

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Use of Molecular Cytogenetic Techniques for the Identification of Chromosomal Abnormalities

Rasime Kalkan

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/67415>

Abstract

Chromosomal analysis is an increasingly important diagnostic procedure in numerous areas of clinical medicine that includes haematology, perinatology or obstetrics. Chromosomal disorders are viewed as a major category of genetic diseases, and sometimes the identification of abnormal chromosomes is not easily applicable. Just like the identification of the marker chromosome or the identification of the complex karyotypes is important in clinics for the evaluation of the patient prognosis as well as the treatment response, needless to say; fluorescence in situ hybridization (FISH) is the most suitable and rapid method in the above-mentioned situations. It gives chance to the rapid analysis of chromosomal aneuploidies in dividing and non-dividing cells. In this chapter, we will discuss the general principles of the chromosomal abnormalities and the molecular cytogenetic techniques that can help the identification of presence or absence of a particular DNA sequence or the evaluation of the number of organization of a chromosome or chromosomal region.

Keywords: FISH, chromosomal abnormalities, marker chromosome, molecular cytogenetics, cytogenetics

1. Introduction

A chromosome is the condensed version of the DNA, and it contains two sister chromatids. The critical parts are consisted of centromere, telomere and nucleolar organizing regions [1]. Depending on the mechanism, chromosomal abnormalities can be classified under two-major groups, numerical and structural abnormalities. The non-disjunction of chromosomes or anaphase lagging is the major cause of the numerical chromosome abnormalities. The structural abnormalities can be classified as balanced and unbalanced abnormalities. Balanced structural

abnormalities include translocations, insertions and inversions. Unbalanced structural abnormalities include deletions, duplications, marker and ring chromosomes [2, 3]. The identification of these numerical and structural abnormalities has an impact on the diagnosis of the syndromes, understanding of the phenotypic effects of chromosomal abnormalities, identification of the diagnosis and prognosis of haematological malignancies or solid tumours [3]. From that perspective, one can say that the identification of chromosome abnormalities has an important role in several conditions.

The diagnosis of chromosomal abnormalities is the most important factor in haematology, prenatal genetics and postnatal diagnosis of genetic conditions [4–6]. The identification of the chromosomal abnormalities in foetus is one of the most important thing in modern perinatology, or the identification of the BCR/ABL translocation in CML (Chronic Myeloid Leukaemia) is the most important diagnostic and prognostic factor in haematology. Chromosomal abnormalities involve the pathogenesis of several clinical conditions like infertility or hematologic malignancies and are important indicators for their diagnosis and prognosis [4, 5, 7]. There are a several methods that can be used to detect the genetic changes in genetic clinics include:

- (a) conventional cytogenetics (karyotyping on cells derived from cell cultures using banding analysis; G-banding);
- (b) molecular cytogenetics, e.g., fluorescence in situ hybridization (FISH), multicolour FISH, locus-specific FISH;
- (c) molecular techniques to analyse DNA, RNA or proteins directly, e.g. the polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), quantitative real-time RT-PCR (RQ-PCR; qRTPCR), array CGH (comparative genomic hybridization), NGS (next generation sequencing), and microarray analysis [8].

Conventional cytogenetics is the golden standard and most comprehensive method to assess chromosomal abnormalities, especially numerical and structural chromosome aberrations. Technical issues like the need for fresh sample, difficulties in identification of masked or cryptic aberrations due to limited resolution by classic banding techniques have resulted in an increased use of molecular cytogenetic techniques, such as FISH, to identify specific abnormalities that are useful in either the diagnosis or management of hematologic disorders and are important tools for the identification of the cryptic translocations and sub-telomeric deletions in dysmorphic or mentally/developmentally retarded patients [8–12].

This chapter summarizes the structural abnormalities and the use of molecular cytogenetics as well as the identification of the chromosomal abnormalities.

1.1. Indications of the chromosome analysis in prenatal, postnatal cytogenetics and haematological malignancies

Chromosomal analysis can be used as a golden standard for pre-natal and post-natal genetic diagnostic testing.

- In prenatal diagnosis, prenatal chromosomal analysis is applied during the following conditions.
- Presence of structural chromosomal or genome abnormality in one of the parents.
- Previous child with de novo chromosomal aneuploidy or another genomic imbalance.
- Higher maternal ages.
- Positive family history.
- Abnormal findings during the maternal serum screening.
- Abnormal USG finding.
- Stillbirth or neonatal deaths [2].

Postnatally chromosome analysis is applied during the following conditions:

- Fertility problems.
- Suspected syndrome identification.
- Mentally or developmentally retarded patients.
- Problems of early growth and development.
- Family history.
- Neoplasm [2].

Prognostic and predictive chromosome analysis in haematological malignancies:

Chromosomal abnormalities are important for the chromosomal and molecular changes as well as the identification of specific hematologic malignancies and syndromes and have important therapeutic and prognostic impacts, which include [13]:

- t(15;17)PML/RAR α is characteristic for acute promyelocytic leukaemia (APL), that is a unique variant of acute myeloid leukaemia (AML) treated with ATRA and arsenic dioxide.
- t(8;21) or inv(16) comprises the favourable risk group of AML.
- Deletions of 5q, monosomy 7, deletion of 7q, trisomy 8, deletion of 9q, trisomy 11, trisomy 13, and trisomy 1 are the unbalanced abnormalities in AML.
- Acute myeloid leukaemia with associated abnormalities of 11q23 has an intermediate survival. The *MLL* gene at 11q23 is involved in a number of translocations with different partner chromosomes. The more common translocations observed in childhood AML are t(9;11)(p21;q23) and t(11;19)(q23;p13).
- The complex karyotype in AML predicts a poor prognosis.

- t(9;22)BCR/ABL is typical for chronic myeloid leukaemia (CML), although it may be seen in AML, where it is associated with a poor prognosis.
- In MDS, the typical abnormalities are partial and complete chromosome loss, most commonly -5, 5q-, -7, 7q-, +8, 11q-, 13q-, 20q-, and -Y [6, 14, 15].

Understanding the role of chromosomal abnormalities in the pathogenesis of haematological malignancies led to the development of a selective treatment options and gives prognosis information [13, 16–20].

1.2. FISH development

The development fluorescence in situ hybridization (FISH) technique increased the resolution of visualization of the chromosome rearrangements which is at the submicroscopic level [12, 21]. The FISH is applied on metaphase chromosomes, interphase nuclei, fixed tissues or cells and solid tumour samples [22]. The procedure contains denaturation, hybridization, post hybridization washes, which removes unbound single-strand DNA and after washing, an anti-fade solution containing DAPI (4',6-diamidino-2-phenylindole) is applied to the slide, and a coverslip must be added (**Figure 1**) [23]. For FISH analysis, epifluorescence microscopes with specific filters and for identifying fluorochromes, a charge-coupled device (CCD) camera that captures the images were needed. A huge range of probes can be used for the identification of the chromosome abnormalities, which includes whole-chromosome painting probes, chromosome-arm painting probes, repetitive centromeric, subtelomeric and locus-specific

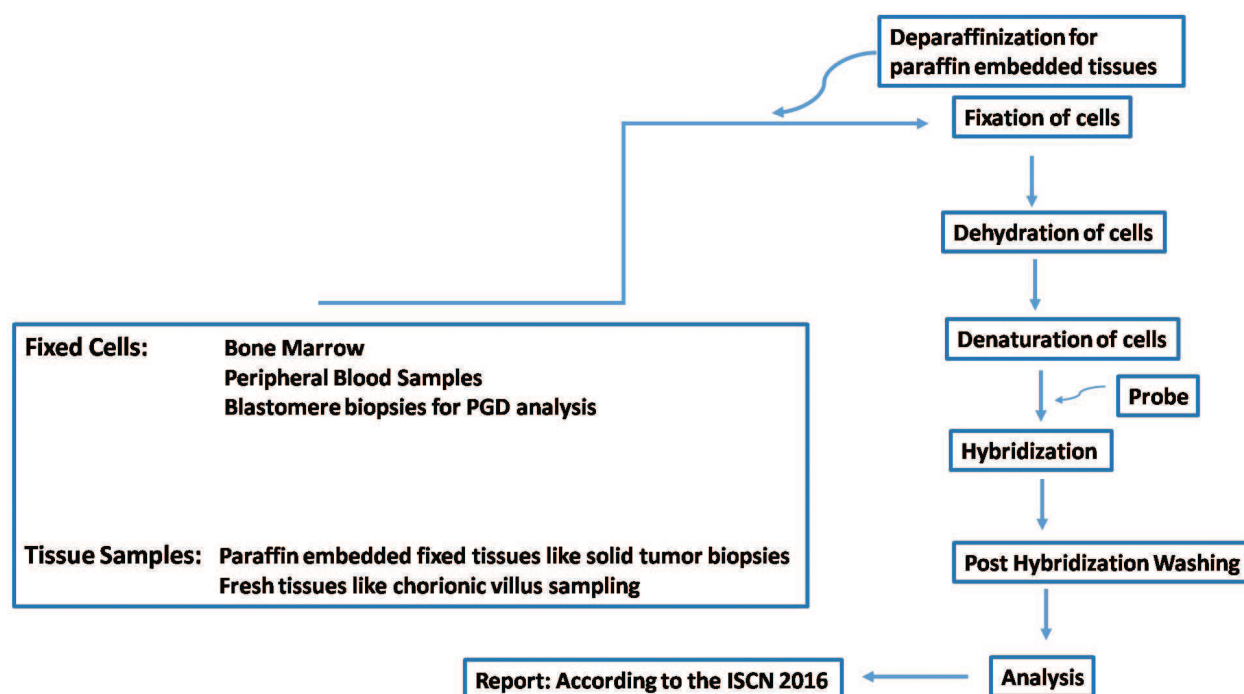


Figure 1. Algorithm for FISH analysis.

probes [24]. First, FISH probes obtained chromosome flow sorting [25] or microdissection [26] using universal degenerate oligonucleotide-primed PCR (DOP-PCR) [26].

There are different applications which used FISH-based methods like reverse-FISH [27], fibre-FISH [28, 29], M-FISH (multicolor FISH) [30], SKY (spectral karyotyping FISH) [31], flow-FISH [32], Q-FISH (quantitative FISH) [33], COBRA-FISH (combined binary ratio labelling FISH) [34], cenM-FISH (centromere-specific M-FISH) [35], podFISH (parental origin determination FISH) [36] and heterochromatin-M-FISH [37]. The most advanced FISH-based approaches included COBRA-FISH, M-FISH and SKY. These techniques give chance to the simultaneous visualization and the detection of all human chromosomes. These three FISH techniques use similar probes to be able to stain each of the 24 human chromosomes with a different colour [38].

Another high-resolution molecular cytogenetic technique for metaphase chromosomes, which gives chance to analyse chromosomes, is called multicolour banding (MCB). This technique involves the microdissection of chromosomal loci to obtain a set of probes that produce multi-colour pseudo-G-banding [39].

1.3. The use of FISH analysis in clinical diagnosis

Fluorescent in situ hybridization (FISH) was used for mapping human genes [40–43], and today, this technology is utilized for the characterization of chromosomal rearrangements and marker chromosomes [25, 44], the detection of microdeletions [45], and the prenatal diagnosis of common aneuploidies [46, 47], the detection of prognostic or predictive chromosomal abnormalities in haematological malignancies in clinical cytogenetic laboratories. At the same time, numerous DNA probes have been commercialized, further promoting the wide-spread clinical applications of molecular cytogenetic. Many new FISH techniques have been developed, including primed in situ labelling (PRINS [48]), fibre FISH [29, 49], comparative genomic hybridization (CGH) [50], chromosome microdissection [51, 52], spectral karyotyping (SKY [31]), multiple colour FISH (M-FISH [30, 53]), colour banding [54], FISH with multiple sub-telomeric probes [55], and array-based CGH [56, 57]. With the current FISH techniques, deletion or rearrangement of a single gene can be detected, cryptic chromosome translocations can be visualized, the copy number of oncogenes amplified in tumour cells can be assessed, and very complex rearrangements can be fully characterized. Using interphase FISH, genomic alterations can be studied in virtually all types of human tissues at any stage of cell division, without the need of cell culture and chromosome preparation. In that case, FISH is a unique technique that gives way to identification of numerical or structural chromosomal abnormalities in 1–3 days. The biggest advantage of the FISH technique is that it is more cost effective and labour intensive than the quantitative PCR (q-PCR) or other molecular genetics techniques.

Depending on the suspected genetic abnormalities type, the FISH probes can be generally subclassified into the following categories:

- (a) Centromere-specific probes.
- (b) Whole chromosome ('painting') probes.

- (c) Single-copy (locus-specific) genomic probes.
- (d) Spectral karyotyping (SKY; multiplex metaphase FISH; multi-colour FISH).
- (e) Translocation fusion probes [58].

2. Identification of the translocations

Translocation involves the exchange of chromosome segments between two chromosomes [2]. The balanced reciprocal translocation carrier individuals are clinically normal; they do have an increased risk for having children with unbalanced karyotypes secondary to meiotic non-disjunction of their translocation [1]. In addition to being inherited, reciprocal translocations can also occur as new or de novo mutations and can be disrupt the proto-oncogenes and can cause uncontrolled cell division and cancer development.

The identification of translocations is mostly used for the evaluation of the haematopoietic malignancies. There are two types of probes, which are used to detect translocations: (single- or dual-) fusion probes and break-apart probes. A dual-fusion probe consists of a pair of probes labelled with two different colours (fluorochromes), green (e.g. FITC) and red (e.g. rhodamine), directed against translocation breakpoint regions in the two different genes involved in a reciprocal translocation. Variant and complex patterns may also be identified and provide additional clinical information on the underlying chromosomal changes. One locus is adjacent to another locus like in a normal cell, but the second pair is separated. This implies some type of rearrangement, which separated two loci that are usually found together, and this kind of probes was called break-apart. Commonly used BA probes in hematologic malignancies include MYC-BA (Burkitt lymphoma; BL), ALK-BA (anaplastic large cell lymphoma; ALCL) and IGH-BA (lymphoma/MM) [8].

Whole chromosome probes (WCPs; chromosome 'painting' probes) consist of numerous overlapping probes that recognize and bind to specific nonrepetitive DNA sequences along the entire length of targeted chromosomes. WCPs can be used to identify marker chromosomes (rearranged chromosomes of unidentified origin) or translocations that are otherwise not evident or difficult to interpret with routine banding cytogenetics. Whole chromosome probes do not give information about the deletion or inversions [8].

3. Duplications

The presence of an extra genomic copy of a chromosomal segment, which causes a partial trisomy, is called duplication. A duplication can be derived as a de novo duplication or as a consequence of the unbalanced chromosomal organizations like isochromosomes, dicentric, derivatives, recombinants and markers [1, 3]. When the duplicated regions contain genes, genomic rearrangements involving the duplicated sequences can result in the deletion of the region between the copies and thus give rise to disease like 22q11.2 duplication or

the 15q11-q13 microduplication [3]. Most cytogenetically detectable tandem duplications in humans appear to be direct [59]. The phenotypes of the duplications are typically less severe than those associated with comparable deletions. Same as the deletions, the locus-specific FISH analysis should be applied when the duplication is suspected.

4. Deletions

The autosomal chromosome deletions can be detected by conventional, high-resolution or molecular cytogenetic methods and produce monosomies that are generally associated with significant disorders [1]. Deletions are classified into two groups: interstitial and terminal deletions. Due to the haploinsufficiency of the regions or the continuous gene deletions, the phenotypes of these patients are highly variable [2].

The deletions, which have a pathological significance, can be detected by routine methodology. Larger deletions have a more severe phenotype and associated with the major malformation than smaller ones. The gene content of the deleted material is also important for the phenotypic severity of the patients and an important point in determining whether a specific deletion is viable [1]. The deletion of the chromosome segment can cause complex birth defects like Cri du chat syndrome, Wolf-Hirschhorn syndrome and DiGeorge Syndrome [2]. The locus-specific FISH analysis should be applied when the deletion is suspected. All stable chromosomes have telomeres at the end of the chromosomes. The sub-telomeric deletions were associated with the severe problems, which include mental retardation, developmental delay, and this terminal deletions cannot detect with the conventional cytogenetic techniques. The sub-telomeric FISH analysis is the appropriate technique to evaluate the abnormalities [10, 11, 21]. Some exceptions occur like loss of the short arm material from acrocentric chromosomes during the formation of Robertsonian translocations has no impact on phenotype [1].

5. Inversions

An inversion is an intrachromosomal rearrangement, which occurs when a single chromosome undergoes two breaks and is reconstituted with the segment between the breaks inverted. Two types of inversions occur: a paracentric inversion which both breaks occur in one arm and pericentric inversion which there is a break in each arm of the chromosomes [1]. The pericentric inversions can be easier to identify cytogenetically when they change the proportion of the chromosome arms as well as the banding pattern. The inversion does not usually cause an abnormal phenotype in carriers because it is a balanced rearrangement. The major problem of these patients is at risk for producing abnormal gametes that may lead to unbalanced offspring [2]. The breakpoints could be identified by visual inspection of the GTG image. However, further molecular cytogenetic analysis would be required to define the exact breakpoints. The locus-specific FISH is a suitable method, and also the break-apart FISH probes or m-banding is the useful molecular cytogenetics techniques for determining the inversions [60].

6. Complex chromosomal abnormalities

The complex chromosomal rearrangements (CCRs) involve two or more chromosomes, and at least three breakpoints are generally considered to be complex [61]. The greater the number of chromosome breaks and the higher the probability that an essential gene has been interrupted or that genetic material has been lost or gained during its formation. These CCRs are rarely seen in constitutional karyotypes and mostly seen in hematologic malignancies, and the identification of the structurally abnormal chromosomes is more important to be evaluated for the prognosis of haematological malignancies and important for the treatment response [62].

7. Identification of the marker chromosome

A marker chromosome is the extra structurally abnormal chromosomes in cytogenetics [2]. The precise characterization of marker chromosomes is important for prenatal and postnatal diagnosis and proper genetic counselling [63].

Mostly, the banding pattern of this abnormal chromosome does not permit for identification of the marker chromosome [62]. The chromosomal origin of marker chromosomes can be identified by using a combination of banding cytogenetics and molecular cytogenetic techniques including diverse fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (array CGH) (Figure 2) [63].

The small structurally abnormal chromosomes, which are called as supernumerary marker chromosomes (sSMCs), are generally equal or smaller in size than a chromosome 20 of the

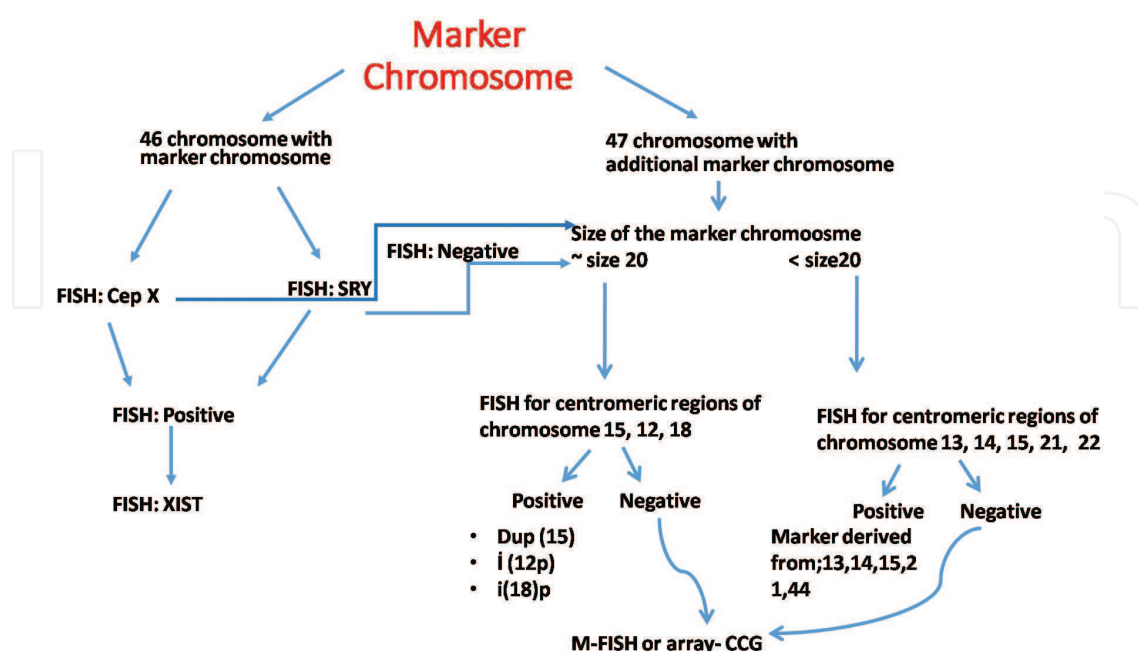


Figure 2. The possible algorithm chart for the identification of the marker chromosome.

same metaphase spread [64], and the chromosomal origin can be identified by conventional banding techniques, and molecular cytogenetic techniques are necessary for their characterization. **Figure 2** could be used for the identification of the marker chromosome.

Due to the effect of two or more chromosomal abnormalities, the conventional cytogenetics is limited to the identification. The m-FISH or the SKY is the best molecular cytogenetics choice to identify these CCRs.

8. Oncogenic amplifications

Amplification refers to the increasing copy number of a gene. Amplification causes gene upregulation of gene expression. The oncogenic amplification has described several solid tumours, which are often associated with progression, therapeutic response and prognostic markers of the cancer [65].

The oncogenes coded proteins, which have a role on control cell proliferation and programmed cell death. These oncogenes are activated by mutation, gene amplification and translocation. The oncogenic amplification mostly occurs in metastatic and low-differentiated tumours and reflects the genetic instability of solid tumour cell [66]. MYC, EGFR and RAS gene families are frequently amplified oncogenes in solid tumours. MYCN amplification in neuroblastoma or Her-2 amplification has been demonstrated, and the locus-specific probes allow accurate enumeration of each locus within individual nuclei [65]. Also, dual colour break apart rearrangement probes were used for the identification of these gene rearrangements [67].

9. Final remarks and conclusion

FISH techniques do not require mitotically active cells for evaluation of the chromosomal abnormalities, do not require culturing and allow disease monitoring in haematological malignancies. FISH can be applied on fixed, fresh tissue and on paraffin-embedded materials like paraffin-embedded solid tumours. It allows the analysis of a large number of cells. The SKY, M-FISH or COBRA-FISH techniques provide an overall evaluation of the whole genome. On the other hand, FISH analysis is not a screening test and cannot detect small intragenic mutations, deletions or insertions. Because it requires chromosome-specific FISH probes and generally less sensitive than the molecular genetics techniques.

Instead of array-CGH technology, in developed countries, the cytogenetic testing is the first line test in the diagnostic investigation detection of novel or rare chromosomal abnormalities like micro-deletions, microduplications or trisomies. With the increased technology, array-based analysis like array comparative genome hybridization can help easily assess the relative copy number of genomic DNA sequences in a comprehensive, genome-wide manner, but the main disadvantage in aCGH is that it cannot detect translocation or balanced abnormalities, and in these conditions, FISH is important to determine the nature of the abnormality and its risk of recurrence.

Author details

Rasime Kalkan

Address all correspondence to: kalkanr@yahoo.com

Department of Medical Genetics, Faculty of Medicine, Near East University, Nicosia, TRNC

References

- [1] Gersen SL, Keagle BM: The Principles of Clinical Cytogenetics. 2nd edition. Humana Press Inc., Totowa, New Jersey, 2005, pp. 1-113.
- [2] Nussbaum RL, McInnes RR, Willard HF: Thompson & Thompson Genetics in Medicine. 8th edition. Elsevier Inc, 2016, pp. 57-102.
- [3] Gardner RJM, Sutherland GR, Shaffer LG: Chromosome Abnormalities and Genetic Counseling. 4th edition. Oxford University Press, 2011. pp. 27-53. doi: 10.1093/ med/ 9780195375336.001.0001
- [4] Wieacker P, Steinhard J: The prenatal diagnosis of genetic diseases. Dtsch Arztebl Int. 2010; 107(48): 857-862. doi: 10.3238/arztebl.2010.0857.
- [5] Vundinti BR, Ghosh K: Chromosomal aberrations in hematological malignancies: a guide to the identification of novel oncogenes. Indian J Hum Genet. 2011; 17(2): 43-44. doi: 10.4103/0971-6866.86168.
- [6] Provan D, Gribben JG: Molecular Hematology. 3rd edition. Wiley-Blackwell, 2010, p. 318. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex
- [7] Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, Apperley J, Cervantes F, Cortes J, Deininger M, Gratwohl A, Guilhot F, Horowitz M, Hughes T, Kantarjian H, Larson R, Niederwieser D, Silver R, Hehlmann R, European LeukemiaNet: Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108:1809-20. doi: 10.1182/blood-2006-02-005686.
- [8] Gorczyca W: Cytogenetics, FISH and Molecular Testing in Hematologic Malignancies. 1st edition. Informa UK Ltd, 2008, pp. 31-108.
- [9] Czepulkowski B: Analyzing Chromosomes. 1st edition. Bios Scientific Publishers Limited. 2001, pp. 1-106. Springer-Verlag New York Inc. 175 Fifth Avenue, New York.
- [10] Mohan S, Koshy T, Vekatachalam P, Nampoothiri S, Yesodharan D, Gowrishankar K, Kumar J, Ravichandran L, Joseph S, Chandrasekaran A, Paul SF: Subtelomeric rearrangements in

Indian children with idiopathic intellectual disability/developmental delay: frequency estimation & clinical correlation using fluorescence in situ hybridization (FISH). *Indian J Med Res.* 2016 Aug;144(2):206-214. doi: 10.4103/0971-5916.195031.

- [11] Charalsawadi C, Khayman J, Praphanphoj V, Limprasert P: Screening for subtelomeric rearrangements in Thai patients with intellectual disabilities using FISH and review of literature on subtelomeric FISH in 15,591 cases with intellectual disabilities. *Genet Res Int.* 2016;2016:9153740.
- [12] Henegariu O, Artan S, John GM, Xiao-Ning C, Julie RK, Gail VH, Lisa S, Bray-Ward P, David WC: Cryptic translocation identification in human and mouse using several telomeric multiplex FISH (TM-FISH) strategies. *Lab Invest* (00236837) 2001;81(4):483.
- [13] Ren R: Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer.* 2005; 5:172-83.
- [14] Durak BA, Akay OM, Sungar G, Bademci G, Aslan V, Caferler J, Ozdemir M, Cilingir O, Artan S, Gülbaş Z: Conventional and molecular cytogenetic analyses in Turkish patients with multiple myeloma. *Turk J Haematol.* 2012 Jun;29(2):135-42. doi: 10.5152/tjh.2011.42.
- [15] Sahin DG, Durak B, Gündüz E, Artan S, Akay OM: A new case of dic(1;15)(p11;p11) in AML M1: apropos of a case and a review of the literature. *Case Rep Genet.* 2013;2013:462896. doi: 10.1155/2013/462896.
- [16] Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M, Druker B, Goldman J, O'Brien SG, Russell N, Fischer T, Ottmann O, Cony-Makhoul P, Facon T, Stone R, Miller C, Tallman M, Brown R, Schuster M, Loughran T, Gratwohl A, Mandelli F, Saglio G, Lazzarino M, Russo D, Baccarani M, Morra E: Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med.* 2002;346:645-52.
- [17] O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ, IRIS Investigators: Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003; 348:994-1004.
- [18] Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM: The biology of chronic myeloid leukemia. *N Engl J Med.* 1999;341:164-72.
- [19] Goldman JM, Melo JV: Chronic myeloid leukemia – advances in biology and new approaches to treatment. *N Engl J Med.* 2003;349:1451-64.
- [20] Jones RJ: Biology and treatment of chronic myeloid leukemia. *Curr Opin Oncol.* 1997;9:3-7.
- [21] Hélias-Rodzewicz Z, Bocian E, Stankiewicz P, Obersztyn E, Kostyk E, Jakubów-Durska K, Kutkowska-Kaźmierczak A, Mazurczak T: Subtelomeric rearrangements detected by FISH in three of 33 families with idiopathic mental retardation and minor physical anomalies. *J Med Genet* 2002;39:e53. doi: 10.1136/jmg.39.9.e53

- [22] Bishop R: Applications of fluorescence *in situ* hybridization (FISH) in detecting genetic aberrations of medical significance. Biosci Horiz. 2010;3:85-95. doi: 10.1093/biohorizons/hzq009 first published online February 27, 2010.
- [23] Shah P, Vedarethinam I, Kwasny D, Andresen L, Skov S, Silahtaroglu A, Tümer Z, Dimaki M, Svendsen WE: FISH prep: a novel integrated device for metaphase FISH sample preparation. Micromachines. 2011;2:116-128. doi:10.3390/mi2020116.
- [24] Riegel M: Human molecular cytogenetics: from cells to nucleotides. Genet Mol Biol. 2014 Mar;37(1 Suppl):194-209.
- [25] Pinkel D, Landegent J, Collins C, Fuscoe J, Segreaves R, Lucas J, Gray JW: Fluorescence *in situ* hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA. 1988;85:9138-9142.
- [26] Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BA, Tunnacliffe A: Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992;13:718-725.
- [27] Carter NP, Ferguson-Smith MA, Perryman MT, Telenius H, Pelmeur AH, Leversha MA, Glancy MT, Wood SL, Cook K, Dyson HM, *et al.*: Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. J Med Genet. 1992; 29:299-307.
- [28] Florijn RJ, Bonden LA, Vrolijk H, Wiegant J, Vaandrager JW, Baas F, den Dunnen JT, Tanke HJ, van Ommen GJ, Raap AK: High-resolution DNA Fiber-FISH for genomic DNA mapping and colour bar-coding of large genes. Hum Mol Genet. 1995 May;4(5):831-6.
- [29] Heiskanen M, Hellsten E, Kallioniemi OP, Mäkelä TP, Alitalo K, Peltonen L and Palotie A: Visual mapping by fiber-FISH. Genomics. 1995;30:31-36.
- [30] Speicher MR, Ballard SG, Ward DC: Karyotyping human chromosomes by combinatorial multifluor FISH. Nat Genet. 1996;12:368-375.
- [31] Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, *et al.*: Multicolor spectral karyotyping of human chromosomes. Science. 1996;273:494-497.
- [32] Rufer N, Dragowska W, Thornbury G, Roosnek E, Lansdorp PM: Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. Nat Biotechnol. 1998;16:743-747.
- [33] Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, Lansdorp PM: Short telomeres on human chromosome 17p. Nat Genet. 1998;18:76-80.
- [34] Tanke HJ, Wiegant J, van Gijlswijk RP, Bezrookove V, Pattenier H, Heetebrij RJ, Talman EG, Raap AK, Vrolijk J: New strategy for multi-colour fluorescence *in situ* hybridisation: COBRA: COmbined Binary RAtio labelling. Eur J Hum Genet. 1999;7:2-11.

- [35] Nietzel A, Rocchi M, Starke H, Heller A, Fiedler W, Wlodarska I, Loncarevic IF, Beensen V, Claussen U, Liehr T: A new multicolor-FISH approach for the characterization of marker chromosomes: centromere-specific multicolor- FISH (cenM-FISH). *Hum Genet.* 2001;108:199-204.
- [36] Weise A, Gross M, Mrasek K, Mkrtchyan H, Horsthemke B, Jonsrud C, Von Eggeling F, Hinreiner S, Witthuhn V, Claussen U, *et al.*: Parental-origin-determination fluorescence in situ hybridization distinguishes homologous human chromosomes on a single-cell level. *Int J Mol Med.* 2008; 21:189-200.
- [37] Bucksch M, Ziegler M, Kosayakova N, Mulatinho MV, Llerena Jr JC, Morlot S, Fischer W, Polityko AD, Kulpanovich AI, Petersen MB, *et al.*: A new multicolor fluorescence in situ hybridization probe set directed against human heterochromatin: HCM-FISH. *J Histochem Cytochem.* 2012;60:530-536.
- [38] Schröck E, Zscheschang P, O'Brien P, Helmrich A, Hardt T, Matthaei A, Stout Weider K: Spectral karyotyping of human, mouse, rat and ape chromosomes—applications for genetic diagnostics and research. *Cytogenet Genome Res.* 2006;114:199-221.
- [39] Liehr T, Heller A, Starke H, Rubtsov N, Trifonov V, Mrasek K, Weise A, Kuechler A, Claussen U: Microdissection based high resolution multicolor banding for all 24 human chromosomes. *Int J Mol Med.* 2002;9:335-339.
- [40] Pinkel D, Straumer T, Gray IW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence in situ hybridization. *Proc Natl Acad Sci USA.* 1986;83:2934-2938.
- [41] Lawrence JB, Villnave CA, Singer RH: Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell.* 1988;52:51-61.
- [42] Fan YS, Davis LM, Shows TB: Mapping small DNA sequences by fluorescence in situ hybridization directly on banded metaphase chromosomes. *Proc Natl Acad Sci USA.* 1990;87:6223-6227.
- [43] Lichter P, Ward DC: High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science.* 1990; 247:64-69.
- [44] Blennow E, Anneren G, Bui T-H, Berggren E, Asadi E, Nordenskjold M: Characterization of supernumerary ring marker chromosomes by fluorescence in situ hybridization. *Am J Hum Genet.* 1993; 53:433-442.
- [45] Ledbetter DH, Ballabio A: Molecular cytogenetics of continuous gene syndromes: mechanisms and consequences of gene dosage imbalances, in Scriver CR, Beaudet A, Sly W, Valle D, eds., *The Metabolic and Molecular Bases of Inherited Diseases*, 7th edition, McGraw Hill, New York. 1995, pp. 811-839.
- [46] Klinger K, Landes G, Shook D., Harvey R, Lopez L, Locke P, Lerner T, Osathanondh R, Leverone B, Houseal T: Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). *Am J Hum Genet.* 1992;51, 55-65.

- [47] Ward BE, Gersen SL, Carelli MP, McGuire NM, Dackowski WR, Weinstein M, Sandlin C, Warren R, Klinger KW: Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization: clinical experience with 4,500 specimens. *Am J Hum Genet.* 1993;52:954-865.
- [48] Koch J, Kovarra S, Gregersen N, Petersen KB, Bolund L: Oligonucleotide-priming methods for the chromosome-specific labeling of alpha satellite DNA in situ. *Chromosoma.* 1989; 98:259-262.
- [49] Heng HHQ, Squire J, Tsui L-C: High resolution mapping of mammalian genes by in situ hybridization to free chromatin. *Proc Natl Acad Sci USA.* 1992; 89:9509-9513.
- [50] Kallioniemi A, Kallioniemi OP, Suder D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science.* 1992;258:818-821.
- [51] Su Y, Trent J, Guan XY, Meltzer P: Direct isolation of genes encoded within a homogeneously staining region by chromosome microdissection. *Proc Natl Acad Sci USA.* 1994; 91:9121-9125.
- [52] Guan XY, Meltzer P, Dalton W, Trent J: Identification of cryptic sites of DNA sequence amplification in human breast cancer by chromosome microdissection. *Nat Genet.* 1994;8:155-160.
- [53] Eils R, Uhring S, Saracoglu, Sätzler K, Bolzer A, Petersen I, Chassery J, Ganser M, Speicher MR: An optimized, fully automated system for fast and accurate identification of chromosome rearrangements by multiplex-FISH (M-FISH). *Cytogenet Cell Genet.* 1998; 82:160-171.
- [54] Müller S, Rocchi M, Ferguson-Smith MA, Wienberg J: Toward a multicolor chromosome bar code for the entire human karyotype by fluorescence in situ hybridization. *Hum Genet.* 1997; 100:271-278.
- [55] Knight SJL, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DL, Flint J, Kearney L: Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. *Eur J Hum Genet.* 1997;5:1-8.
- [56] Pinkel D, Segreaves R, Sudar D, *et al.*: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet.* 1998;20: 207-211.
- [57] Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO: Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet.* 1999; 23:41-46.
- [58] Weise A, Liehr T: In Thomas L. ed., *Fluorescence In Situ Hybridization (FISH) — Application Guide.* 2009 Springer-Verlag Berlin Heidelberg, pp. 113-143

- [59] Van Dyke DL: Isochromosomes and interstitial tandem direct and inverted duplications. In Daniel A, ed., *The Cytogenetics of Mammalian Autosomal Rearrangements*, Alan R. Liss, New York. 1988, pp. 635-666.
- [60] Schmidt S, Claussen U, Liehr T, Weise A: Evolution versus constitution: differences in chromosomal inversion. *Hum Genet.* 2005;117:213-219.
- [61] Pai GS, Thomas GH, Mahoney W, Migeon BR: Complex chromosome rearrangements; report of a new case and literature review. *Clin Genet.* 1980;18:436-444.
- [62] Kalkan R, Özdağ N, Bundak R, Serakinci N: A unique mosaic Turner syndrome patient with Androgen Receptor gene derived marker chromosome. *Syst Biol Reprod Med.* 2016;62(1):77-83. 10.3109/19396368.2015.1109007.
- [63] Jang W, Chae H, Kim J, Son JO, Kim SC, Koo BK, Kim M, Kim Y, Park IY, Sung IK: Identification of small marker chromosomes using microarray comparative genomic hybridization and multicolor fluorescent in situ hybridization. *Mol Cytogenet.* 2016 Aug 8;9:61. doi: 10.1186/s13039-016-0273-5. eCollection 2016.
- [64] Liehr T, Claussen U, Starke H: Small supernumerary marker chromosomes (sSMC) in humans. *Cytogenet Genome Res.* 2004;107:55-67.
- [65] Bagci O, Kurtgöz S: Amplification of Cellular Oncogenes in Solid Tumors. *N Am J Med Sci.* 2015 Aug;7(8):341-6. doi: 10.4103/1947-2714.163641.
- [66] Fan Y-S: In Yao-Shan ed. *Molecular Cytogenetics*. Humana Press. 2003, pp. 11-12.
- [67] Yoon JH, Baek YS, Jeon J, Oh CH, Song HJ: Dual-color, break-apart fluorescence in situ hybridization probe for distinguishing clear cell sarcoma of soft tissue from malignant melanoma. *Int J Dermatol.* 2014 Dec;53(12):1464-7. doi: 10.1111/j.1365-4632.2011.05009.x.

