Chromosome painting in farm, pet and wild animal species

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Abstract. Among the advanced karyotype analysis approaches embraced by animal cytogenetics during the past decade, chromosome painting has had the greatest impact. Generation of chromosome specific paints is considered pivotal to his development. Additionally, ability to use these paints across species (referred to as Zoo-FISH or comparative painting) is undisputedly the most important breakthrough that has contributed to our ability to compare karyotypes of a wide range of evolutionarily highly diverged species. This review introduces the readers to the basic principles underlying chromosome painting, and makes them aware of the tools/resources available to carry out this research in a variety of animal species. An overview of the current status of comparative chromosome painting results across closely as well as distantly related species is presented. Findings from different studies show how some segmental syntenies are more conserved as compared to others. The comparisons provide insight into the likely constitution of a vertebrate/mammalian ancestral karyotype and help understand some of the intricacies about karyotype evolution. Importance of comparative painting in setting the stage for rapid development of gene maps in a number of economically important species is elaborated.

Key words: Chromosome painting, Comparative genomics, Karyotype evolution, Zoo-FISH

Molecular cytogenetics has revolutionised the way we presently perceive chromosomes in the animal kingdom. Introduction of a number of new molecular approaches during the 80s and the 90s has enabled us to study the chromosomes at the DNA level. One of the techniques central to this transformation is in situ hybridization (ISH), especially the non-radioactive or the fluorescent version (FISH). In combination with resources generated during the past decade, the technique has been instrumental not only in overcoming the limitations encountered in studying chromosomes through traditional cytogenetics, but has been a major contributor to a strong and emerging branch - comparative genomics, where ‘chromosome painting’ is a very familiar term.

Background

Chromosome painting implies FISH mediated highlighting of chromosomes in metaphase or interphase preparations using composite probes specific for a whole chromosome (whole chromosome paints: WCPs) or a region thereof (partial chromosome paints: PCPs). The probe does not represents a single genomic site but is a cocktail of numerous sites from the originating chromosome, such that the whole chromosome or part thereof is almost completely represented. Hence, the observed signal is in fact an aggregation of several hybridization sites uniformly covering the chromosome, and giving a visual impression of ‘painting’. It is from this term that ‘chromosome painting’ has been coined.

There are two terms concurrently floating in the literature, viz., ‘chromosome painting’ and ‘comparative chromosome painting’. Though with regards to methodology the two approaches do not differ significantly from each other, their applications and implications differ widely. In the simplest possible way, the former represents use of the painting probe(s) on chromosomes of the species from which they originate (species specific chromosome painting), while the latter refers to their use across evolutionarily distantly related species. It is relatively difficult to define ‘distantly related’. Hence, for the sake of clarity and convenience in discussion, any use of painting probes on species other than their origin will be referred to as comparative chromosome painting. Application and significance of the two categories of chromosome painting will be discussed at length under individual sections. However, because the probes or ‘paints’ (WCPs or PCPs) are the same for both types of painting, they will be discussed commonly in the following section.
Source and generation of painting probes

Broadly speaking, the probes used for chromosome painting (CP) may originate from two different sources: i) from flow sorted chromosomes and ii) from microdissected chromosomes. Both approaches have now been fairly widely applied in a variety of animal species, however, the balance is tilted more in favor of the former. The extent of representation of the chromosomal content in both types of probes, as judged from the paints, is fairly uniform along the length of the chromosome. The only advantage of the latter type is that they can be rapidly and conveniently prepared usually with minimal or no contamination of other chromosomal material. Also, using this approach, one can readily decide about the size of the chromosomal segment, which is to be used in the preparation of the probe.

Over the years, flow sorted chromosomes have indisputably emerged as the primary source of chromosome specific paints. Initially, for a considerably long period of time, only human chromosome specific libraries generated from flow sorted chromosomes [20, 22] were available as the main probe source for chromosome painting (species specific as well as cross-species). However, during the past 6–7 years, significant breakthroughs in the ability to resolve individual chromosomes of a large variety of species with complex karyotypes has enabled construction of chromosome paints for a wide range of mammals (e.g., see [7, 51, 74]). The breakthrough is primarily attributed to improved resolution of flow cytometry obtained by adopting new procedures for chromosome preparation. Species for which chromosome specific paints are currently available, is shown in Tables 1a and 1b.

The other approach – chromosome microdissection – is a technique that was first applied to polytene chromosomes of Drosophila [13, 90] and mouse [86]. The technique has since been successfully used to obtain whole or partial human chromosomal DNA (see [87]) to generate paints and construct libraries. The past few years have witnessed a steep surge in the application of this technique to the chromosomes of a number of other species (see Table 1b for details and references). Chromosome specific paints thus obtained have provided interesting clues about karyotype evolution in some of the evolutionarily related species studied [19, 77].

Following flow sorting or microdissection, the obtained DNA is PCR amplified and labelled for use in fluorescent in situ hybridization (FISH) experiments. If, e.g., biotin or digoxigenin tagged dNTPs are used for labelling, indirect detection of the signal is carried out (see [18]). However, during recent years it has been possible to do direct detection by the use of fluorescent conjugated dNTPs. A brief overview of the actual process of FISH for chromo-