MURINE MULTICOLOR BANDING

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Multicolor banding approach, first introduced for human chromosomes only, was established as an optimal approach for karyotyping of murine chromosomes. Here we present the established mcb probe sets for all murine autosomes and the X-chromosome and review their potential application.

Key words: murine autosomes, X-chromosome, multicolor banding.

Laboratory mouse remains the leading experimental model for studying human biology and disease, with multiple murine cell lines being used in research worldwide. However, the karyotype structure and stability of mouse strains, and especially of murine cell lines is rarely checked due to the difficulties in G-banding and R-banding. Murine chromosomes are all telocentric, many of them being similar in size; thus, their chromosomal morphology is hard to distinguish. This also limits cytogenetic evolutionary studies in *Muridae* species and *Rhodentia*, which are thought to be very good models to study karyotype evolution in vertebrates due to the high karyotype variability.

In general, murine chromosomal rearrangements can only be characterized in detail using molecular cytogenetic approaches. Previously, whole chromosome paint (wcp) probes for mouse chromosomes were obtained using chromosome microdissection and flow sorting followed by whole genome amplification (Liechty et al., 1995; Rabbitts et al., 1995). FISH (fluorescence *in situ* hybridization) methods using murine wcps were applied in multicolor-FISH approaches first in 1996 (Liyanage et al., 1996). Evolutionary FISH studies using wcps permitted a revision of several ancestral karyotypic reconstructions, and a more accurate depiction of rodent chromosomal evolution; for review see Romanenko et al. (2012). However, wcp-based FISH fails to detect exact breakpoints and does not allow characterization of intrachromosomal rearrangements, such as inversions, deletions or duplications. These limitations can be easily overcome by FISH banding methods, such as multicolor banding (MCB) (Chudoba et al., 1999; Liehr et al., 2010). The first attempt to establish mouse multicolor banding probes was done by our group in 2002 (Karst et al., 2006). In 2003 another group also established a corresponding probe set for chromosome 11 (Benedek et al., 2004).

Here we present the first MCB sets for all 19 murine autosomes and the X-chromosome. All region-specific chromosome paints were obtained by glass-needle microdissection (Yang et al., 2010) followed by DOP-PCR (Telenius et al., 1992) with 6MW or anti-6MW primer (Rabbits et al., 1995), or followed by whole genome amplification using GenomePlex® Single Cell Whole Genome Amplification Kit (Sigma-Aldrich, USA). Murine chromosome spreads of different origin were used for microdissection: mouse embryonic and adult fibroblasts cultures, short-term cultivation of murine spleen tissue or somatic cell hybrids. All obtained preparations were checked for the number and morphology of chromosomes prior to dissection. Different strategies to reliably identify target chromosomes were applied: dissecting from mouse/human somatic cell hybrids harboring only one murine chromosome, each (Trifonov et al., 2005), FISH-microdissection method (Weimer et al., 2000) based on mouse wcp probes, and chromosome identification by size (applied for chromosomes 1 and 19). Murine wcp probes for FISH-microdissection were previously established in the Institute of Human Genetics (Jena) using chromosome microdissection technique from one single murine metaphase. 10 to 20 copies of the target region were dissected to generate each MCB DNA library. In total 115 region-specific partial chromosome painting (PCP) libraries were produced covering all 19 mouse autosomes and the X-chromosome. Each PCP probe was tested by reverse-FISH on control mouse metaphases, and assigned cytogenetically based on inverted DAPI bands. Three to eight (depending on chromosome's size) overlapping PCPs correspond to each chromosome. The changing fluorescence intensity ratios along the chromosomes were used by mBAND software (MetaSystems Hard & Software GmbH, Altlussheim, Germany) to assign different pseudocolors to specific chromosomal regions. The only mouse chromosome we were not able to establish a proper MCB probe set, was the Y-chromosome. Irrespectively of which chromosome segment was microdissected and amplified, the generated FISH probe resulted in homogeneous staining the entire Y-chromosome. This result might be explained by the fact that the ampliconic portion of the mouse Y has expanded enormously, taking up to 95 % of the chromosome (Alföldi, 2008). The organization of genes on mouse Y-chromosome is very different to the one on human Y-chromosome; i. e. in mouse most of the genes on Y-chromosome are located in the small pericentric region

(Bishop, Mitchell, 1999). Thus, this pericentric region might be just too small to be mechanically microdissected, so all of dissected DNA libraries contained repeat units which might got preferentially amplified during original amplification step.

We have applied the generated mcb probes to karyotypically characterize several cell lines. Some of the results have been already published before: mcb probes for chromosomes 3, 6, 18 and X were used to study in detail the chromosomal rearrangements previously found by wcp-based mFISH techniques in WMP2 cell line (Karst et al., 2006). Recently, we cytogenetically characterized the NIH3T3 cell line, which was used as a model system in thousands of studies since its first description in 1963. Surprisingly, detailed analysis of the NIH3T3 karyotype was never really done besides a GTG-banding analysis in 1989 (Kasid et al., 1989). Our MCB data allowed to describe previously uncharacterized chromosome markers, determine balanced and unbalanced translocations, inverted duplications, deletions or complex rearrangements and to characterize breakpoints on cytogenetical level (Leibiger et al., 2012).

Murine MCB approach found its application in evolutionary studies, as well. MCB probe sets for mouse chromosomes 3, 6, 18 and 19 were used to analyze karyotypes in 9 muroid species from subfamilies Murinae, Cricetinae, and Arvicolinae. The obtained results pointed out new segments of homology, helped to clarify the segment distribution and to describe the segment order in detail (Trifonov et al., 2010).

In spite of limitations of the mcb technique, such as lower resolution in comparison with BAC (Bacterial Artificial Chromosome)-FISH studies or sequencing, mcb provides an amazing opportunity to resolve a murine karyotype. Application of MCB technique has demonstrated a high potential of MCB probes in detecting intrachromosomal rearrangements and cryptic aberrations. Cytogenetic characterization using MCB libraries proved to be effective for both evolutionary studies and for characterization of laboratory models.

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МНОГОЦВЕТНЫЙ БЭНДИНГ ХРОМОСОМ МЫШИ

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Многоцветный бэндинг был впервые разработан только для хромосом человека. В настоящей работе мы сообщаем о разработке подобного набора для всех аутосом Х-хромосомы мыши и обсуждаем возможное применение полученных зондов.

К л ю ч е в ы е с л о в а: многоцветный бэндинг, аутосомы, Х-хромосомы мыши.