International Journal of Molecular Medicine and Advance Sciences 2 (2) : 128-133, 2006

Molecular Cytogenetics Analysis: As A Technique and its Applications

[1,2]H.H. Musa,, [1]B.C.Li, [1]G.H. Chen and [1]J.H. Cheng
[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China
[2]Department of Animal Production, Faculty of Veterinary Science, University of Nyala, Sudan

Abstract: Cytogenetic analysis always based on examination of chromosome. Before karyotype of an organism including their number, size, shape and internal arrangements, was determined from images taken from cells in metaphase. When banding techniques were developed, the individual chromosomes were identified. In 1960, in-situ hybridization utilized probes labelled with radioisotopes was used. Additionally, spectral karyotyping, multiplex fluorescence in-situ hybridization, comparative genomic hybridization and more recently array comparative genomic hybridization have proven to be useful for the characterization of structural chromosome aberrations found in conventional cytogenetics. In this review, we summarize the developments of chromosome analysis techniques and layout the applications for each technique in different area of science.

Key words: Cytogenetics, choromosome, molecular, hybridization

INTRODUCTION

Conventional cytogenetics is the main technique in routine genetic diagnostic for the analysis of genotypic or phenotypic correlations. Additionally, fluorescence in-situ hybridization, spectral karyotyping, multiplex fluorescence in-situ hybridization, comparative genomic hybridization and more recently, array comparative genomic hybridization has proven to be useful for the characterization of structural chromosome aberrations found in conventional cytogenetics. The application of these techniques for the analysis of specimens from humans or mouse models of human diseases enables one to reliably identify and characterize complex chromosomal rearrangements resulting in alterations of the genome[1].

The use of Fluorescence In-Situ Hybridization (FISH) technology has enabled the rapid analysis of cytogenetic specimens as an adjunct to classical cytogenetic analysis. Spectral Karyotyping (SKY) is a 24-colour, multi-chromosomal painting assay that allows the visualization of all human chromosomes in one experiment[2]. Comparative Genomic Hybridization (CGH) is a molecular cytogenetic FISH-approach developed for the detection of genomic imbalances with cytogenetic resolution. CGH allows the genome-wide assessment of relative DNA copy number changes using extracted specimen DNA as a probe[3]. DNA micro-arrays have been developed to exploit the huge amount of sequence data generated by large-scale sequencing program. The aim of this paper was to review the development of chromosome analysis techniques and it is application in different fields of science.

BASIC KARYOTYPE

Chromosomes are structures that exist in the nucleus of the cell in a diploid state. However, during cell division they sometimes split into a haploid state. There are two types of cellular division where chromosomes appear: mitosis in somatic cell (the diploid number of chromosomes is retained) and meiosis in gamete cells (sperm and egg) with a haploid number of chromosomes). Rearrangements of chromosomal structures are most likely to occur during crossing-over including deletions, duplications, inversion and translocations. A set of chromosomes was present in form of polyploidy or aneuploidy.

Cytogenetic analysis always based on examination of chromosome fixed during mitotic metaphase. Metaphase chromosome differs from one to another in size, shape as well as in length. However, the relative position of centromere is constant and this ratio is vital for chromosome identification. Before karyotype of an organism chromosome were determined from images taken from cells in metaphase[4]. When banding techniques were developed in the early 1970s, the individual chromosomes were unambiguously identified[5].

BANDING TECHNIQUES

Since Hungerford *et al.*[6] made the first karyotype from peripheral blood cultures and demonstrated the

Corresponding Author: B.C.Li, College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China

value of chromosome analysis in both the clinical and research laboratories. Chromosome banding has become a standard and indispensable tool for cytogenetic analysis and several banding techniques have been developed:

G-banding produces a high resolution and high contrast image of chromosomes. They are digested with trypsin before staining with Giemsa. Q-banding uses quinacrine as a dye and R-banding shows the reverse pattern of G-banding. By carefully application of these dyes, sub-bands and multi-sub-bands can be identified. In C-banding, chromosomes are treated with acid and base and then stained with Giesma stain. Chromosomes treated in this way have to be viewed with UV-fluorescence[7]. By the late 1980s, cytogenetics was a mature discipline and underwent more extensive technological changes as molecular genetic technologies were applied to cytogenetic preparations. Early karyotype analysis of many types of malignancies identified complex structural rearrangements and extra-chromosomal structures that were left unidentifiable and simply termed marker chromosomes[8].

FLUORESCENCE IN-SITU HYBRIDIZATION (FISH)

The identity of specific molecule was first demonstrated using antigen-antibody interaction. In 1940s, antibodies were conjugated to flurochromes without loss of their epitope-binding specificity. Later antibody dependent fluorescent detection of nucleic acid hybrids was achieved and then replaced by advent fluorescent nucleic acid probes[9]. In 1960, in-situ hybridization utilized probes labeled with radioisotopes[8]. The method of isotopic detection employed in random incorporation of radioactive modified bases into growing cells followed by autoradiography. The drawbacks of isotope such as inconsistent of probe activity, high sensitivity of radiography, limitation of resolution, time needs to get result and relatively hazardous materials were developed new techniques.

Fluorescence In-Situ Hybridization (FISH) quickly replaced radioactive in-situ assays by the late 1980s[10], which actually opened new considerable research opportunities for reasons such as: Their possibility to obtain cytogenetic results on interphase cells, easy to analyze the colour images, possible to study archived material and paraffin-embedded tissues and can also be combined with other techniques to improve the results obtained. The applications of fluorescent in-situ detection were used for detection of DNA and mRNA targets[11,12]. The advancement of recombinant DNA technology permitted newly identified genes to be mapped to chromosomal regions[13]. Likewise, Polymerase Chain Reaction (PCR), is enables the DNA to be amplified in sufficient quantities for genetic analysis. FISH quickly replaced PCR-based method, which had led to misdiagnoses, for sexing of embryos[14]. FISH is a powerful technique for detecting probes from known genes and specific chromosomal loci enabled the genome to be localization of specific DNA sequences within interphase chromatin and metaphase chromosomes and the identification of both structural and numerical chromosome changes[15]. FISH is currently the method of choice for evaluating sex selection procedures because it accurately identifies the sex chromosome of individual spermatozoa using specific probes for the X and Y-chromosomes and a two-colour detection system; and through FISH, large numbers of spermatozoa can be screened within minimal time[16]. In-situ hybridization technique provides information complementary to those provided by immuno-histochemistry. It is possible to detect the nucleic acid sequences coding for the expression of specific proteins. The combination of immuno-histochemistry and in-situ hybridization allows complete and detailed analysis of gene expression in-situ including the identification of specific chromosomes.

SPECTRAL KARYOTYPING (SKY)

Several multicolor karyotyping procedures, such as Multiplex-FISH (M-FISH), Spectral Karyotyping (SKY) or Colour-Changing Karyotyping (CCK)[17,18] were introduced in the past several years. These techniques were applied in prenatal diagnosis[19], peripheral blood cultures and leukemia and solid tumors[20], especially in cases where G-banding was not sufficient to identify the chromosome of origin. There are two methods of performing M-FISH, one based on the use of specific filter sets[18] and the other based on the spectral signature of the fluorochromes or dyes used and termed Spectral Karyotyping (SKY)[17]. The most popular method of Spectral Karyotyping (SKY), involves the use of 24-colour, whole chromosome-painting permitting visualization of each chromosome in one experiment. This technology is based on the principles of spectral imaging[21] and Fourier spectroscopy[22]. Flow sorted chromosomes are PCR-labeled[23], either directly or indirectly, with fluorochromes or haptens. Five pure dyes that were spectrally distinct are used in combination to create the unique chromosome cocktail of probes.

The limitation of SKY depends on its inability to detect deletions or other intra-chromosomal structural changes such as inversions. However, there are some reasons for using SKY in the analysis of abnormal

chromosome preparations. It is that, does not need to have a highly experienced metaphase analyst to perform the microscopy to interpret the clearly assigned color patterns. In addition the subtle translocations of DNA can be detected[24]. SKY therefore provides a method for rapid high-resolution screening of the cancer karyotype and has applications both in the research and clinical cytogenetics laboratories[20]. Many cytogenetic changes in carcinomas are resulted of segregation defects during mitosis leading to increase in the number of numerical changes in the karyotype[25]. SKY can provide more precise information concerning both numerical and structural changes when genomic instability is suspected. SKY has been applied to various tumor groups including hematological malignancies, sarcomas, carcinomas and brain tumors, with the intent of identifying specific chromosomal abnormalities that may provide insight to the genes involved in the disease process[20]. SKY has also been applied for the mouse genome, enabling investigators to extrapolate information from mouse models of cancer to their human counterparts.

MULTIPLEX FLUORESCENCE IN-SITU HYBRIDIZATION (M-FISH)

Multicolor- karyotyping and labeling strategies have been recently reviewed[26]; multicolour techniques have been particularly applied to chromosome panting probes, an important limitation of painting probe is their poor sensitivity for detection of intrachromosomal rearrangements. Deletions or duplications will be detected only if they result in significant size differences of the two homologous chromosomes.

The novel M-FISH diagnostic procedures is called centromeric M-FISH and used for one step diagnosis of chromosomal aneuploidies. Two separate methods were developed, first was metaphase assay or (M-FISH) where the human centromeric probes labeled using combinatorial labeling, mixed together and hybridized on the same cytogenetic preparation it is used for small marker chromosome identification in the presence of metaphases. Second is interphase assay (iCM-FISH), here the centromeric probes were divided into three groups, this allows identification of aneuploidies in interphase nuclei and doses not require cell culture before FISH[27]. The advantages of CM-FISH was that the probes dose not required competitor DNA during hybridization, FISH signals are strong and all probes are available as plasmids[28]. Hybridization time was range between 30 min to 2 hrs. The disadvantage is the lack of separation between the centromeres of chromosomes 13 and 21[29] and the inability to detect marker chromosome lacking α-satellite sequences[30].

COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

One of the technically most demanding of a modified in-situ hybridization technique is Comparative Genomic Hybridization (CGH)[31], provides superior resolution to traditional karyotype analysis. In this type of analysis, two differentially labeled genomic DNAs are co-hybridized to normal metaphase spreads or to micro-array. CGH allows detection and mapping of DNA sequence copy differences between two genomes in a single experiment. Comparative genomic hybridization is a powerful tool for the detection and identification of unbalanced chromosomal abnormalities in prenatal, postnatal and pre-implantation diagnostics[32]. Unbalanced translocations caused severe abnormalities or non-viability of the embryos, so CGH is able to identify more subtle abnormalities known as translocations, but it is not able to detect balanced translocations[33]. CGH will allow us to select the embryos with the highest pregnancy potential in order to transfer them first and to avoid the transfer of embryos with chromosome abnormalities.

Embryo biopsy followed by CGH is a way of screening embryos for some common chromosome faults that are known to lead to pregnancy loss. Hopefully the selection of better quality embryos will achieve pregnancies more quickly. This allows determining of complete and partial chromosome gains and losses[31,34]. CGH results will thus allow the classification of many tumor cell lines and together with other complementary techniques such as micro-dissection-FISH and primed *in-situ* hybridization will increase the possibility to select appropriate treatment for cancer patients.

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (A-CGH)

The development in CGH technology was heralded in 1997 by (Solinas-Toldo *et al.* [35] they described as matrix-CGH. Compared with traditional CGH experiments, Matrix-CGH is able to detect genome gains and losses. There are several names for this technology: DNA micro-arrays, DNA arrays, DNA chips, Gene chips, etc. Two different micro-array technologies are available, the oligo micro-arrays and the cDNA micro-arrays that differ with the length of DNA sequences (from 25 oligomeres to several hundred oligomeres, respectively) synthesized or grafted on the matrix, the type of the matrix (glass, nylon, membranes and other formats) and, finally, the data processing. Arrays are customizable in DNA species and in number of genes represented. When using two different samples (treated and controlled), we can compare the gene expression profiles between them and then determine how

the cell or tissue regulates its genes in a specific environment. DNA micro-arrays are like powerful automatic RNA differential display experiments, without the need for both sequence and quantify the bands of interest.

DNA micro-arrays are suitable tools for endocrinology studies, such as the analysis of the cellular response to a specific stimulus. For example, Feng *et al.*[36], identified from mouse liver 45 genes not previously identified as thyroid hormone-responsive genes. In a related work, Dupont *et al.*[37] have used cDNA micro-array technology to define the specificity of insulin vs. IGF-1 signaling. Expression arrays can be used to understand mutagenic diseases such as many cancers[38]. The ratio of gene expression in a treated sample over the control sample is used as quantitative measurements of the differential expression, to generate a clustering of genes[39]. Another application of DNA micro-arrays is the finding of new functions of genes by association of gene expression.

DNA micro-arrays are useful for the identification of genes that are markers of multigenic diseases. Furthermore, DNA micro-arrays will be very efficient tools to detect the response to therapy, such as the prostate tumor response to androgen withdrawal and to plan more appropriate medical treatments[40]. The physical chemistry of hybridization is oligonucleotides micro-array is used to detect *point mutations* (the missing, adding or changing of a single base) in a known DNA sequence. Furthermore, oligo micro-array raw original data have to be processed for bias corrections like multiplicative effects (*e.g.* difference in the total mRNA concentration of samples), additive effects (*e.g.* background) and position effects on the micro-array and nonlinear effects (saturation of detectors of the hybridization intensity).

The raw data produced from micro-array experiments are hybridized micro-array images. These images should be analyzed, each spot on the array identified its intensity measured and compared to the background. This is called image quantification and is done by image analysis software. To obtain the final gene expression matrix from spot quantifications, all the quantities related to some gene (either on the same array or on arrays measuring the same condition in repeated experiments) have to be combined and the entire matrix has to be scaled to make different arrays comparable. Many micro-array data are now available on public online databases where they can be queried, compared and analyzed by different computer software programs. The European Bioinformatics Institute (EBI) as well as the National Center for Biotechnology Information (NCBI) is establishing a public repository for micro-array gene expression data analogous to banks for DNA sequence data.

SUMMARY OF APPLICATIONS

FISH was used in radiation biology[41]. The ability of FISH to perform interphase cytogenetics makes it suitable for prenatal diagnosis. Some syndromes and specific mutated loci can be detected by PCR, but in cases of a micro deletion FISH probes was recommended. As FISH can be performed with a single cell, preimplantation diagnosis can be used to detect aneuploidies[42]. Diagnosis of the presence of certain viruses has been reported using probes for specific DNA of the infectious agent[43]. Cytogenetics analysis was applied in hereditary genetics to detect syndromes passed from the generations. Similarly, mutation caused by radiation and other contaminants, can cause genetic damage[44]. In the evolution process FISH technique proved that related species have common fragments of chromosomes[45]. The development of probes for animal research models and for specific diagnosis in species of agricultural and animal economic importance will provide many new applications of molecular cytogenetics, as every species has its own banding pattern[46].

REFERENCES

1. Dorritie, K., C. Montagna, M.J. Difilippantonio and T. Ried, 2004. Advanced molecular cytogenetics in human and mouse. Expert. Rev. Mol. Diagnosis. 45: 663-676.
2. Bayani, J.M. and J.A. Squire, 2002. Applications of SKY in cancer cytogenetics. Cancer Invest. 20: 373-386.
3. Tonnies, H., M. Stumm, R.D. Wegner, I. Chudoba, V. Kalscheuer and H. Neitzel, 2001. Comparative genomic hybridization based strategy for the analysis of different chromosome imbalances detected in conventional cytogenetic diagnostics. Cytogenet Cell Genet., 93: 188-194.
4. Bender, M.A., 1960. X-ray-induced chromosome aberrations in mammalian cells *in vivo* and *in vitro*. Intl. J. radiation Biology and related Studies in Physics, Chemistry and Medicine, pp: 103-118.
5. Strachan, T. and A.P. Read, 1999. Human Molecular Genetics 2nd Ed Bios.
6. Hungerford, D.A., H.S.Chandra, R.L. Snyder and F.A.Ulmer, 1966. Chromosomes of three elephants, two Asian (*Elephas maximus*) and one African (*Loxodonta africana*). Cytogenetics 5:243-246.
7. Ronne, M., 1989. Chromosome preparation and high resolution banding techniques. A Review. J. Dairy Sci., 72: 1363-1377.

8. Levsky, J.M. and R.H. Singer, 2003. Fluorescence *in-situ* hybridization: past, present and future. J. Cell Sci., 116: 2833-2838.
9. Rudkin, G.T. and B.D. Stollar, 1977. High-resolution detection of DNA-RNA hybrids in situ by indirect immunofluorescence. Nature, 265: 472-473.
10. Bauman, J.G., J. Wiegant, P. Borst and van P. Duijn, 1980. A new method for fluorescence microscopical localization of specific DNA sequences by in-situ hybridization of fluorochrome labeled RNA. Exp. Cell Res., 128: 485-490.
11. Singer, R.H. and D.C. Ward, 1982. Actin gene expression visualized in chicken muscle tissue culture by using *in-situ* hybridization with a biotinated nucleotide analog. Proc. Natl. Acad. Sci., USA, 79: 7331-7335.
12. Wiegant, J., T. Ried, P.M. Nederlof, M. van der Ploeg, H.J. Tanke and A.K. Raap, 1991. In situ hybridization with fluorescenated DNA. Nucleic Acids. Res., 19: 3237-3241.
13. Nath, J. and K.L. Johnson, 2000. A review of Fluorescence In-Situ Hybridization (FISH): Current status and future prospects. Biotech Histochem. 75: 54-78.
14. Matsuda Y., V.M. Chapman, 1995. Application of fluorescence in-situ hybridization in genome analysis of the mouse. Electrophoresis, 16: 261-272.
15. Poddighe, P.J., O. Moesker, D. Smeets, B. Awwad, C. Ramaekers and A. Hopman, 1991. Interphase cytogenetics of hematological cancer: Comparison of classical karyotyping and *In-situ* Hybridization using a panel of eleven chromosome specific DNA probes. Cancer Res., 51: 1959-1967.
16. Flaherty, S.P. and C.D. Matthews, 1996. Application of modern molecular techniques to evaluate sperm sex selection methods. Mol. Human Reprod., 2: 937-942.
17. Schrock, E., S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M.A. Ferguson-Smith and Y. Ning, 1996. Multicolor spectral karyotyping of human chromosomes. Science, 273: 494-497.
18. Speicher, M.R., S. Gwyn-Ballard and D.C. Ward, 1996. Karyotyping Human Chromosomes by Combinatorial Multi-Fluorescence FISH. Nat. Genet., 12: 368-375.
19. Uhrig, S., S. Schuffenhauer, C. Fauth, A. Wirtz, C. Daumer-Haas, C. Apacik and M. Cohen, 1999. Multiplex-FISH for pre-and postnatal diagnostic applications. Am. J. Hum. Genet., 65: 448-462.
20. Haddad, B.R., E. Schrock, J. Meck, J. Cowan, H.Young, M. A. Ferguson-Smith and S. du Manoir, 1998. Identification of de novo chromosomal markers and derivatives by spectral karyotyping. Hum. Genet., 103: 619-625.
21. Malik, Z., D. Cabib, R.A. Buckwald, A. Talmi, Y. Garini and S.G. Lipson, 1996. Fourier transform multipixel spectroscopy for quantitative cytology. J. Microsc. 182: 133-140.
22. Bell, R., 1972. Introductory Fourier Transform Spectroscopy. London, Academic Press.
23. Telenius, H., A.H. Pelmear and A. Tunnacliffe, 1992. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. Gene Chromosomes. Cancer, 4: 257-263.
24. Fan, Y.S., V.M. Siu, J.H. Jung and J. Xu, 2000. Sensitivity of multiple color spectral karyotyping in detecting small inter-chromosomal rearrangements. Genet. Test., 4: 9-14.
25. Lengauer, C., K.W. Kinzler and B. Vogelstein, 1998. Genetic instabilities in human cancers. Nature, 396: 643-649.
26. Maierhofer, C., I. Jentsch, G. Lederer, C. Fauth and M.R. Speicher, 2002. Multicolor-FISH in two and three dimensions for clastogenic analyses. Mutagenesis, 17: 523-527.
27. Henegariu, O., P.B. Ward, S. Artan, G.H. Vance, M. Qumsyieh and D.C. Ward, 2001. Small marker chromosome identification in metaphase and interphase using centromeric multiplex-FISH (CM-FISH). Lab. Inves. 81: 475-481.
28. Durm, M., I. Sorokine-Durm, F.M. Haar, M. Hausmann, H. Ludwig, P. Voisin and C. Cremer, 1998. Fast-FISH technique for rapid, simultaneous labeling of all human centromeres. Cytometry 31:153-162.
29. Maratou, K.Y. A.M. Siddique, Kessling, and G.E. Davies, 1999. Novel Methodology for the Detection of Chromosome 21-specific Alpha-Satellite DNA Sequences. Genomics, 57: 429-432.
30. Wandall, A., L. Tranebjaerg and N. Tommerup, 1998. A neo-centromere on human chromosome 3 without detectable alpha-satellite DNA forms morphologically normal kinetochores. Chromosoma, 107: 359-365.
31. duManoir, S., M.R. Speicher, S. Joos, E. Schroeck, S. Popp, H. Doehner, G. Kovacs, M. Robert-Nicoud, P. Lichter and T. Cremer, 1993. Detection of complete and partial chromosome gains and losses by comparative genomic *in situ* hybridization. Hum. Genet., 90: 590-610.
32. Lapierre, J.M. and G. Tachdjian, 2005. Detection of chromosomal abnormalities by comparative genomic hybridization. Obstet. Gynaecol., 17: 171-177.
33. Hermsen, M.A., G.A. Meijer, J.P. Baak, H. Joenje and J.J. Walboomers, 1996. Comparative Genomic Hybridization: A New Tool in Cancer Pathology. Hum. Pathol., 27: 342-349.

34. Kallioniemi, A., O.P. Kallioniemi, D. Sudar, D. Rutovitz, J.W. Gray, F. Waldman and L.D. Pinke, 1992. Comparative Genomic Hybridization for Molecular Cytogenetic Analysis of Solid Tumors. Sci., 258: 818-821.
35. Solinas-Toldo, S., S. Lampel, S. Stilgenbauer, J. Nickolenko, A. Benner, H. Dohner, T. Cremer, P. Lichter, 1997. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes. Chromosomes Cancer, 20 : 399-407.
36. Feng, X., Y. Jiang, P. Meltzer, P.M. Yen, 2000. Thyroid hormone regulation of hepatic genes *in vivo* detected by complementary Dna Micro-Array. Mol. Endocrinol., 14: 947-955.
37. Dupont, J., J. Khan, B.H. Qu, P. Metzler, L. Helman and D. LeRoith, 2001. Insulin and IGF-1 Induce Different Patterns of Gene Expression in Mouse Fibroblast NIH-3T3 cells: Identification by cDNA Micro-Array Analysis. Endocrinol., 142: 4969-4975.
38. Martin, R.D., 1990. Primate Origins and Evolution: A Phylogenetic Reconstruction (Princeton Univ. Press, Princeton).
39. Kolnicki, R.L., 1999. Kinetochore reproduction in animal evolution: Cell biological explanation of karyotypic fission theory. Symbiosis, 26: 123-141.
40. Bubendorf, L., A. Nocito, H. Moch and G. Sauter, 2001. Tissue micro-array (TMA) technology: Miniaturized Pathology Archives for High-Throughput *In-situ* Studies. J. Pathol., 195: 72-79.
41. Edwards, A.A. 1997. The use of chromosomal aberrations in human lymphocytes for biological dosimetry. Radiat. Res., 148: 39-44.
42. Coco, R., A. Gallo, M. Mühlmann and S. Provenzano, 2000. Estudio preconcepcional en un heterocigota de translocación t(1;4)(p32;q25). Análisis de segregación y aneuploidía por FISH doble y triple color. J. Bras. Reprod. Assist., 4: 51-59.
43. Pillai, S.D., 1997. Rapid molecular detection of microbial pathogens: breakthroughs and challenges. Arch. Virol., 13: 67-82.
44. Ulsh, B.A., M.C. Mühlmann-Diaz, F.W. Whicker, J.D. Congdon, T.G. Hinton and J.S. Bedford, 2000. Chromosome translocations in turtles: A biomarker in a sentinel animal for ecodosimetry. Radiat. Res., 153: 732-759.
45. Wienberg, J. and R. Stanyon, 1997. Comparative painting of mammalian chromosomes. Genet. Dev., 7: 784-791.
46. Musa, H.H., B.C.Li, G.H. Chen, T.P. Lanyasunya, Q. Xu and B.W. Ben, 2005. Karyotype and banding patterns of chicken breeds. Int. J. Poult. Sci., 4: 741-744.