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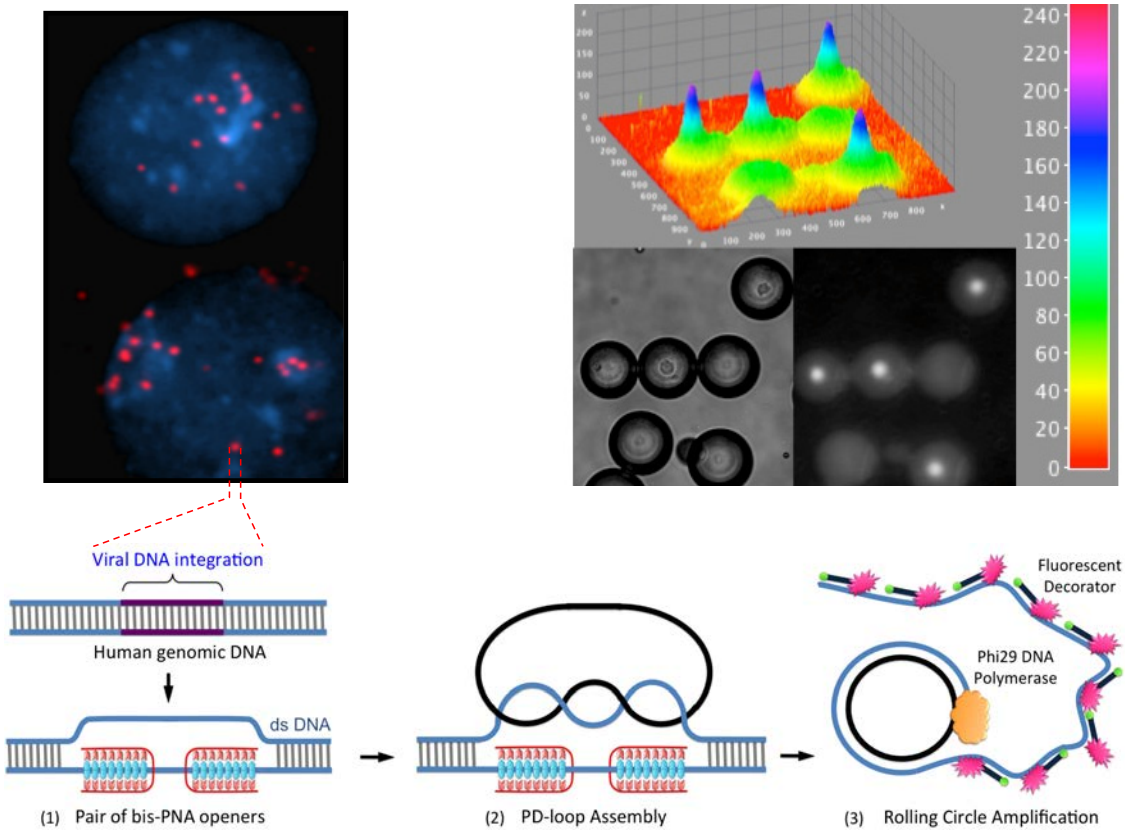
Target DNA detection and quantitation on a single cell with single base resolution

Being able to analyze single-cells is important to many fields of research and diagnostics; particularly in the context of morphologically preserved fixed cells. The main goal of this progressive multidisciplinary research is in the development of a novel molecular diagnostic technology for genomic double-stranded DNA analysis in its native state in single cell. This novel diagnostic method based on isothermal peptide nucleic acid (PNA) and rolling circle amplification (RCA) allows ultrasensitive detection and quantitation of short specific DNA sites in single cells with single base resolution in a cells-in-flow format.



Assay demonstration on fixed cells and in nano droplet based format

A team of researchers from Boston University, Northeastern University, Massachusetts General Hospital (MGH)/ Harvard Medical School (HMS) report a novel isothermal method based on PNA and RCA for ultrasensitive detection and quantitation of short specific DNA sites in single cells with single base resolution in a cells-in-flow format. In addition this method allows for quantitative detection of genetic variations down to single-nucleotide polymorphisms (SNPs). In comparison to a Flow-FISH technique which was previously used to analyze in flow short DNA sequences in chromosomes, our technique does not require any pre-treatment to denature cellular DNA such as high temperatures and high concentrations of formamide.

Encouragingly, the researchers have demonstrated the effectiveness of this method by detecting short, oncoviral DNA inserts in human cells. The team was able to visualize the viral genome in each infected cell and determine the number of oncoviral DNA sites per cell. When fully developed, the technology promises to be a solution to a class of problems, which require detection of mutations, transpositions, insertions and deletions and become particularly critical for understanding tumor material and prognostic cancer diagnostics.

This is an extremely versatile technique that is only limited by the selection of the DNA target. It can be employed in countless clinical and research applications", says Prof. Irina Smolina, of Boston University. Smolina's research is focused on developing approaches to fluorescently detect specific double-stranded DNA sequences under non-denaturing conditions, which is advantageous for certain applications. Smolina's team has been developing a method that facilitates ultra-specific and very sensitive detection of short unique sequences (20−30 nucleotides) within genomic DNA in its native, double-stranded form. Smolina hopes that such approaches will potentially provide with much higher specificity than methods based on global DNA denaturation.

"It is a fascinating application" says Prof. Tania Konry, of Northeastern University. Prof. Konry's team is working on developing microfluidic approaches for miniaturization of bioassays and diagnostic tools. "We were able in the past to adapt droplet based approach to cell secretion and surface studies. This microfluidic droplet technology is particularly advantageous when single-cell/single-molecule analysis is required and it is particularly important in understanding cell population heterogeneity effects. In this paper, we were able to simplify the flow component of the assay by adapting the PNA-RCA method to accommodate a one-step nanoscale isothermal assay using a droplet-based microfluidic system developed in our laboratory."

This groundbreaking research aims to develop a novel technique for screening of mammalian cells for genetic variations in the range of few tenths of nucleotides, Smolina believes. "The study will equip conventional FACS analyses with the exciting possibility to discriminate even single-base changes or point mutations in the context of the entire genome on the morphologically preserved whole cell level," she asserts. The new DNA-based diagnostic method the research team is working on is based on recent advances in the PNA field and the droplets microfluidic to achieve a highly specific and sensitive assay. Key advantages include low reaction volumes and isothermal conditions that allow for preservation of cell structure. The group anticipates that their work will equip biomedical and clinical researchers with a new powerful tool for the detection of genetic alterations that convey specific DNA sequence variability, responsible for the predisposition of individuals to a specific malignancy.

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