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Review Article

A Review on Probes used for Fluorescent *in-situ* Hybridization in Bovines

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Abstract

The choice of a probe is one of the most important considerations in fluorescent in situ hybridization technique. There are three types of FISH probes- whole chromosome painting probes, repetitive sequence/centromeric probes and locus-specific probes. Each type of probe has its own importance in the field of research, which is discussed in this review. Probes used for the diagnosis of different chromosomal abnormalities, genetically inherited diseases, gene mapping and localization of specific genes on chromosomes are reviewed. This helps the researchers in selection of probes based on their purpose of an experiment. This review also focuses on software tools for the construction of probes; companies providing readily available and customized probes according to the researcher's needs; laboratories offering their services in the field of molecular cytogenetics for the diagnosis of genetically inherited diseases, chromosomal anomalies and cancer. All data and materials collected in this review are related to references in this published article.

Keywords: Bovines, FISH laboratories, FISH probes, Software

Introduction

The cytogenetic analysis is an important measure for accurate selection of genetically healthy breeding animals (Kosarcic et al. 2006). so, karyotyping have emerged as an effective tool to screen the chromosomal abnormalities at an early age. Then karyotyping analysis is refined by use of chromosome banding analysis. The resolution of banding analysis is limited to mitotically active cells and chromsomal aberrations involves >3Mb of DNA (Kearney 2001 and Bishop 2010). The detection of the specific molecular entities was first demonstrated by using antigen-antibody interactions Later, discovered the first antibody dependent fluorescent detection of nucleic acids (Rudkin and Stollar 1977). This technology was replaced by the use of probes labeled with radioisotopes called in-situ hybridization technique. Owing to the drawbacks like non-specific labeling strategies, isotopes decay over time so, the specific activity of the probe is unstable, low resolution, costly and hazardous material with radioisotopes lead to the discovery of fluorescently labeled probes that resulted in the development of a technique called fluorescent in situ hybridization (Levsky and Singer, 2003).

Fluorescent *In-Situ* Hybridization probes are generally assigned by where they hybridize in the genome or by the type of chromosome abnormality; they detect (Naha *et al.* 2016). With the commercialization of FISH probes, standardized and efficient labeling of probes and best resolution imaging system, FISH has been revamped for research analysis for nuclear structures and gene functions (Ward *et al.* 1993 and Cui *et al.* 2016). So, this review is focused on types of fluorescent probes, probes so far used for research in humans and livestock species, software tools used to design FISH probes and laboratories working on FISH technique.

Types of FISH Probes

The first step in the fluorescent *in-situ* hybridization is choice of probe. A wide range of probes can be used from whole genomes to the small cloned probes (1-10Kb) (Bishop *et al*. 2010). There are broadly three different categories of probes, each with the different range of applications (Kearney 2001). They are -

1. Whole chromosome painting probes
2. Centromeric or Repetitive sequence probes
3. Locus-specific probes

Whole Chromosome Painting Probes

Chromosome painting was developed independently by research teams at Lawrence Livermore National Laboratories (Pinkel *et al.* 1988) and at Yale University **(**Cremer *et al.* 1988; Lichter *et al.* 1988). The use of FISH to colorize entire genome and can distinguish all chromosomes with a specific color (Ried *et al.* 1993; Schrock *et al.* 1996). Chromosome painting refers to the hybridization of fluorescently labeled chromosome specific, composite probe pools to cytological preparations. Painting probes are DNA probes derived from a single type of chromosome (Kearney 2001). These probes are flow sorted or microdissected chromosomes amplified and labeled by degenerate oligonucleotide polymerase chain reaction (DOP-PCR) to generate a paint which highlights the entire chromosome homogenously along its length (Telenius *et al.* 1992 and Gribble *et al.* 2004). Micro dissection of probes enables to generate region-specific probes for chromosomal arms or chromosomal bands (Guan *et al.* 1993; Ried *et al.* 1993 and Guan *et al.* 1996). Microdissection of chromosomes using micromanipulators and glass needles is time consuming so, attempts were made to improve the protocol by reducing the number of chromosomes used (Gribble *et al.* 2004) and Guan *et al.*, (1993) generated high intensity microdissected FISH probes from a single DOP-PCR amplified microdissected chromosome. First generation chromosome painting probes were based on chromosome specific phage libraries, were rather cumbersome to use because of low insert -to -vector ratios which result in relatively high background staining. But these limitations were overcome with the plasmid vectors which improved insert-to-vector ratios and easier probe generation enhanced the painting quality (Ried *et al.* 1993). Gribble *et al.*, (2004) used Omniplex^{TM} to construct chromosome painting probes from single copies of flow-sorted chromosomes. The advantage of using these probes is the probe will be specific to the sorted chromosome and will not suffer from contamination from other chromosomes.

Applications

Chromosomal painting allows the visualization of individual chromosomes in metaphase cells and localization of chromosomal numerical and structural aberrations with high specificity and sensitivity (Bishop *et al.* 2010). FISH with readily available chromosome paint probes offers the possibility to assess the disomy rate of each chromosome individually (Chandley *et al.* 1996). Whole chromosome painting probes enables whole genome-wide screening for the aberrations, identification of pattern of chromosomal rearrangements, understanding the chromosomal changes that occurred during the evolution and identification of homologous chromosomal segments in different species can be possible with color karyotyping (Weinberg *et al.* 1990; Ried *et al.* 1993 and Ried *et al.* 1998). Multiple chromosomal painting probes tagged with different specific fluorochromes can be hybridized to multiple chromosomes simultaneously, resulted into differential color display of human or non-human chromosomes called color karyotyping or spectral karyotyping. Spectral karyotyping allows the detection of small chromosomal translocations and classification of marker chromosomes. Colour karyotyping can be applicable to the non-human species also for easy and rapid analysis of karyotypes (Ried *et al.* 1998). But whole chromosome painting probes are not suitable for the identification of chromosomal abnormalities in interphase cells (Kearney 2001) because the signal domains are so large and diffuse (Bishop *et al.* 2010).

Centromeric or Repetitive Sequence Probes

Repetitive sequence probes hybridize to specific chromosomal regions or structures that contain many thousands of copies of short sequences (Gozzetti *et al.* 2000; Kearney 2001 and Bishop *et al.* 2010). Centromeric probes targeting the α and β satellite sequences and pan-telomeric probes targeting the tandemly repeated sequences are repetitive sequence probes. Alpha-satellite probes are specific to particular chromosomes means probes derived from one chromosome will hybridize to that chromosome only, whereas pan-centromeric probes that hybridize all chromosome centromeres are also available (Gozzetti *et al.* 2000). Alpha satellite repetitive probes have good sensitivity but not specificity because of high homology of alpha DNA sequences (Lebo *et al.* 1992; Pellestor *et al.* 1995 and Rives *et al.* 1999).

Applications

Satellite DNA probes are suitable for detection of trisomy, monosomy and other aneuploidies in tumor cells and numerical chromosomal abnormalities because when these probes hybridize with multiple copies of repeat units present at centromeric regions will give very bright fluorescent signal both in metaphase and interphase cells (Bishop *et al.* 2010 and Gozzetti *et al.* 2000). Highly repetitive sequences are considered suitable FISH probes that can avoid many potential problems of using unique sequences as FISH probes. Unique sequences can also be used as FISH probes, but such FISH probes are hard to obtain (Li *et al.* 2010). When these probes labeled and hybridized at sufficient stringency, produce intense and compact zones in heterochromatic regions of chromosomes or near the centromere regions and compact domains in interphase chromatin (Trask 1991).

Locus specific Probes

Locus-specific probes are genomic clones which vary in their size depending on the cloning vector nature, from the plasmid (1-10 kb) to the larger PAC, BAC and YAC vectors (80kb-1 Mb). These probes are specifically useful for the detection of deletions, inversions, chromosomal translocations and structural rearrangements (Kearney 2001 and Gozzetti and Beau 2000).

A List of Probes Used for FISH Technique - Application Wise

Selection of the probe for fluorescent in-situ hybridization depend on the purpose of experiment, such as identification of chromosomal anomalies in metaphase spreads/ interphase nuclei/embryos, localization of specific genes on the chromosomes, sexing of semen samples, and embryos, identification of conserved regions between different species etc.

Diagnosis of Chromosomal Anomalies

The high sensitivity, specificity and speed with which assay can be performed made FISH technique a pivotal

molecular cytogenetic technique for the diagnosis of genetic abnormalities like gene fusions, aneuploidy, deletions and translocations (Sen 2014). The probes used for the identification various kinds of chromosomal abnormalities were listed in the Table 1

The study of sperm chromatin has started with using quinacrine mustard or quinacrine dihydrochloride to stain heterochromatin region of Y chromosome (Barlow and Vosa 1970). Human sperm chromosomes were prepared by using the capacity of the spermatozoa to penetrate the zona-free hamster oocytes (Rudak *et al.* 1978). Later these techniques were replaced by the use of chromosome specific DNA probes labeled with radioactive compounds (Joseph *et al.* 1984) and later with fluorochromes (Guttenbach and Schmid 1990 and Pieters *et al.* 1990). Then the multi-colour FISH technique either on sperm nuclei or sperm-derived pronuclei has come into light (Martínez-Pasarell *et al.* 1997). The FISH technique on decondensed sperm head allows an accurate analysis of incidences of aneuploidies in spermatozoa and this technique has been incorporated in protocols of many laboratories for the study of infertile males (Egozcue *et al.* 1991). Screening of the donor cells and nuclear transfer embryos for chromosomal anomalies is one of the steps needed to improve the success rate of the cloning procedures (Bureau *et al.* 2003). FISH technique has been used to identify the chromosomal aberrations at embryo, sperm and oocyte level and the review of probes used were presented in the Table 1.

Table 1: Probes used in identification of structural and numerical aberrations of chromosomes in Metaphase spreads, semen samples and embryos

Purpose of experiment	Name of probe	Type of the probe	Author(s)
Detection of chr Y in bovine gonadal hypoplasia (XY female)	BC1.2 and btDYZ-1	Locus specific probes	Kawakura *et al*. (1997)
Identification of chimerism of bulls born in heterosexual twinning	cosmid PL 44	Locus specific probe	Rejduch *et al*. (2000)
Identification of chromosomal anomalies in bovine pre-implanted embryo's	E1A (EcoRI)	Repetitive sequence probes	Slimane *et al*. (2000)
	E4A (EcoRI)		
	Ba (BamH1)		
	W18 (SacI)		
	W22 (EcoRV)		
	W5 (Stu1)		
	H1A (HindIII)		
Determination of Autosomal reciprocal translocations	HSA3, HSA12, HSA21 and HSA22	Chromosome painting probes	Iannuzzi *et al*. (2001a)
Identification of Y-autosome reciprocal translocation	Goat BAC clones containing AMD1, CGA, IGF2R, cattle BAC clone containing SRY and bovine cosmid IDVGA50	BAC and cosmid probes	Iannuzzi *et al*. (2001b)
Detection of chromosomal abnormalities in bovine nuclear transfer embryos	XY painting probes	Chromosome painting probes	Bureau *et al*. (2003)
Detection of XXY trisomy in bull	X-specific	Chromosome painting probes	Ewa *et al*. (2003)
	Y-specific		
Detection of robertsonian translocations in embryos	chromosomes 16 (Spectrum Green)	Chromosome painting probes	Ryber *et al*. (2005)
	Chromosomes 20 (Spectrum Orange)		
	chromosomes 1 (Spectrum Orange)		
	Chromosomes 29 (Spectrum Green)		
Identification of robertsonian translocation in semen sample	BTA1	Chromosome painting probes	Bonnet-Garnier *et al*. (2006)
	BTA29		
Rob (1;29)- determination of complex chromosomal rearrangements	BAC clone-INRA143 and bovine satellite DNA-I, III,IV	Centromeric probes	Di Meo *et al*. (2006)
Balanced reciprocal translocations, rcp(9;11)(q27;q11)	163E12, 474A12, 293G09, 035D03 and 286F08	BAC probes	De Lorenzi *et al*. (2007)

Diagnosis of bovine freemartinism	BC1.2	Locus specific probe	Sohn *et al*. (2007)
Centric fusion, rob(21;23)	355A04 and 712H08 harboring *IGH* on chr21 and *BOLA-DYA* on chr23	BAC probes	De Lorenz *et al*. (2008a)
Robertsonian translocation (14;17)	BACs-111B7 and 1004F9	BAC- gene specific probes	De Lorenz *et al*. (2008b)
Azoospermic boar with (Y;14) translocation	Probes for chr-X,Y and 14were prepared by microdissection and DOP-PCR	Chromosome painting probes	Pinton *et al*. (2008)
Identification of reciprocal translocations	Chromosome specific probes prepared by laser microdissectiona nd labeled by DOP-PCR	Chromosome painting probes	Switonski *et al*. (2008)
Identification of (1;29) translocation in bull sperm	Rob(1;29) specific probe	Pan-cenromeric specific probes	Vodzova *et al*. (2008)
	locus btD6Z1	locus specific probe	
FISH on cattle chromosomes with cloned human fragile X- DNA	Cosmid clones 23H10, pE5.1and c22.3 labeled with digoxigenin	Cosmid probes	Ali *et al*. (2009)
Determination of XY sperm aneuploidy in cattle	Xcen	Chromosome painting probes	Nicodemo *et al*. (2009)
	Y chromosome specific		
Reciprocal translocation t(4;7)	BTA4 and BTA7 were laser microdissected and labeled by DOP-PCR	Chromosome painting probes	De Lorenzi *et al*. (2010)
Detection of aneuploidy in in vitro matured oocytes	Prepared by microdissection and DOP-PCR	Chromosome painting probes	Nicodemo *et al*. (2010)
Identification of diploid spermatozoa	X-Y paint set BC1.2	Chromosome painting probes	Revay *et al*. (2010)
Detection of chromosomal aberrations	BTA1	Chromosome painting probes	Martina *et al*. (2011)
	BTA5		
	BTA7		
Detection of X-Y aneuploidy	Xcen-Y	Chromosome painting probes	Paucillo *et al*. (2011)
Y-autosomal reciprocal translocation	Y- BAC harboring *ANT3* and *SRY* gene Chr 21- BAC (RP42–82D5) harboring *SPC18* gene	Locus specific probes	Switonski *et al*. (2011)
Detection of chromosomal aberrations in sperm and oocytes	Xcen probe(Xp11-14)	Centromeric probe	Paucillo *et al*. (2012a, b)
	Microdissected and DOP-PCR, labeled with biotin-16-dUTP	Chromosome painting probe	
	Microdissected and DOP-PCR, labeled with biotin-16-dUTP	Chromosome painting probe	
Diagnosis of inherited reciprocal translocation (13;26)	BTA13 and BTA26	Chromosome painting probes	Biltueva *et al*. (2014)
Identification of reciprocal translocation t(5;6)	BACs- 382A08, 808F01, 866F10 and 752C12	BAC probes	De Lorenzi *et al*. (2014)

Gene Mapping

Cytogenetic map using FISH technique is a type of physical mapping and it is useful in research applications, such as for gene mapping, comparative mapping or the identification of novel oncogenes or genetic aberrations that contribute towards various cancers.

Di Meo *et al* (2008), Farhadi *et al* (2013), Iannuzzi *et al* (2015) and Kolesarova *et al* (2015) has reconfirmed that gene mapping using FISH technique revealed high conservation of autosomal chromosomes in the bovidae. Iannuzzi

et al (2001c) have done comparative FISH mapping by using caprinae BAC probes and identified a small chromosomal translocation between bovinae chromosome 9 and caprinae chromosome 12 during the evolution and proved that bovinae type is ancestral to the caprinae type. The list of probes used for mapping of specific genes on the chromosomes were listed in Table 2.

Table 2: Chromosomal localization of specific genes on chromosomes

Purpose of experiment	Name of the probe	Type of the probe	Author(s)
Localization of MHC complex	BL3-7 cDNA clone (Biotynilated)	cDNA probe	Iannuzzi *et al*. (1993)
Mapping of bovine TP53 gene	cloned bovine TP53 probe	Locus specific probe	Coggins *et al*. (1995)
Mapping of ZNF164, ZNF146, GGTA1, SOX2, PRLR, EEF2	ZNF164, ZNF146, GGTA1, SOX2, PRLR, EEF2 probes labeled with biotin-11-dUTP	Gene specific probes	Hayes *et al*. (1996)
Comparative FISH-mapping of *VIL* gene	λ-66.2 and λ-113.5 genomic clones	Gene specific probes	Iannuzzi *et al*. (1997)
Mapping of bovine cosmids of chr-X	clOBT 314	Cosmid FISH probes	Prakash *et al*. (1997)
	clOBT 945		
	clOBT 1489		
Location of TSPY gene on chr Y	bTSPY-specific primers TV11 and FD07	Gene specific probe	Vogel *et al*. (1997)
Mapping of prion genes	*PrP* gene (GAAGTCATCATGGTGAAAAGC and TCACATCTCTAAACAATGTCAAA)	Gene specific probe	Castiglioni *et al*. (1998)
Comparative mapping of thirteen type-I markers in river buffalo and sheep	BTA6	Chromosome painting probes	Di Meo *et al*. (2000)
	BTA8		
	BTA26		
	BTA29		
Comparative mapping of river buffalo and sheep chromosomes	Caprinae BAC clones	BAC probes	Iannuzzi et al. (2001c)
Mapping of ED1 gene to chr X	BBI_B750N22235Q2	genomic DNA	Kuiper *et al*. (2001)
Assignment of TRO gene to chr X	BAC clone 0577G05 labeled with biotin-16-dUTP	BAC probe	Asai *et al*. (2004)
Mapping of *C14orf4* gene to BTA10q36	BAC library RPCI-42	Gene specific probes	Drogemuller *et al*. (2004)
Mapping of SRY, ANT3 and CSF2RA genes to chr Y	BAC clones 95D10, 402H11, and 282L01 containing bovine SRY, ANT3, and CSF2RA genes, respectively	BAC probes	Liu *et al*. (2004)
Mapping of FHIT gene	bI0876E06 BAC clone	BAC probe	Di Meo *et al*. (2005)
Mapping of PHACTR1 gene	CH240-229B8	Bovine genomic BAC clone	Drogemuller *et al*. (2005a)
Mapping of TYK2 and PDE4A genes	RP42-545G13	bovine genomic BAC clone	Drogemuller *et al*. (2005b)
Mapping of FGF10 gene	BBI_B750O1689	Bovine genomic BAC clone	Kuhn *et al*. (2005)
Mapping of PAX6 gene	CH240-442K7	Bovine genomic BAC clone	Kuiper *et al*. (2005)
Mapping of SLC27A1 gene	BAC clone, 927A3, containing the bovine SLC27A1 gene	BAC probe	Ordovas *et al*. (2005)
Comparative FISH mapping of *MUC1* gene	BACs- 0494F01 and 0060B06	Gene specific probes	Perucatti *et al*. (2006)
Assigment of genes to chr 5 and 16	TAMBT BAC library screened with PCR and BAC clones were cultured	BAC probes	Hansen *et al*. (2007)
Assignment of 68 autosomal loci	Bovine and caprine BAC clones labeled with biotin or digoxigenin	BAC probes	Di Meo *et al*. (2008)
Comaparative FISH mapping of fecundity genes in cattle,	BtINRA-152G11		Farhadi *et al*. (2013)
	BtINRA-748C10 +BtINRA-320H10		

river buffalo, sheep and goat	BtINRA-544F11	BAC FISH probes	
	BtINRA-152G11		
	BtINRA-748C10 +BtINRA-320H10		
	BtINRA-544F11		
FISH mapping of fecundity genes in cattle, river buffalo, sheep and goat	BtINRA-81C03	BAC FISH Probe	Iannuzzi *et al*. (2015)
	BtINRA-243A01		
	BtINRA-448A07		
Comparative mapping of LCA5L gene in cattle, sheep, and goats	BAC CH240-118J20 BAC clone labeled with digoxigenin	BAC probe	Kolesarova *et al*. (2015)

Sexing of Bovine Spermatozoa and Embryos

Recently the dairy industry was revolutionized with sorted semen to meet the increased demands for milk production. Currently, the only commercially available technique for sperm sorting technique is flow cytometric fluorescence activated cell sorting (FACs). The purity of the sexed semen can be validated by molecular technique like quantitative polymerase chain reaction and Fluorescent in-situ hybridization technique. FISH allows to identify the sex of sperms at single cell level, cost-effective, simple and fast technique (Reinsalu *et al* 2019).In embryo transfer programmes, pre-selection of the gender and survivability of an embryo has great value for economy and management of herd. FISH can detect sex of the embryo and chromosomal abnormalities related to embryo viability at the Pre-implantation stage (Kobayashi *et al* 1998). The probes used for the sexing of the sperms and embryos were listed in Table 3.

Table 3: Probes used in sexing of spermatozoa and embryos

Purpose of experiment	Name of probe	Type of the probe	Author
Quantification of chr-Y bearing spermatozoa	BC1.2	Repetitive sequence probe	Schwerin *et al*. (1991)
Detection of male specific DNA sequences in embryos	BC1.2	Locus specific probes	Kobayashi *et al*. (1998)
Detection of X and Y bearing spermatozoa	Chr X- cosmid PL 44 Y- Y fragments were amplified by PCR and labeled with digoxigenin	X- cosmid probe Y- chromsome specific probe	Hassanane *et al*. (1999)
Determination of percentage of spermatozoa bearing Y chromatin	BC1.2 BC1.34	Locus specific probes	Kobayashi *et al*. (1999)
Determination of sex ratio of bovine sperm samples	X- specific Goat BAC probes	Repetitive sequence probes	Piumi *et al*. (2001)
	BRY4a	Repetitive sequence probes	
Validation of cattle sperm sorting procedures	X-Y paint set	Chromosome painting probes	Rens *et al*. (2001)
Determination of viability and sexing of bovine spematozoa	X-Y paint set	Chromosome painting probes	Revay *et al*. (2002)
Identification of X and Y bearing spermatozoa in water buffalo	X-Y paint set and BC1.2 (Y)	Chromosome painting probes	Revay *et al*. (2003)
Sexing of river buffalo, and cattle spermatozoa	Xcen	Chromosome painting probes	Di Berardino *et al*. (2004)
	Y		
Validation of sorted semen	6(locusD6Z1)	Locus specific probe	Habermann *et al*. (2005)
	Y(locusDYZ1)		
Assessment of X and Y bearing spermatozoa	BC1.2 (Y)	Locus specific probe	Kobayashi *et al*. (2004a)
Sexing of embryos	BC1.2 (Y)	Locus specific probe	Kobayashi *et al*. (2004b)
Sexing of IVF bovine embryos	BtY2-L1	Locus specific probes	Lee *et al*. (2004)
Sexing of bovine embryos	BtY2 gene	Gene specific DNA probe	Cenariu *et al*. (2008)
Routine validation of *Bos taurus* sexed semen	X and Y specific probes prperaed by DOP-PCR amplification	Gene specific probes	Reinsalu *et al*. (2019)

Evolutionary Studies

Cytogenetic analysis plays an important role in the field of evolutionary biology and taxonomy. The relationship between different species karyotypes has been studied to elucidate phylogeny and the process of species evolution (Nath and Johnson 2000). FISH also applied to establish the homologies between species (Sherlock 1993). ZOO-FISH analysis has been used for the comparative karyotype studies, which reveals evolutionary conservation or divergence between the species (Solinas-Toldo *et al* 1995 and Scalzi and Hozier 1998). List of the probes used for the evolutionary studies were given in Table 4.

Table 4: Identification of conserved and divergent chromosomal regions between different species

Purpose of experiment	Name of the probe	Type of the probe	Author(s)
Assessment of X chromosome homologies in cattle, sheep and goat	Xp and Xq	Chromosome painting probes	De Leon *et al*. (1996)
Comparative analysis of chr-Y structure in *Bos taurus* and *Bos indicus*	BC1.2	Locus specific probes	Goldammer *et al*. (1997)
	λES6.0		
	BTA Yp-12	Region specific probes	
	BTA Yp-12.1ter		
Mapping of conserved human chromosome regions on river buffalo chromosomes	HSA painting probes	Chromosome painting probes	Iannuzzi *et al*. (1998)
Comparison of pig and cattle karyotype	Probes generated by flow sorting of chromosomes and PARM-PCR	Chromosome painting probes	Schimtz *et al*. (1998)
Comparative mapping of chr X between bovinae and caprinae	24 goat BAC clones labeled with biotin	BAC probes	Iannuzzi *et al*. (2000)
Define the rate of karyotype changes between cattle and pig	Probes prepared by flow sorting of chromosomes and DOP-PCR	Chromosome painting probes	Fronicke and Weinberg (2001)
Determination of chromosomal rearrangements between human and cattle X chromosome	HSAXp HSAXq	Chromosome painting probes	Rubes *et al*. (2005)
Characterization of conserved Y-specific DNA sequence in zebu and taurine cattle	Zebu male specific OPA-06 sequence	Region specific painting probe	Alves *et al*. (2006)
Cetartiodactyla ancestral karyotype: comparative cross species chromosome painting	Chromosome specific probes prepared by flow-sorting and DOP-PCR	Chromosome painting probes	Balmus *et al*. (2007)
Comparative gene mapping in cattle, Indian muntjac and Chinese muntjac	Cosmid clones- 27A, 20K and 36H	Cosmid probes	Murmann *et al*. (2008)
Phylogenetic position of Pseudoryx nghetinhensis from eleven species of bovidae	Ribosomal 28S and telomeric probes	Ribosomal and telomeric probes	Nguyen *et al*. (2008)
Identification of conserved male specific repeat sequences in wild Bovidae	Probe prepared by DOP-PCR method	chromosome painting probe	Cabelova *et al*. (2012)
Establishment of heterosome synteny groups in ruminantia	Bovine IDetectTM Chr X Point Probe GREEN	Chromosome painting probes	Kozubska-Sobocińska *et al*. (2012)
	Bovine IDetectTM Chr Y Point Probe RED		
Comparative analysis of X chromosome in river buffalo, cattle, sheep and human	22 BACs from cytogenetic OARX map	BAC probes	Perucatti *et al*. (2012)
Detection of cryptic chromosome divergence between cattle and goat	Bacterial artificial chromosomes	BAC probes	De Lorenzi *et al*. (2015)
Determination of karyotype relationship between cattle and deer species	Flow sorted painting probes and BAC clones	Whole chromosome painting probes and BAC probes	Frohlich *et al*. (2017)
Validation of segmental duplications and associated copy number variations in water buffalo	Clones form CHORI-240 bovine BAC library	BAC probes	Liu *et al*. (2019)

Software Tools to Construct FISH Probes

HD FISH (*High definition DNA fluorescence in-situ hybridization)*

HD FISH can operate at the resolution limit of conventional optical microscopy due to the systematic design of unique probes with short span enabling visualization of virtually any human or mouse genomic locus. It is a cost effective genome scale PCR based method for high definition DNA FISH and is a combination of HD-FISH with single molecule RNA FISH can visualizing gene loci with diffraction-limited resolution, chromosomes as spot clusters and single genes together with transcripts. Rapid and flexible generation of probes with a database of 4.3 million primer pairs targeting human and mouse genome. Combination of HD-FISH and RNA FISH allows in situ analysis of the association between chromosome organization and gene expression and it has broad applications from the diagnosis of chromosomal aberrations to chromosomal architecture studies (Bienko *et al* 2013).

PROBER *(Oligonucleotide FISH probe design software)*

PROBER produces tiling oligonucleotide probes (TOP), which are an alternative to bacterial artificial chromosomes (BACs), by masking genomic repetitive sequences and delineating essentially unique regions that can be amplified to yield small DNA probes (100-2000bp) that can produce a single, strong fluorescent signal. PROBER can be applied to any genomic locus but it should contain at least 10kb of unique blocks. The software was already tested by designing the number of probes for genomic amplifications and hemizygous deletions that were detected by representational oligonucleotide microarray analysis of breast cancer tumors (Navin *et al* 2006).

mathFISH

The mathematical models reduce the number of cycles of trial and error to reach the right probe and optimal hybridization conditions. mathFISH is a tool, which design RNA targeted FISH probes using mathematical models. This tool calculates all modeled thermodynamic properties of a single probe for any target molecule provided (Perfect match or with mismatches). This web based tool is freely available and was developed to provide a user friendly environment for the simulation of RNA targeted FISH with oligonucleotide probes. mathFISH is expected to maximize the probability of successful hybridization with respect to probe sensitivity and specificity (Yilmaz *et al.,* 2011).

OligoArray

OligoArray 2.0 a programme that design specific oligonucleotides at genomic scale. It computes gene-specific and secondary structure free probes for genome scale oligonucleotide microarray construction. It uses the thermodynamic approach to predict the secondary structures and to calculate specificity of targets on chips for a unique probe in a mixture of probes. It can adjust the oligonucleotide length to fit narrow T_m range suitable with hybridization requirements. This programme does not depend on the cDNA or oligonucleotide libraries and makes it feasible to do gene expression analysis on a genome scale for any organism for which genome sequence is known (Roulliard *et al* 2003).

Oli2go

It is an efficient, user friendly and fully automated multiplex oligonucleotide design tool. This programme combines several steps in an all-in-one solution for high quality probe and primer design for a variety of biological experiments. Oli2go specificity has been checked for several species like mouse, bacteria, virus, fungi, invertebrates, plants, protozoa, archaea and sequences from the whole genome shotgun sequence projects and environmental samples, at once. The software is freely accessible to all users at http://oli2go.ait.ac.at/. (Hendling *et al* 2018).

H-MAPD (*Human MLPA probe design, Implemented in Perl-CGI and hosted on Linux server)*

MLPA is multiplex ligation dependent probe amplification, an efficient and reliable method for gene dosage analysis. Software packages available for the only electrophoresis based MLPA probe design. H-MAPD programme automates the generation and selection of probes for human genomic MLPA. This web based tool uses UNAFold software to tests physicochemical properties and UCSC genomic browser for the uniqueness tests. H-MAPD

supports both traditional electrophoresis based assays and FlexMAP bead-coupled MLPA probe design. It is freely available to the non-commercial users at URL http://genomics01.arcan.stonybrook.edu/mlpa/cgibin/mlpa.cgi. (Zhi and Hatchwell 2008).

OligoMiner

A web tool used to generate genome scale design of oligo FISH probes that affords the scientist exact control over the parameters of each probe. This software introduces a method for evaluating the specificity of each probe molecule that connects simulated hybridization energetics to rapidly generated sequence alignments using supervised machine learning. The ability of the software has been demonstrated by performing genome-scale probe discovery in numerous model organism genomes and displayed the performance of the resulting probes with diffraction-limited and single-molecule super resolution imaging of chromosomal and RNA targets. This pipeline will make the FISH probe design process much more accessible and will more broadly facilitate the design of pools of hybridization probes for a variety of applications. (Beliveau *et al* 2018).

kmasker (*An in silico prediction of single copy FISH probes)*

This is used for barley to construct unique genomic sequences of large genomic fragments for FISH technique. This method used to integrate genetic and cytogenetic maps in plants and other species with large and complex genomes if the probe sequence (e.g. BACs, sequence contigs) and low coverage (8-fold) of unassembled sequences of the species of interest are available. Kmasker has been made publicly available as a web tool at http://webblast.ipk-gatersleben.de/kmasker (Schmutzer *et al* 2014).

ProbeMaker

A computer-assisted design and analysis of oligonucleotide probe sequences. The tool helps in the design of probes for target sequences by incorporating sequence motifs for purposes such as amplification, visualization, or identification. An extension system allows the framework to be equipped with application-specific components for evaluation of probe sequences, and provides the possibility to include support for importing sequence data from a variety of file formats (Stenberg *et al.*, 2005).

Companies Providing FISH Probes

Cytocell (*Oxford Gene technology)*

It is an European manufacturer of FISH probes and has well established world-wide network for its products. Cytocell manufacture directly labeled FISH probes for constitutional cytogenetics and oncology. Cytocell product ranges: Aquarius range, Multiprobe range, myprobe custom FISH probes, ZOO-FISH

Empire Genomics

It is a clinical molecular diagnostics company offering comprehensive menu of products and services that are used in guiding precise treatments for patients. Empire genomics is offering over 1 million FISH probes like Gene specific, chromosome specific, disease specific, region specific, clinical FISH panels, fusion probes, break apart probes, human BAC probes, mouse BAC probes and custom FISH probes.

Abbott Vysis

A molecular laboratory aims to patient care in diagnosing and managing the most serious and complex diseases. It is offering all CEP probes, Vysis CEP 12 spectrum orange labelled fluorescent labeled DNA, Vysis CEP 8 spectrum orange labelled fluorescent labeled DNA, probes for the diagnosis of microdeletion syndromes, telomeric regions, rearrangements, deletions, additions, trisomies and sex chromosomal abnormalities.

ThermoFischer Scientific

It is offering Invitrogen™ FISH tag detection kits for routine analysis and Invitrogen™ super boost kits for very low or rare abundance targets. FISH tag detection kits provide labelling reagents and buffers to generate optimal

FISH probes. The nick translation is used to enzymatically incorporate amine-modified nucleotides, followed by chemical labelling with amine-reactive Invitrogen™ Alexa Fluor™ dyes.

EXIQON (*A QIAGEN Company)*

One can design own LNA™ FISH probes through QIAGEN LNA™ custom oilgo shop or experts helps to design probes. LNA enhanced oligonucleotides shows high affinity towards complementary sequences results in a improved specificity and sensitivity, when compared to Normal DNA and RNA oligonucleotides. These can distinguish the sequences differing by only a single nucleotide, which is difficult for success of many experiments.

Kreatech FISH Probes *(Leica Biosystems)*

These probes are latest advancement in the FISH probes, which eliminates the use of Cot-1 or blocking DNA, providing clear background and bright signal with enhanced signal to noise ratio. Currently providing more than 400 probes and custom probes are also available upon request.

Abnova

Abnova is providing FISH probes from bacterial artificial chromosome library with specific sites. It is offering chromosome specific probes, subtelomeric FISH probes, genotype probes to identify specific gene amplification or deletion, split probes to identify gene breaks, translocation probes, custom probes and pre-natal ISH probes to identify chromosomal abnormalities.

MetaSystems Probes

MetaSystems probes has been designing and manufacturing systems for automated microscopic imaging. In 1997, presented the first commercial software for the filter based 24 color FISH (mFISH) and in 1998, multicolour banding (mBAND) was introduced. This company is providing chromosome painting probes, enumeration probes, locus specific probes, multicolour FISH, banding and probes specific to the diseases (cancer diagnosis).

InteGen LLC

It was started with a patented technology called Interphase chromosome profiling means the uncondensed individual chromosomes are studied in an interphase nucleus using a panel of FISH probes. It is providing multiplex interphase chromosome profiling, in which basic panel will detect aneuploidy of all chromosomes and common micro deletions and comphrehensive panel detects arm specific structural changes for all chromosomes. It is also offering chromosome wise high resolution probes and probes for cancer diagnosis.

Agilent **(**SureFISH probes)

SureFISH DNA FISH probes are designed in silico and synthesized by oligonucleotide library synthesis technology. This eliminates the limitations of FISH probes manufactured with bacterial artificial chromosome (BAC) technology. This is the only company not using BAC technology in probe manufacturing. SureFISH probes are repeat free and produce a clean signal, eliminates the need for blocking and signal suppression produced by blocking agents. It is providing chromosome enumeration probes, telomere probes and customized FISH probes.

CytoTest

A biotechnology company providing high quality, innovative and affordable molecular cytogenetics products and services. CytoTest offering probes for cancer diagnosis, also chromosome specific counting probes, locus specific probes, whole chromosome painting probes, subtelomere probes and custom probes.

Conclusion

Fluorescent *in-situ* hybridization technique has broad applications in diagnosis and research. The increasing availability of commercial probes making the FISH as a regular diagnostic technique in clinical laboratories. Chromosome painting probes will give ultimate diagnosis of structural and numerical chromosomal abnormalities

with high sensitivity and specificity because of their ability to visualize individual chromosomes. Repetitive sequence probes hybridize with complementary sequences more rapidly than highly conserved unique sequences and particularly suitable for diagnosis of aneuploidies in interphase and metaphase. Locus specific probes are useful to determine the position and how many copies of a specific gene on a chromosome in a particular genome. These probes are suitable in the detection of structural rearrangements such as translocations, deletions and inversions in both interphase and metaphase. Locus specific probes when used in combination with Repetitive sequence probes determine the missing genetic material from a particular chromosome.

Conflict of Interests

There is no conflict of interest.

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