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IT-7-P-3342 Applying of 6-carboxyfluorescein (6-FAM) to cytogenetics

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6-carboxyfluorescein (6-FAM) is one of the most commonly employed and simplest fluorescent reagents to use in oligonucleotide synthesis. 6-FAM is highly reactive, water-soluble single isomer of fluorescein, with absorbance/emission maxima in the visible region of the electromagnetic spectrum (492/517 nm respectively). 6-FAM plays a particularly important role in real-time PCR and SNP-analysis, being used in TaqMan probes, Scorpion primers and Molecular Beacons. Oligonucleotides labeled with 6-FAM at the 5'-end are widely adopted as PCR and DNA sequencing primers to generate fluorescently-labeled products for sequencing and genetic analysis. Per se 6-FAM-labeled oligonucleotides can be used as hybridization probes for fluorescent in situ hybridization (FISH), for example for a direct visualization of microorganisms in human and animal clinical samples (e.g. Behrens et al., 2004; Lin et al., 2011; Fontenete et al., 2013).

We used 6-FAM-labeled oligonucleotide probes specific for various chicken tandem repeats to detect RNA-transcripts and to localize them on giant transcriptionally active lampbrush chromosomes dissected from growing chicken oocytes. Lampbrush chromosomes have distinctive chromomere-loop patterns that enable high-resolution cytogenetic mapping of unique and repeat nucleotide sequences. We report that due to the high brightness and relatively long lifetime, the 6-FAM is found to be well suited for FISH proceeded accordingly with a DNA/(DNA+RNA) hybridization protocol.

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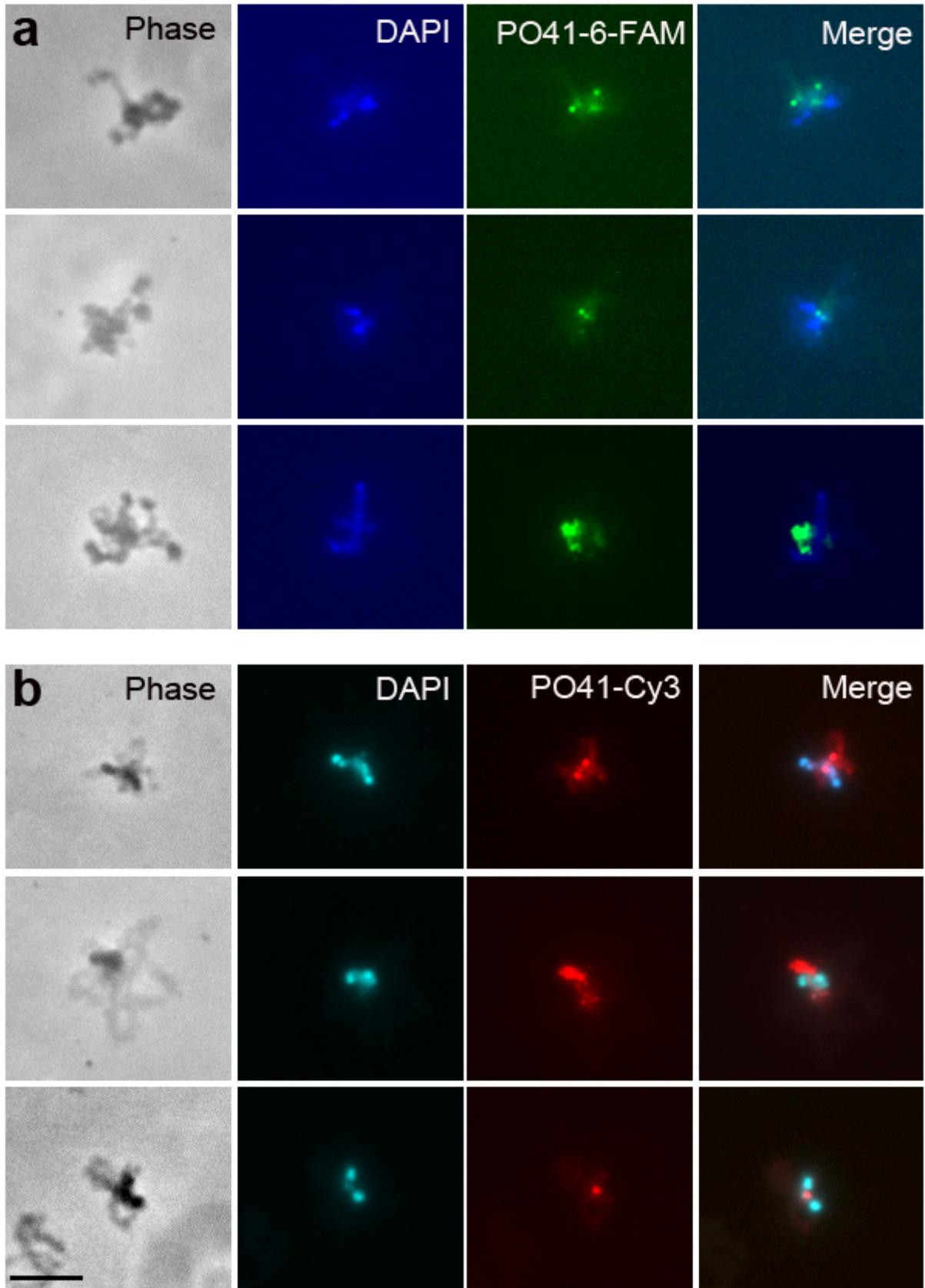


Fig. 1: FISH with oligonucleotide probe PO41 on chicken lampbrush microchromosomes. (a) Representative smallest microbivalents probed with PO41 labeled with 6-FAM (green signal); (b) microbivalents probed with PO41 labeled with Cy3 (red signal). Chromosomes are counterstained with DAPI. Left panels - phase contrast images. Scale bar 5 μ m.