unmet need for a sensitive and robust chromogenic DNA-ISH assay that can enable high-resolution detection of genomic DNA targets and chromosomal aberrations using bright-field microscopy.

NOTES

WHERE SCIENCE
INTERSECTS INNOVATION™

bio-techne®

bio-techne.com

R&D SYSTEMS | NOVUS BIOLOGICALS | TOCRIS | protein simple | ACD | exosome dx

Global info@bio-techne.com bio-techne.com/find-us/distributors TEL +1 612 379 2956 North America TEL 800 343 7475
Europe | Middle East | Africa TEL +44 (0)1235 529449 China info.cn@bio-techne.com TEL +86 (21) 52380373

MK 51-162 WP_DNAscope_STRY0122453