SHORT COMMUNICATION

A Mouse Chromosome-Specific YAC Probe Collection for *in Situ* Hybridization

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To facilitate the identification of mouse metaphase chromosomes by fluorescence *in situ* hybridization (FISH), a complete collection of mouse chromosome-specific markers has been established. Yeast artificial chromosome libraries were screened by polymerase chain reaction using primers for known loci. DNAs from positive clones were then tested by FISH. One probe per chromosome was selected on the basis of high specificity (nonchimerism) and strong fluorescence.

Fluorescence *in situ* hybridization (FISH) is a commonly used procedure for gene mapping. In mouse, the identification of metaphase chromosomes is rather difficult given the fact that mouse chromosomes are all telocentric and of rather similar size. To facilitate karyotyping, a complete collection of chromosome-specific markers cloned in yeast artificial chromosomes (YACs) was established. No specific marker was selected for the Y chromosome, which can be easily identified on the basis of its specific shape and size. Primers specific for selected loci (listed in Table 1) were designed and used to screen mouse YAC libraries by polymerase chain reaction (PCR). Positive clones were subsequently tested by FISH on metaphase chromosomes.

The PCRs were performed in a total volume of 50 μl containing 1.5 m*M* $MgCl_2$, 0.125 m*M* dNTP, 50 m*M* KCl, 10 m*M* Tris, pH 8.8, 0.1% Triton X-100, 50 ng of each primer, 1.5 units of *Taq* DNA polymerase (from Perkin–Elmer/Cetus), and 10 ng of template DNA. Reactions were performed in a Peltier effect thermal cycler (MJ Research) for 35 cycles (each cycle: 30 s at 94°C, 30 s at 55°C, 30 s at 72°C) with an initial denaturation of 3 min at 94°C and a final extension at 72°C for 3 min. PCRs were used to screen YACs from the two YAC libraries established by Larin *et al.* (5) and Kusumi *et al.* (4) at the Généthon screening center using a straightforward screening procedure based on sequential PCR screening of pooled material (2). This procedure made it possible to identify the YACs that correspond to any PCR primers selected using a limited number of PCR amplifications.

Positive YACs were analyzed by FISH performed on methanol-acid acetic fixed metaphases from C57BL/6JIco mouse. Total yeast DNA (250 ng), labeled by nick-translation with either biotin (BRL) or digoxigenin (Boehringer) modified nucleotides, was hybridized with 5 μg of mouse Cot-1 DNA (BRL) and 10 μg of salmon sperm DNA (Sigma). *In situ* hybridization was then performed as described (6). Chromosomes were coun-

TABLE 1

Summary of Selected Markers and Cytogenetic Positions Observed in This Work

Chromosome	Band	Marker	Genetic position	Reference
1	B	D1Mit18	29.7/112	(3)
2	A3–B	D2Mit80	9.0/120	(3)
3	A2–A3	D3Mit1	11.2/95	(3)
4	A4–C2	MUP	27.8/90	(1)
5	G	EPO	81.0/95	(1)
6	E	D6Mit55	49.5/80	(3)
7	D	Tyr	44.0/74	(1)
8	B3–C2	D8Mit25	32.0/78	(3)
9	A5–B	D9Jpk1	32.0/78	(9)
10	D	D10Mit14	63.0/78	(3)
11	B2–B4	Trp 53.1	39.0/85	(1)
12	D	D12Mit226	31.0/86	(3)
13	D1	D13Mit53	60.0/78	(3)
14	C2–D3	Ctla-1	20.5/69	(1)
15	E	D15Mit71	40.9/72	(3)
16	C3–C4	D16Mit7	44.0/72	(3)
17	A2–B	Tcr1	7.7/94	(1)
18	E1–E3	D18Mit9	42.0/60	(3)
19	D	D19Mit4	48.0/55	(3)
X	F4–F5	Amel	72.0/76	(8)

Note. Previously determined genetic map positions (10) are given in centimorgans from the centromere/total length of chromosome in centimorgans.

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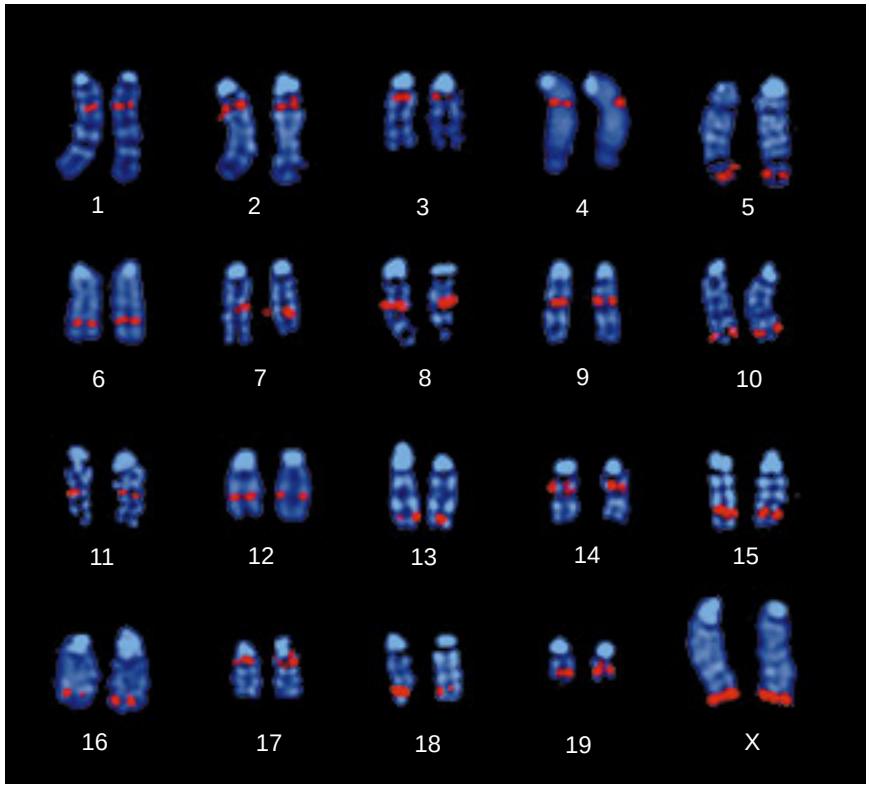

FIG. 1. Pseudokaryotype obtained by hybridization of selected YAC clones.

terstained with DAPI and imaged with a microscope equipped with a cooled CCD camera (Hamamatsu). To improve the DAPI G-banding, digital images were further processed using a deconvolution procedure (7); the sharpness of the resulting banding is amenable to karyotype analysis.

A total of 104 YAC clones were tested by FISH, 39 of which were isolated from the MIT library and 65 from the ICRF library. The percentage of chimerism was found to be analogous in both libraries: 41% (MIT) and 49% (ICRF). By nonchimeric, we mean a clone that gives no additional secondary signal. It is impossible, however, to exclude the possibility of chimerism involving a very short portion of the cloned sequence. Only markers of high specificity (nonchimerism) and good signal/noise ratio and for which chromosomal and genetic positioning were in good agreement were selected (Fig. 1) (10). We do not expect large variation in the chromosomal positions of these markers in standard laboratory strains since genetic distances seem similar.

Our results do not give precise information concerning the representativity of each library. In this study, 36 sets of primers pairs were used. For 8 sets of primers, PCR-positive YACs could be selected from only one of the two libraries.

Certain of the selected markers map to different portions of the chromosome. Depending on their position along the chromosome, the selected markers can be used in association and identified even when labeled with the same fluorochrome.

In conclusion, the use of these markers may be an alternative to the identification by chromosome painting and to labor-intensive chromosome banding techniques. One major advantage is that no competition between the markers and the probe to be mapped is expected. They should be useful for mouse genome mapping and analysis of transgene integration, especially with chromosomes showing a poor-quality banding. All requests concerning this set of markers should be addressed to the authors.

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REFERENCES

1. Abbott, C. (1992). Characterization of mouse–hamster somatic cell hybrids by PCR: A panel of mouse-specific primers for each chromosome. *Mamm. Genome* **2**(2): 106–109.
2. Chumakov, I., Rigault, P., Guillou, S., Ougen, P., Billaut, A., Guasconi, G., Gervy, P., LeGall, I., Soularue, P., Grinas, L., *et al.* (1992). Continuum of overlapping clones spanning the entire human chromosome 21q. *Nature* **359**(6394): 380–387.
3. Dietrich, W., Katz, H., Lincoln, S. E., Shin, H. S., Friedman, J., Dracopoli, N. C., and Lander, E. S. (1992). A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* **131**(2): 423–424.

4. Kusumi, K., Smith, J. S., Segre, J. A., Koos, D. S., and Lander, E. S. (1993). Construction of a large-insert yeast artificial chromosome library of the mouse genome. *Mamm. Genome* **4**(7): 391–392.
5. Larin, Z., Monaco, A. P., Meier-Ewert, S., and Lehrach, H. (1993). Construction and characterization of yeast artificial chromosome libraries from the mouse genome. *Methods Enzymol.* **225:** 623–633.
6. Lichter, P., Tang, C. J., Call, K., Hermanson, G., Evans, G. A., Housman, D., and Ward, D. C. (1990). High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* **247**(4938): 64–69.
7. Monier, K., Usson, Y., Mongelard, F., Szepetowski, P., Robert-Nicoud, M., and Vourc'h, C. (1996). Metaphase and interphase mapping by FISH: Improvement of chromosome banding and signal resolution in interphase nuclei by means of iterative deconvolution. *Cytogenet. Cell Genet.* **72:** 200–209.
8. Snead, M. L., Zeichner-David, M., Chandra, T., Robson, K. J., Woo, S. L., and Slavkin, H. C. (1983). Construction and identification of mouse amelogenin cDNA clones. *Proc. Natl. Acad. Sci. USA* **80**(23): 7254–7258.
9. Suda, T., Oyanagi, M., Wakana, S., Takarashi, Y., Kanada, H., Yonekawa, H., Miyashita, N., Shiroishi, T., Moriwaki, K., and Kominami, R. (1994). Novel mouse microsatellites: Primer sequences and chromosomal location. *DNA Res.* **1:** 169–174.
10. Mouse Genome Database (MGD) 3.1, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine. URL:http://www.informatics.jax.org/ (February, 1996).