Practice Point

Fluorescent in situ hybridisation: A must for delineating subtle chromosomal alterations

Mahrukh Hameed Zargar, Tahir Mohiuddin Malla

Advanced Centre For Human Genetics, SKIMS

Suspected chromosomal disorders comprise the major portion of the referrals for any genetics laboratory. The conventional cytogenetic analysis remains the most widely used method to establish an aetiology in these disorders[1]. However, a large portion of the cases remain undiagnosed because conventional cytogenetics cannot detect chromosomal changes of less than 5Mb. Therefore, Fluorescent in situ hybridisation (FISH) remains a method of choice to detect submicroscopic chromosomal alterations using specific probes.

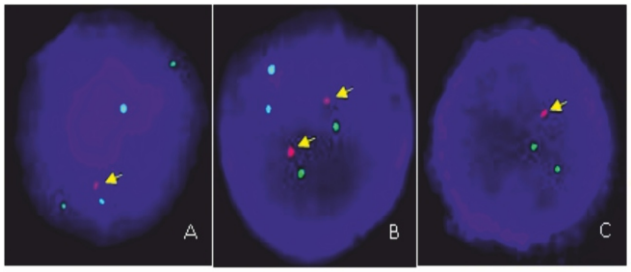
Fluorescent in situ hybridisation (FISH)

FISH is a technique that uses fluorescently labelled locus-specific DNA probes to detect a specific chromosomal alteration. The probe is a short fragment of DNA, fluorescently labelled and complementary to a region of interest of a DNA. The metaphase or interphase chromosome DNA is co-denatured with a specific fluorescently labelled probe using a hybridizer. The hybridizer hybridises the FISH probe at the target sequence while it re-anneals into a double-stranded DNA. The specific colour signal can be detected using a fluorescence microscope equipped with an appropriate set of filters, and the presence or absence of the signal in the metaphase or an interphase cell depicts the presence or absence of the region of interest on a specific chromosome. This makes this technique an ideal one for detecting chromosomal aneuploidies, deletions, duplications and translocations using centromeric enumeration, specific, dual colour break-apart and fusion probes[2]. The FISH can be performed on both metaphase and interphase cells simultaneously[3]. Moreover, tissue sections from fresh and formalin-fixed, paraffin-embedded sections and cytology preparations are also used. The result turn out time for FISH is just 24 hours, and it is time-saving. FISH probes are of many types and are prepared from bacterial artificial chromosome (BAC) clones and are commercially available.

FISH has given birth to another technique known as Spectral Karyotyping (SKY) that uses multicolour FISH probes simultaneously, thereby remarkably improving the stratification of complex chromosome alterations[4]. In this technique, each chromosome is painted differently using whole chromosome painting probes, making it easy to detect complex chromosomal exchanges.

Technical considerations in FISH

We utilised FISH with locus-specific probes to detect microdeletions in children with developmental delays and intellectual disabilities (Figure 1). However, certain technical challenges must be addressed during the reporting process.



Correspondence:
Dr. Mahrukh Hameed Zargar, PhD
Additional Professor, Advanced Centre For Human Genetics SKIMS
Email: mahrukh.hameed@skims.ac.in

Access this article online

Website: www.jmsskims.org

DOI: https://doi.org/10.33883/jms.v28i1.1427

How to cite this article: Zargar M, Malla TM. Fluorescent in-situ hybridization: A must for delineating subtle chromosomal alterations. JMS 2025: 28(1)38-39
Received: 2025-01-25 Accepted: 2025-03-04

This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(a) The fluorescence emitted by the fluorochrome on a probe diminishes quickly when exposed to light under a fluorescent microscope. This can make it challenging to detect signals, particularly when using small deletion probes. To address this, capture the image immediately using a camera attached to the microscope, ensuring the signals are recorded before the fluorescence fades.

(b) Selecting an appropriate area on a FISH slide is crucial for accurate interpretation. Focus on regions with low background noise, well-dispersed cells, and bright signals for analysis. To draw a conclusion, analyse at least 200 interphase nuclei.

(c) To minimise bias, the slides should be independently examined by two well-trained individuals.

Figure 1 (A)-Representative interphase nucleus showing 15q deletion (loss of orange signal) in a suspected Angelman syndrome, (B)-Normal interphase nucleus showing two red signals for the 1p36 region, (C)- Interphase nucleus showing 7q31 deletion in a suspected Williams syndrome.

1. Ghazaey S, Mirzaei F, Ahadian M, Keifi F, Semiramis T, Abbaszadegan MR. Pattern of Chromosomal Aberrations in Patients from North East Iran. Cell J Yakhteh. 2013;15(3):258–65.

2. Qiu HR, Miao KR, Wang R, Qiao C, Zhang JF, Zhang SJ, et al. The application of fluorescence in situ hybridisation in detecting chronic myeloid leukaemia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2009;26:207-210.

3. Li MM, Andersson HC. Clinical application of microarray-based molecular cytogenetics: an emerging new era of genomic medicine. J Pediatr. 2009;155:311-317.

4. Chandran RK, Geetha N, Sakthivel KM, Aswathy CG, Gopinath P, Raj TVA, et al. Genomic amplification of BCR-ABL1 fusion gene and its impact on the disease progression mechanism in patients with chronic myelogenous leukaemia. Gene. 2018;686: 85-91.