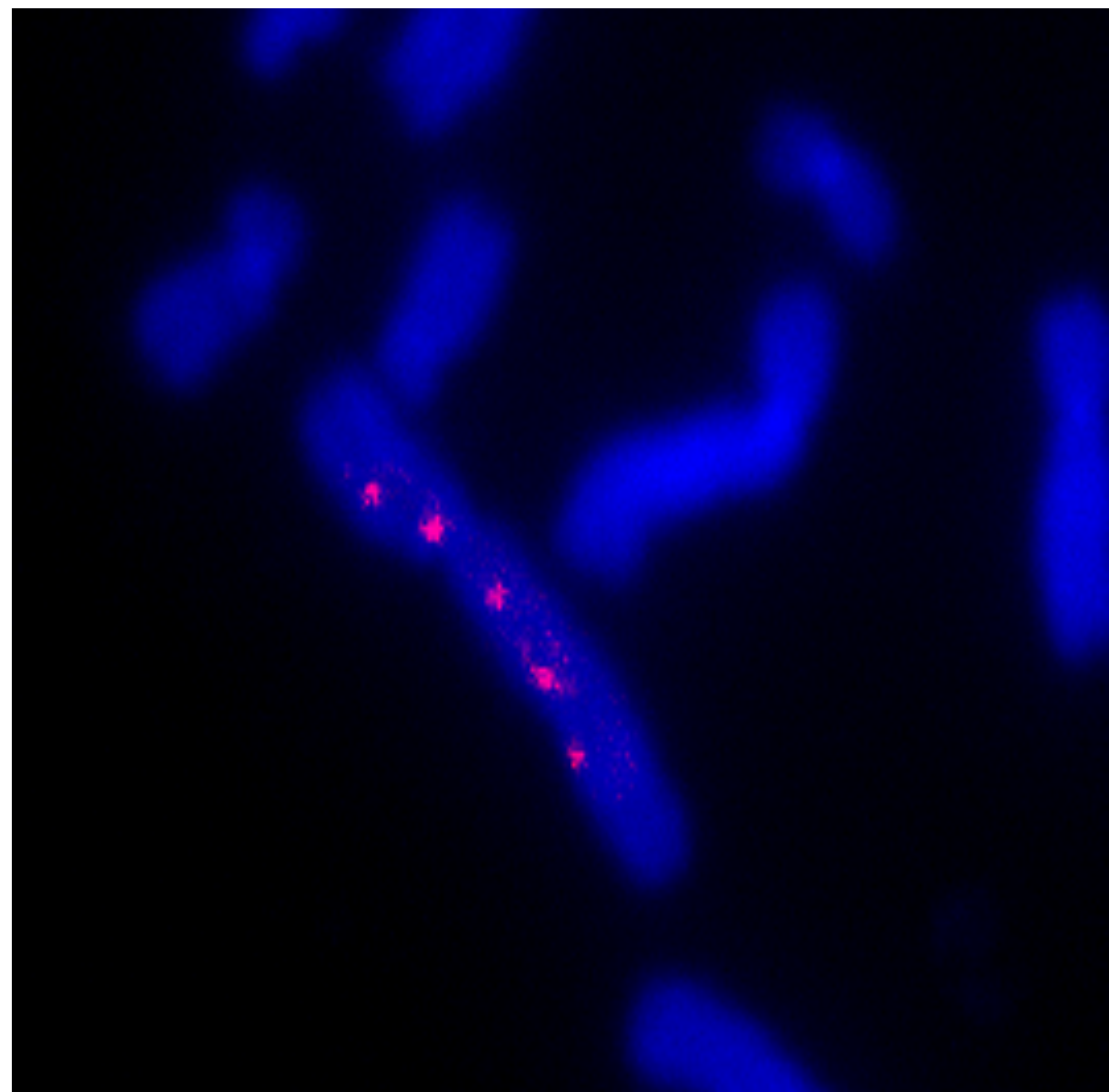


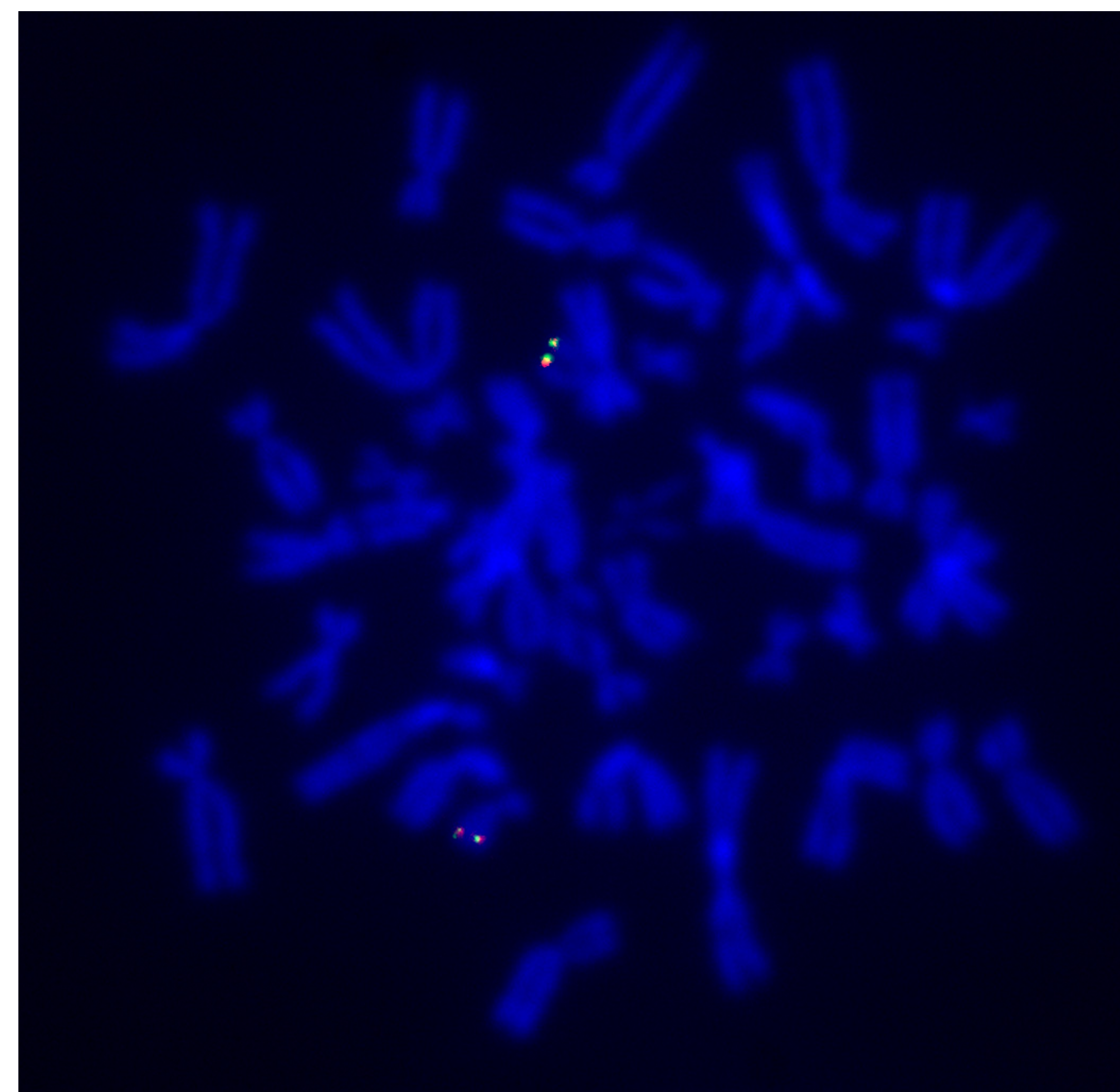
Small Target Detection Using Pinpoint FISH™

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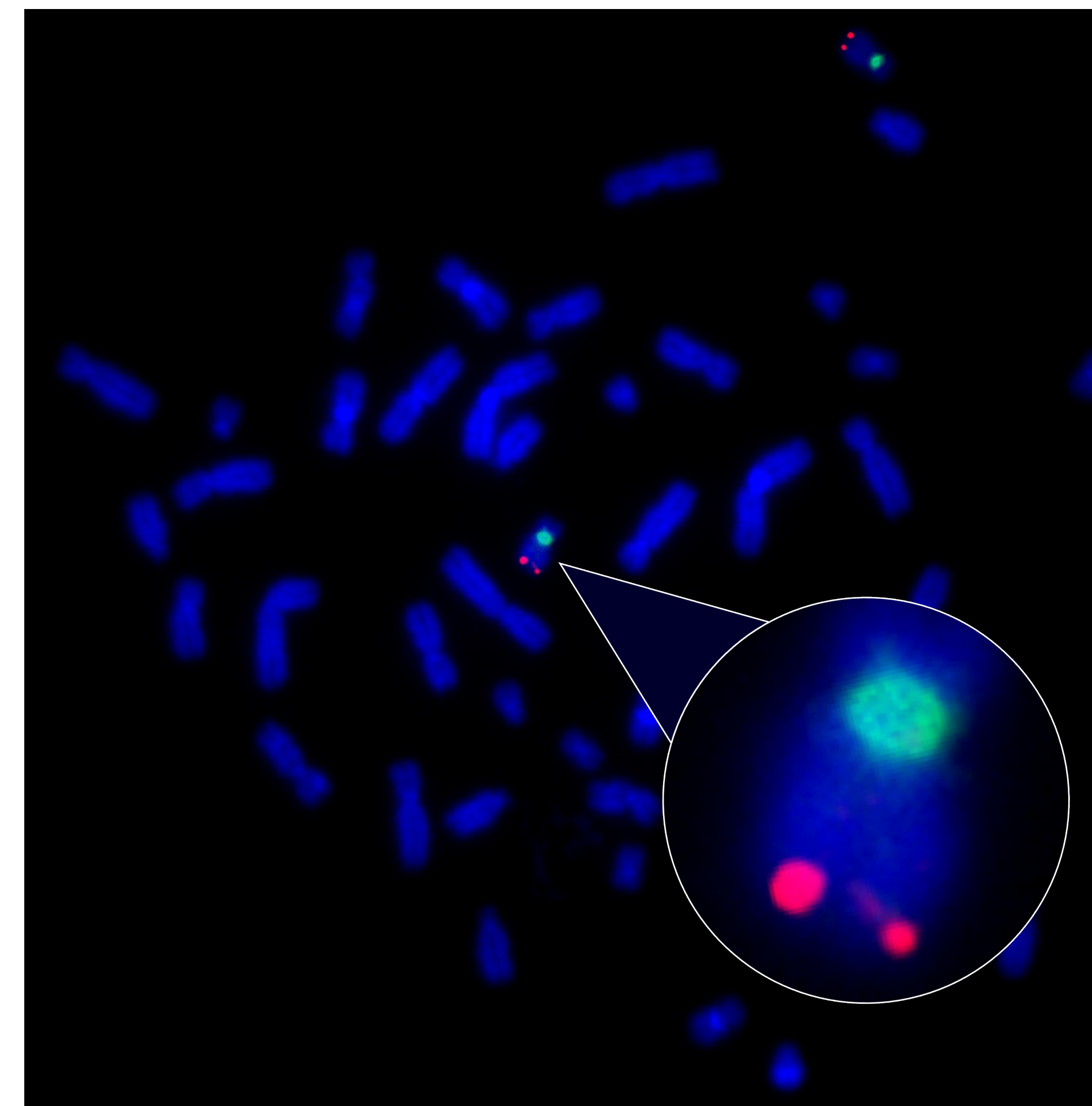
Pinpoint FISH™ is an application of KromaTiD's Directional Genomic Hybridization (dGH) technology, used specifically in fixed samples. By leveraging the bioinformatics and assay design tools developed for dGH, KromaTiD can design specific probes for applications in FISH, enabling visualization of very small targets. Design flexibility allows Pinpoint FISH probes to avoid areas of homology, or repeats, which can generate off target signals or non-specific binding and high background from BAC FISH probes. Here we discuss Pinpoint FISH probes used to demonstrate our limit of detection, to elucidate chromatin structure, and to detect a specific loss of function associated with an oncologic outcome. The various applications illustrate the range of utility for Pinpoint FISH to detect small, specialized targets.



Limit of Detection test. Signals corresponding to different sized targets are shown on a single chromosome. Routine detection of a 10Kb target was demonstrated



Pinpoint FISH probes designed to determine co-location of a binding site to a reference location. The binding site probe (red) is 10KB, and the reference location probe (green) is 100KB.



The same 10KB red probe, combined with Ch17 Control Probe from Empire Genomics, in Empire's standard overnight hybridization buffer and method.

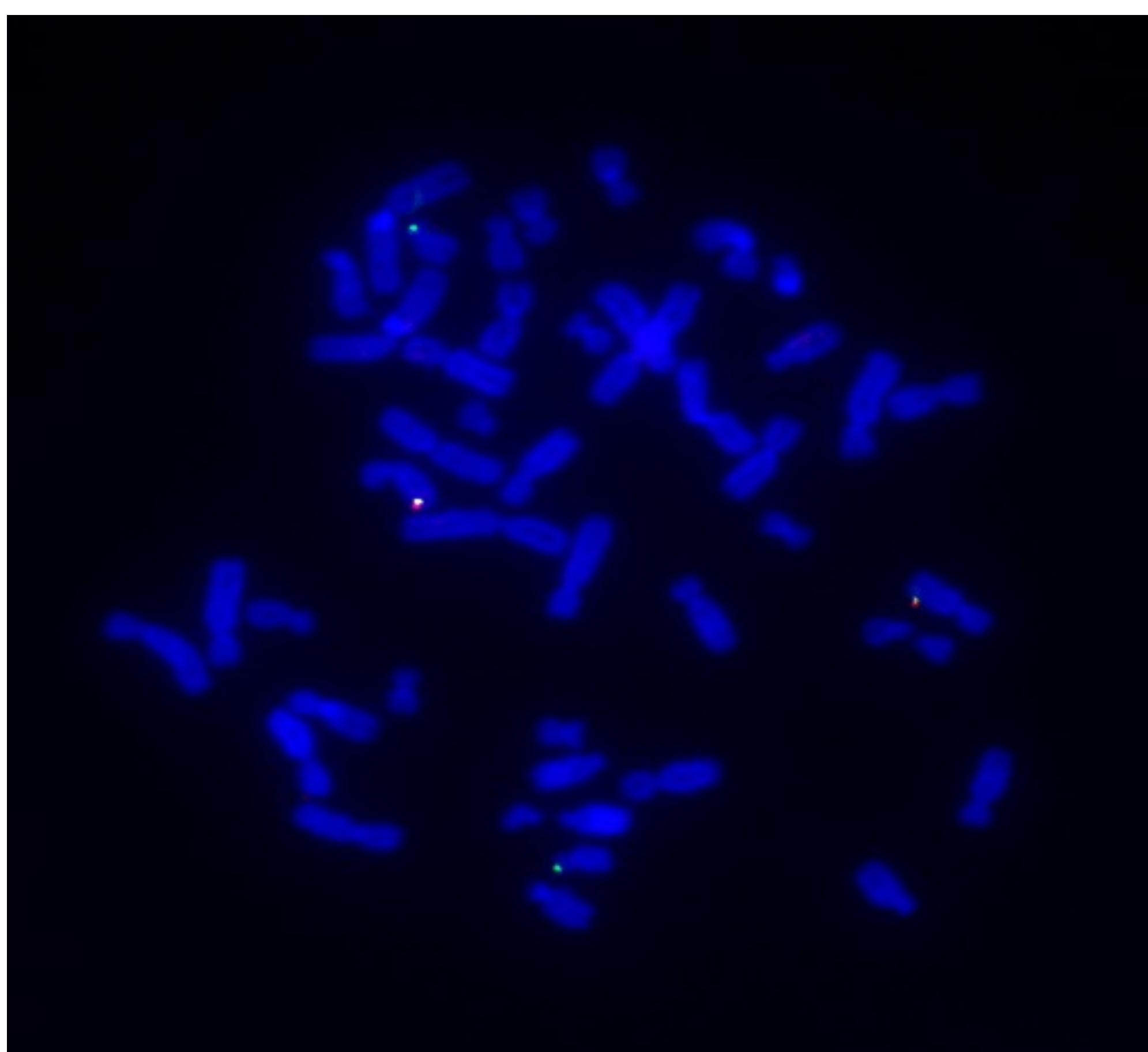
KromaTiD

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KromaTiD Pinpoint FISH probes can be combined in the same assay with BAC probes, in standard FISH buffers. Our probes have been tested using protocols from Empire Genomics and Abbott Molecular.

The assay below is designed to detect rearrangements to NFKB2 on Chromosome 10, with a breakpoint in exon 13. The red probe covers exons 1-12 (4945bp), and the yellow probe covers the 3' end of the gene (4928bp). The assay is also designed to detect the involvement of a potential translocation partner which contains a pseudogene on Chromosome 18. The green probe targets ANKRD62, adjacent to the pseudogene (34.1Kb).

The image on the left shows the assay in all imaging channels in BJ-1 (normal male fibroblast) control cells. The set of 3 images on the right shows the individual channels on the same image, in cell line JK6L (Lymphoma).



Assay in BJ-1 cell line.

