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Evolutionary characterization of a Y chromosomal sequence conserved in the genus *Mus*

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Summary

The extent of accumulation of mouse *Y* chromosomal repetitive sequences generally correlates with the known phylogenetic relationships in the genus *Mus*. However, we describe here a *M. musculus* *Y* chromosomal repetitive sequence, designated as ACC1f1, whose accumulation patterns among eight *Mus* species do not correspond to their phylogenetic relationships. Although male-specific hybridization bands were present in all the species examined, significant accumulation ($\geqslant$ 200 copies) in the *Y* chromosomes was found in *M. minutoides* (subgenus *Nannomys*), *M. pahari* (subgenus *Coelomys*) and *M. saxicola* (subgenus *Pyromys*) as well as in the three closely related species *M. hortulanus*, *M. musculus* and *M. spretus* that belong to the subgenus *Mus*. Unexpectedly, the *Y* chromosomes of *M. caroli* and *M. cookii* (both subgenus *Mus*) had considerably reduced amounts of ACC1f1-related sequences. Furthermore, in rats (*Rattus norvegicus*) the major accumulation sites appear to be autosomal. These observations suggest that caution must be taken in the interpretation of data obtained with repetitive sequences that have evolved quickly.

1. Introduction

Recently several groups have isolated mouse (*Mus musculus*) *Y* chromosomal repetitive sequences (Bishop *et al.* 1985; Eicher *et al.* 1983; Lamar & Palmer, 1984; Nallaseth & Dewey 1986; Nallaseth *et al.* 1983; Nishioka & Lamothe, 1986, 1987a), some of which have been used as molecular probes to examine the phylogenetic relationships among *Mus* species (Nishioka & Lamothe, 1986, 1987a). These comparative studies demonstrated that the extent of accumulation of *Y* chromosomal repetitive sequences reflects the currently accepted phylogenetic relationships: namely, the male specific accumulation observed in *M. musculus* is also evident in species closely related to it such as *M. hortulanus* and *M. spretus*, whereas the *Y* chromosomes of distantly related species including *M. caroli*, *M. cookii*, *M. pahari* and *M. saxicola* show very weak hybridization.

From these results we concluded provisionally that (1) *Y* chromosomal repetitive sequences evolved quickly and (2) they appear to be useful molecular tools to estimate phylogenetic distances among *Mus* species. However, since neither mode nor rate of evolution of *Y* chromosomal repetitive sequences is well understood, the latter conclusion requires confirmation by extending the comparative work to other *Y* chromosomal sequences. We have isolated over 50 mouse (*M. musculus*) *Y* chromosomal sequences (Nishioka & Lamothe, 1987a). To date, 32 DNA fragments were generated from 11 original isolates and their conservation in the genus *Mus* has been studied. All fragments but one showed accumulation patterns similar to those obtained with the published clones. Here we describe the exceptional DNA fragment whose conservation patterns do not correspond to the known phylogenetic relationships among the *Mus* species examined.

2. Materials and methods

(i) *Animals*

Wild mice (*M. musculus musculus*, *M. m. domesticus*, *M. caroli*, *M. cookii*, *M. hortulanus*, *M. minutoides*, *M. pahari*, *M. saxicola* and *M. spretus*) were obtained from Litton Bionetics (Kensington, Maryland) through Dr M. Potter of the National Cancer Institute. Note that *M. saxicola* was previously described as *M. platythrix*. Descendants of house mice caught by Coppock, in Peru (Wallace, 1985) were gifts from Dr F. Biddle, University of Calgary. Inbred strains ABP/Le and CBA/FaCam were from Dr F. Biddle and 020 from Dr V. Chapman, Roswell Park Memorial Institute (Buffalo, New York). Other inbred strains (C57BL/6J and PERA/Ei) were

purchased from the Jackson Laboratory (Bar Harbor, Maine). Rats, hamsters and guinea pigs were purchased from Charles River Canada Inc. (St. Constant, Quebec).

(ii) *Isolation of ACC1*

Previously we reported the isolation of a 3·8 kb mouse *Y* chromosomal sequence designated as AC11 (Nishioka & Lamothe, 1986). Using AC11 as the probe, we isolated over 50 positive clones from a male mouse genomic library, two of which have been characterized (Nishioka & Lamothe, 1987a). Here we describe another clone designated as ACC1.

(iii) Southern blot analysis

High-molecular-weight DNAs were isolated from the liver as described by Maniatis *et al.* (1982), digested with *Eco*R1 and electrophoresed in a 0·9% agarose gel in a buffer consisting of 40 mM Tris (pH 7·5), 80 mM sodium acetate and 2 mM-EDTA. DNA fragments were transferred to membrane filters (Gene Screen, New England Nuclear Canada, Lachine, Quebec) by the method of Southern (1975). Hybridization conditions were as recommended by the supplier (NEN). Filters were then washed 3 times in 0·1 × SSC (1 × SSC is 0·15 N NaCl and 0·015 N Na-citrate) at 50 °C for 30 min each and exposed to Fuji RX films at −70 °C with Cronex intensifying screens.

(iv) *Estimation of copy number*

The approximate copy numbers of DNA fragments in the mouse genome were determined by dot–blot analysis as previously described (Nishioka & Lamothe, 1986).

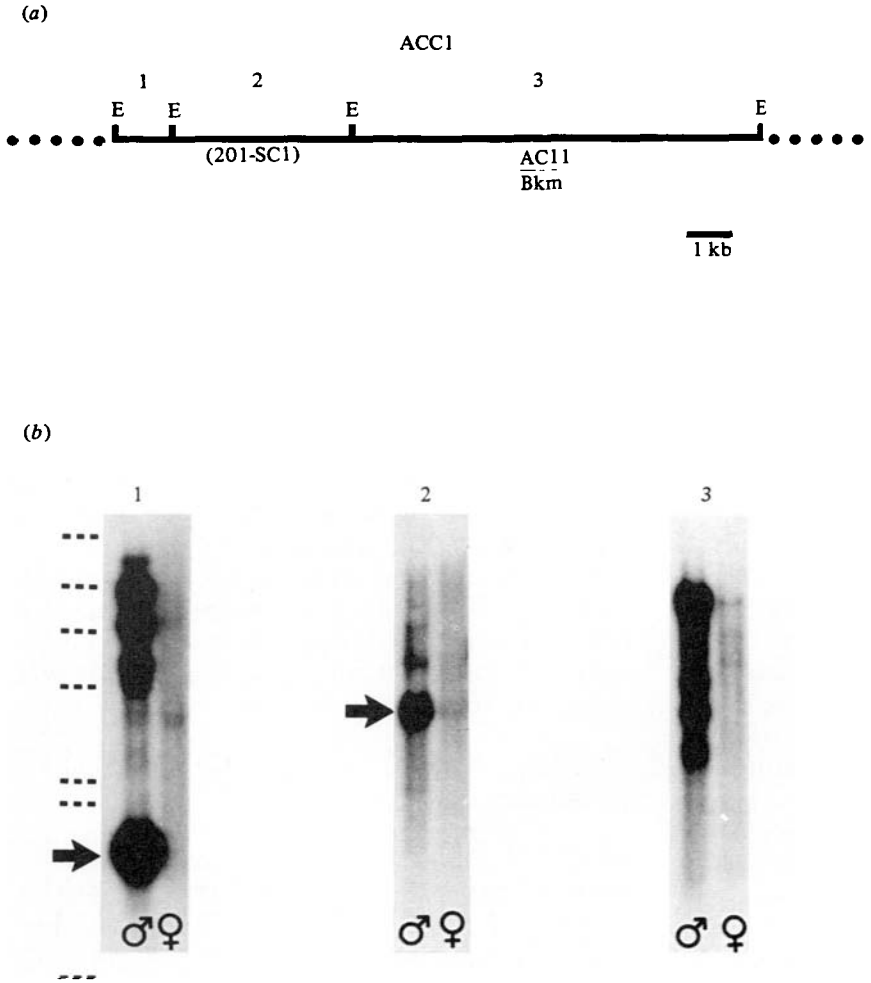

Fig. 1. Characterization of ACC1. (*a*) Restriction map of ACC1. Male BALB/cJ DNA was partially digested with *Hae*III and cloned into Charon 4A. The *Eco*R1 sites at the junctions represent the former *Hae*III sites converted to the *Eco*R1 sites by the addition of *Eco*R1 linkers. Fragment 2 cross-hybridizes to 201SC1 (to be described elsewhere). Fragment 3 contains both a Bkm-related and an AC11-related sequence. Dotted line: phage DNA; E, *Eco*R1 site. (*b*) Male-specific hybridization of fragments 1, 2 and 3. Fragments were purified from agarose gel, labelled with [^{32}P]dCTP and hybridized to male or female C57BL/6J DNA digested with *Eco*R1. *Panel 1* (fragment 1). The arrow indicates the 1·5 kb band. The size markers are, from top to bottom, 23·0, 9·6, 6·8, 4·3, 2·3, 2·0 and 0·5 kb. *Panel 2* (fragment 2). The arrow indicates the 3·8 kb band. *Panel 3* (fragment 3). Since this fragment contains a Bkm-related sequence, the male-specific bands become visible if hybridization is carried out in the presence of eukaryotic DNAs such as herring sperm DNA which effectively absorbs the Bkm-related sequence. In the presence of *E. coli* DNA both male and female DNAs produce smears (not shown).

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Results and discussion

The restriction map of ACC1 is presented in Fig. 1*a*. The insert is a 14·5 kb *Hae*III fragment cloned into Charon 4A (Blattner *et al.* 1977). The *Eco*R1 sites at the junctions represent the former *Hae*III sites which were converted to *Eco*R1 sites by the addition of *Eco*R1 linkers. Upon digestion with *Eco*R1, the insert generated 3 fragments: fragment 1 (1·2 kb), fragment 2 (3·8 kb) and fragment 3 (9·5 kb). Fragment 3 hybridized strongly to AC11 and contained a Bkm-related sequence (Singh *et al.* 1980) (data not shown). Fragment 2 defined a cognate 3·8 kb band in the male lane and cross-hybridized to 201SC1 (to be described elsewhere) (Fig. 1*b*). Fragment 1 did not hybridize to either fragment 2 or fragment 3 and defined several male-specific bands ranging from 1·5 to 16·0 kb, with

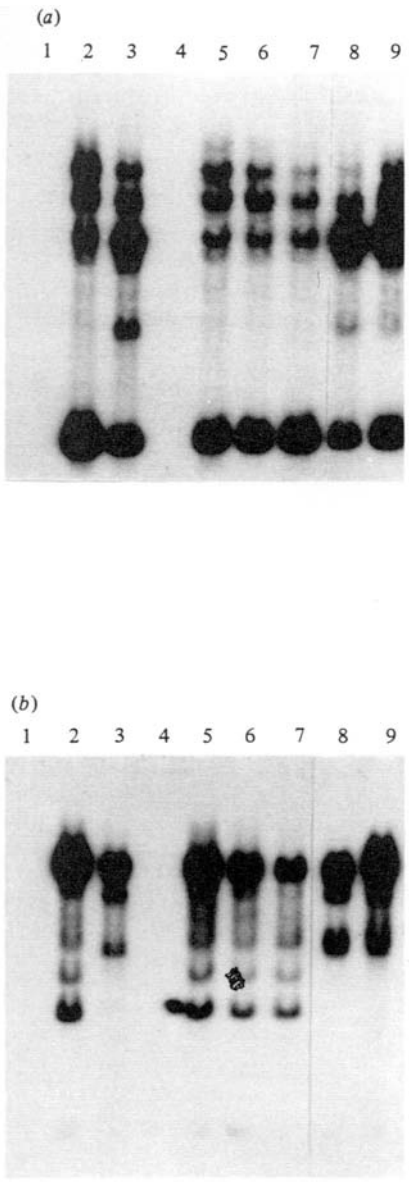

Fig. 2. *Y* chromosomal polymorphism in inbred strains. (*a*) DNAs were isolated from 5 inbred strains as well as authentic *M. m. musculus* and *M. m. domesticus*, disgested with *Eco*R1 and probed with ACC1f1. 1, *M. m. musculus* (female); 2, *M. m. musculus* (male); 3, *M. m. domesticus* (male); 4, *M. m. domesticus* (female); 5, ABP/Le (male); 6, 020 (male); 7, CBA/FaCam (male); 8, PERA/Ei (male); 9, Peru-Coppock (male). (*b*) The filter was stripped of the probe (ACC1f1) and rehybridized to AC11 (Nishioka & Lamothe, 1986). Lanes, 2, 5, 6 and 7 show *M. m. musculus*-type hybridization patterns, while lanes 3, 8 and 9 are *M. m. domesticus* type.

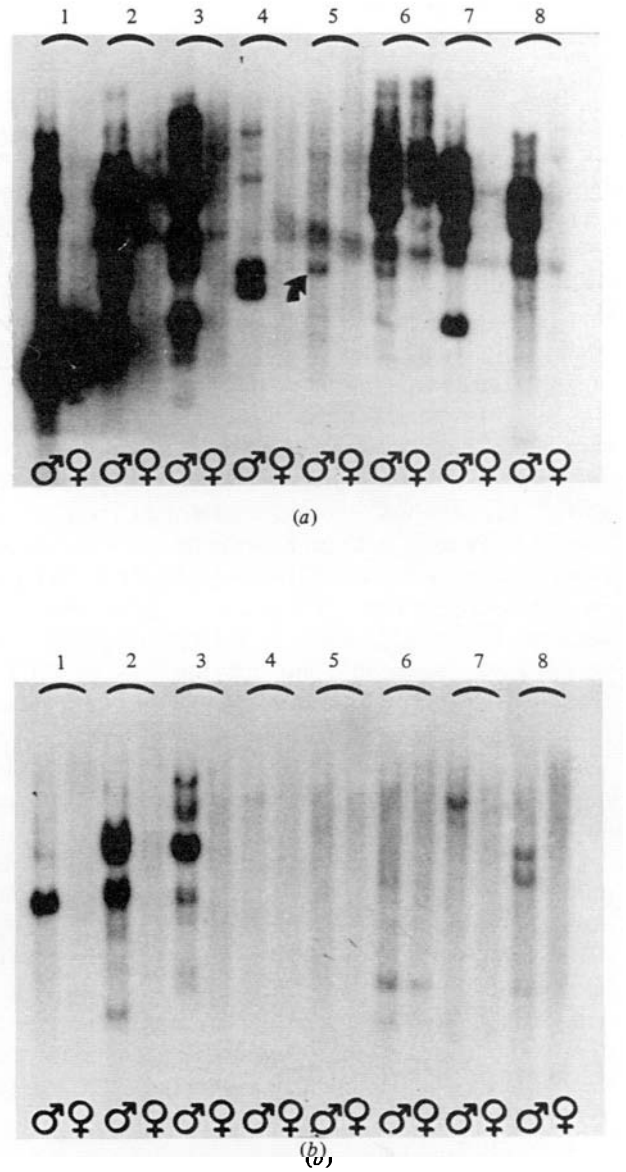

Fig. 3. Phylogenetic conservation of ACC1f1-related sequences in the genus *Mus*. (*a*) DNAs were isolated from 8 mouse species, digested with *Eco*R1 and probed with ACC1f1. Approximate copy numbers are indicated in parentheses. 1, *M. musculus* (*musculus*) (200); 2, *M. hortulanus* (300) (also known as *M. spicilegus*); 3, *M. spretus* (300); 4, *M. caroli* (20); 5, *M. cookii* (2); 6, *M. pahari* (200); 7, *M. saxicola* (200); 8, *M. minutoides* (150). The arrow indicates a male-specific band faintly seen in *M. cookii*. (*b*) The filter was stripped of the probe (ACC1f1) and rehybridized to fragment 2 of ACC1. Note that classification of the genus *Mus* is still in debate. Marshall (1986) subdivided it into 4 subgenera; *Coelomys*, *Pyromys*, *Nannomys* and *Mus* and classified *M. pahari*, *M. saxicola* and *M. minutoides* under subgenera *Coelomys*, *Pyromys* and *Nannomys*, respectively, while Bonhomme (1986) has proposed that *Coelomys*, *Pyromys* and *Nannomys* be considered as independent genera.

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a major hybridization band at 1·5 kb. Since this hybridization pattern is remarkably similar to that of pY353/B (Bishop & Hatat, 1987), it is likely that fragment 1 (herein referred to as ACC1f1) represents a member of the pY353/B-related sequence family. In fact, both sequences agree in copy number (> 200/*Y* chromosome), absence in XX *sxr* (sex-reversed) mice and presence of transcripts in the testis (data not shown), supporting further the possibility that ACC1f1 and pY353/B are virtually identical.

Some *Y* chromosomal sequences differentiate between the *Y* chromosomes of *M. m. musculus* and *M. m. domesticus* (Bishop *et al.* 1985; Lamar & Palmer, 1984; Nishioka & Lamothe, 1986) and over 40 inbred strains were classified into these two groups (Nishioka, 1987; Nishioka & Lamothe, 1987b). ACC1f1 also distinguishes between the *M. m. musculus* and *M. m. domesticus* types of *Y* chromosomes as shown in Fig. 2*a*. The *M. m. musculus*-type *Y* chromosome was present in ABP/Le, 020 and CBA/FaCam. In America, there were no aboriginal house mice and the predominant species found today is *M. m. domesticus* (Sage, 1981). As expected, PERA/Ei, an inbred strain established from mice caught by Atteck, in Peru, had the *M. m. domesticus*-type *Y* chromosome as did descendants of mice caught by Coppock, in Peru (Wallace, 1985). In order to confirm the results with ACC1f1, the filter was stripped of the probe and rehybridized to AC11 (Nishioka & Lamothe, 1986). As shown in Fig. 2*b*, the results were exactly as expected.

Previously we studied the evolutionary conservation of three *M. musculus Y* chromosomal repetitive sequences in seven *Mus* species (*M. caroli*, *M. cookii*, *M. hortulanus*, *M. musculus*, *M. pahari*, *M. saxicola* and *M. spretus*) and found the male-specific accumulation only in *M. hortulanus*, *M. musculus* and *M. spretus* (Nishioka & Lamothe, 1986, 1987). Subsequently, we extended the comparative study to over 30 *M. musculus Y* chromosomal sequences and obtained similar hybridization patterns; namely the accumulation in the *Y* chromosome was limited to these three closely related species (Y. Nishioka, unpublished results). Recently Tucker *et al.* (1987) applied another *M. musculus Y* chromosomal repetitive sequence (pYB10) to a variety of *Mus* species and observed male specific hybrizidation only in *M. musculus* and its closely related congeners. These results are in agreement with the currently accepted phylogenetic relationships among *Mus* species and we gained the impression that *Y* chromosomal repetitive sequences are useful molecular tools to understand genome evolution and phylogenetic relationships.

Since ACC1f1-related sequences are transcribed, their conservation patterns might be different from the sequences already examined and thus we carried out Southern blot analysis against DNAs isolated from the eight *Mus* species, *M. caroli*, *M. cookii*, *M. hortulanus*, *M. minutoides*, *M. musculus*, *M. pahari*, *M. saxicola* and *M. spretus*. All species examined showed male specific hybridization to ACC1f1 (Fig. 3*a*). However, the extent of accumulation in the *Y* chromosome did not correspond to their relatedness to *M. musculus*. Significant accumulation was observed in the distantly related species, *M. minutoides*, *M. pahari* and *M. saxicola* as well as in the closely related species, *M. hortulanus* and *M. spretus*. Unexpectedly, *M. caroli* and *M. cookii*, both of which are more closely related to *M. musculus* than are *M. minutoides*, *M. pahari* and *M. saxicola* showed little accumulation of ACC1f1-related sequences in their *Y* chromosomes. Dot–blot analysis indicated that about 20 and 1–3 copies of ACC1f1-related sequences are present in the *Y* chromosomes of *M. caroli* and *M. cookii*, respectively, compared with about 200 or more copies in the other species. These results are summarized in Fig. 4. As a control, the filter was stripped of ACC1f1 and rehybridized to fragment 2 of ACC1. As shown in Fig. 3*b*, the adjacent DNA fragment showed a typical accumulation pattern of *M. musculus Y* chromosomal repetitive sequences; namely significant accumulation was observed only in *M. hortulanus*, *M. musculus* and *M. spretus*.

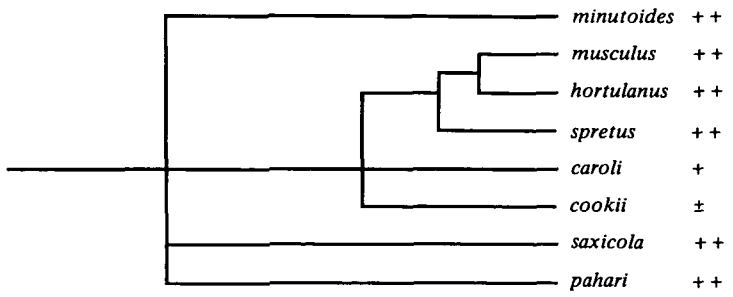

Fig. 4. Phylogenetic relationships among *Mus* species and accumulation of ACC1f1 related sequences. This dendrogram is reconstructed from Bonhomme (1986), Bonhomme *et al.* (1984) and Hammer & Wilson (1987). For the quantitative differences among the species, see Bonhomme *et al.* (1984). According to the estimates made by Bonhomme *et al.* (1984), Hammer & Wilson (1987), Martin *et al.* (1985) and Moriwaki *et al.* (1982), the approximate divergence times for these species are $10–12 \times 10^6$ years for *M. saxicola* and *M. musculus*, $3–4 \times 10^6$ years for *M. spretus* and *M. musculus* and $2–3 \times 10^6$ years for *M. hortulanus* and *M. musculus*. ++, $\geqslant$ 150 copies/*Y* chromosome; +, 10–100 copies/*Y* chromosome; ±, < 10 copies/*Y* chromosome.

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Next we extended the survey to rat, hamster, guinea pig and human DNAs, among which only the rat DNA hybridized to ACC1f1 (Fig. 5). There were at least 7 well-defined bands (ranging from 1·7 to 15·0 kb) and most of them, if not all, appear to be autosomal. Comparing the intensity of hybridization to that obtained with *M. caroli*, we estimated that ACC1f1-related sequences were repeated about 50↖75 times in the rat genome.

From these observations, we conclude that ACC1f1-related sequences evolved in quite distinct ways in the family Muridae. In *Mus*, they amplified in the *Y* chromosome, while in *Rattus* the major amplification sites are autosomal and/or pseudo-autosomal (*X–Y* pairing region). Two possibilities can account for the atypical accumulation patterns observed in the genus *Mus*; (1) lineage specific amplification during speciation and (2) amplification prior to speciation, followed by elimination in the lineages that led to *M. caroli* and *M. cookii*. The latter possibility is viable, if amplification had occurred without dispersion, because then a subsequent deletion in the *Y* chromosome could have effectively reduced the copy number. It has been shown that a *M. musculus* major satellite sequence is poorly represented in the genome of *M. spretus* (Brown & Dover, 1980). ACC1f1 provides another good illustration of the instability of certain repetitive sequences whose application to phylogenetic studies must be treated with caution. A study on the structure and organization of ACC1f1-related sequences in the *Y* chromosome is in progress.

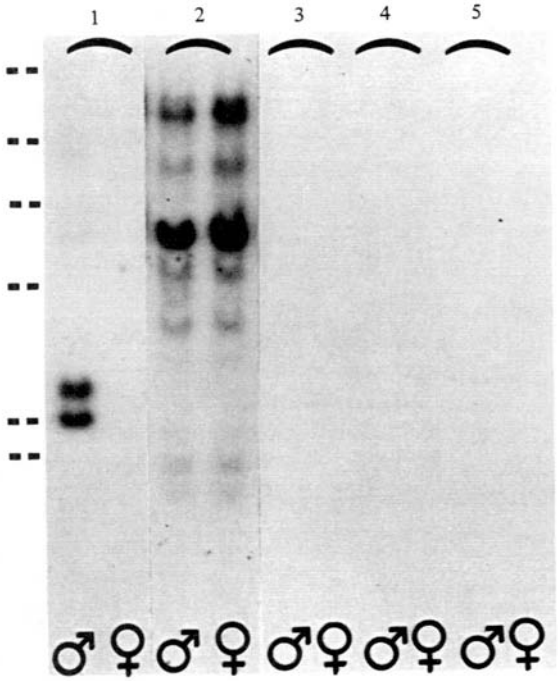

Fig. 5. Cross-hybridization of ACC1f1-related sequences to rat DNA. DNAs were isolated from rat (*Rattus norvegicus*), hamster (*Mesocricetus auratus*), guinea pig (*Cavia porcellus*) and human being (*Homo sapiens*), digested with *Eco*R1 and probed with ACC1f1. *M. caroli* is included as the control. 1, *M. caroli* 2, rat; 3, hamster; 4, guinea pig; 5, human being.

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