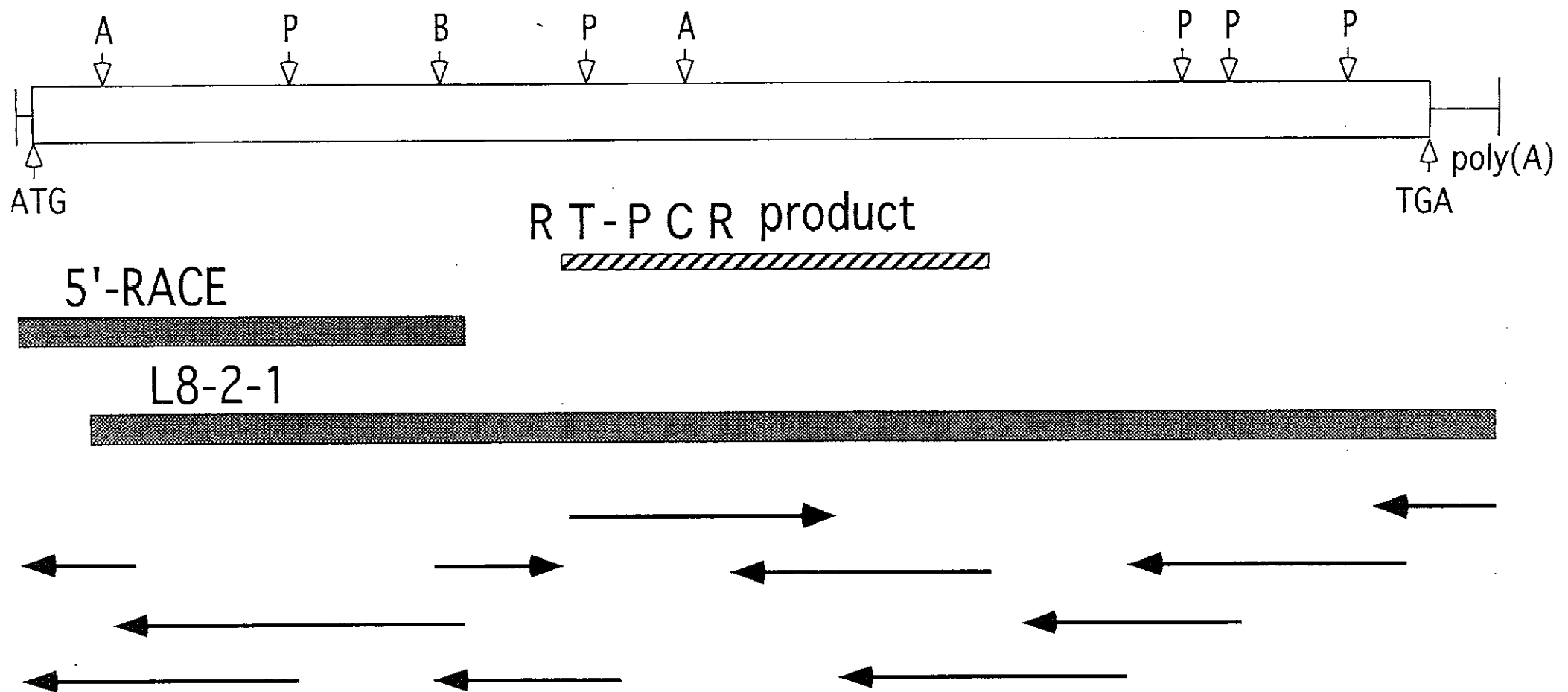


**Fig. 1. The contents of mRNA for the MAN2B2 homologue were analyzed by RT-PCR method.**

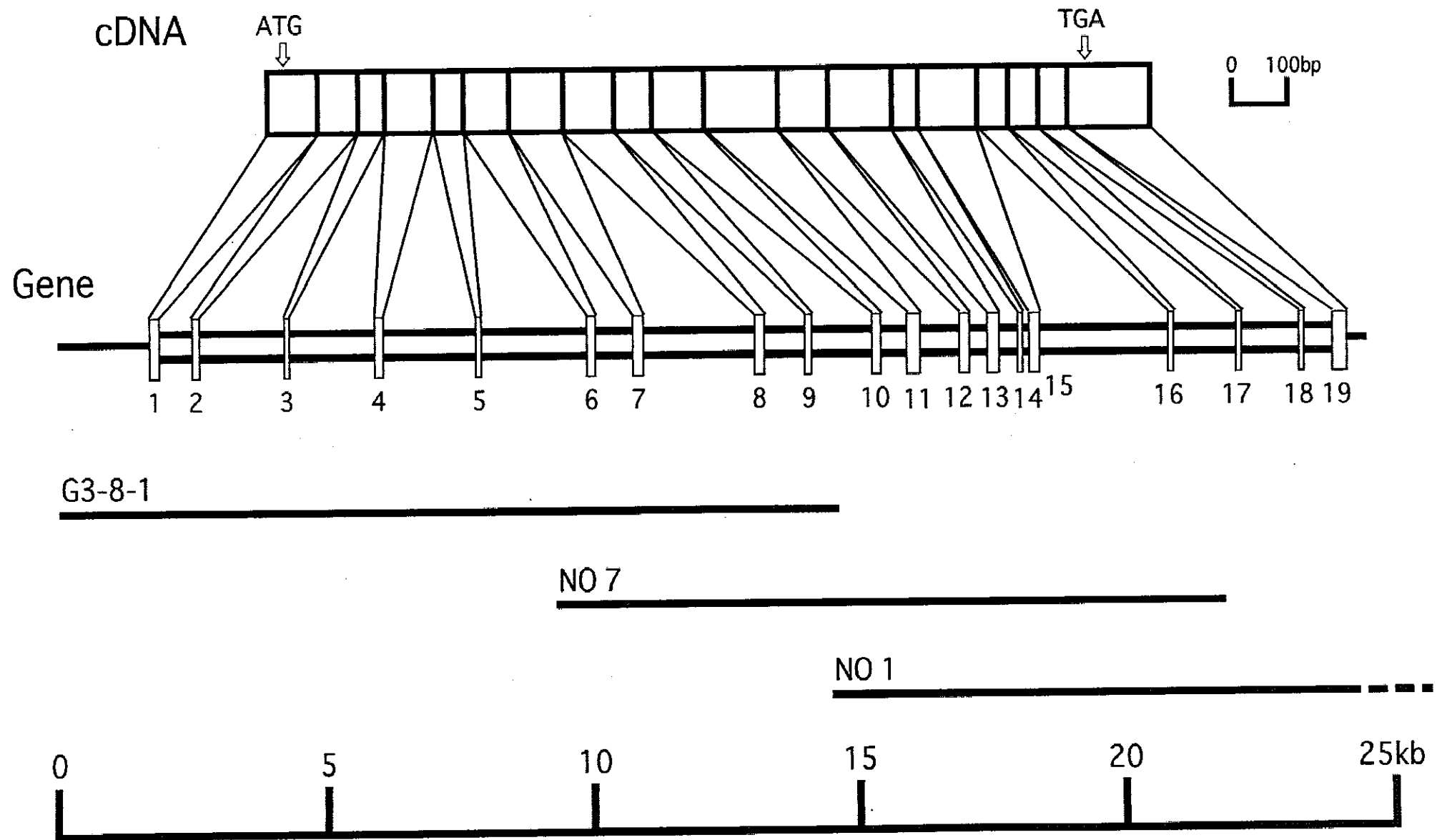
(a) 5  $\mu$ g of total RNAs prepared from the porcine testis (PTes), caput, corpus or cauda epididymis (PE). (b) 0.2  $\mu$ g of mRNAs prepared from the mouse testis (MTes), caput, corpus or cauda epididymis (ME).



**Fig. 2 Partial restriction map of cDNA coding the mMAN2B2 and the sequencing strategy.** Clone L8-2-1, RT-PCR product and 5'-RACE product were sequenced. The coding portion is shown by box. A:Acc I, B:BamH I, P:Pst I.

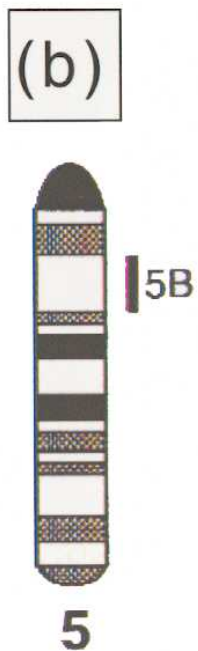




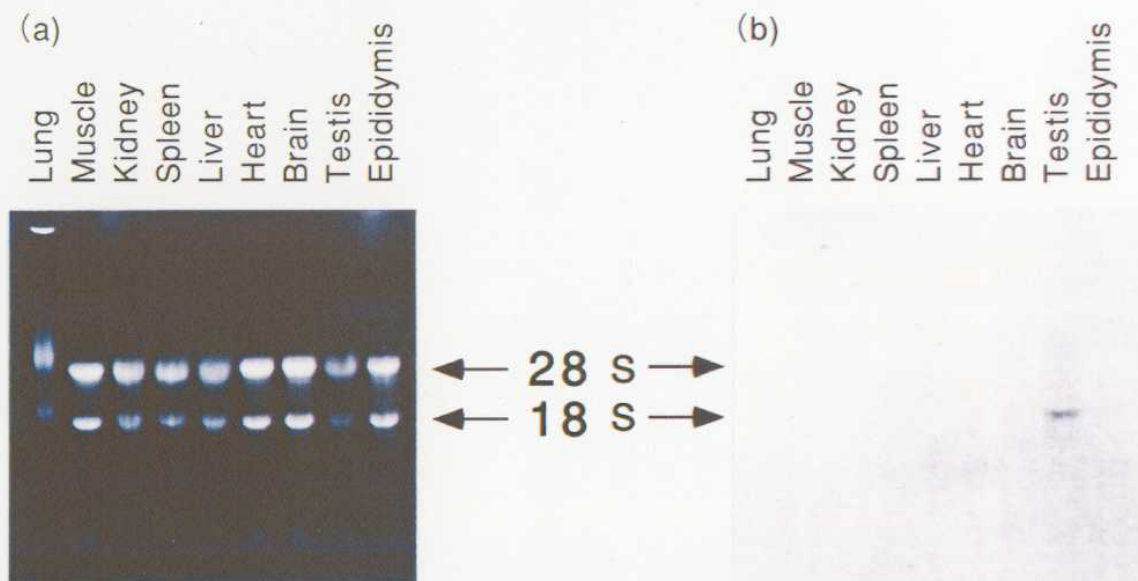


**Fig. 5 Organization of the mMAN2B2 gene.** We isolated and characterized three overlapping phage clones covering the entire length of the MAN2B2 gene and its immediate 5' and 3' flanking sequences. The MAN2B2 gene is consisted of 19 exons.

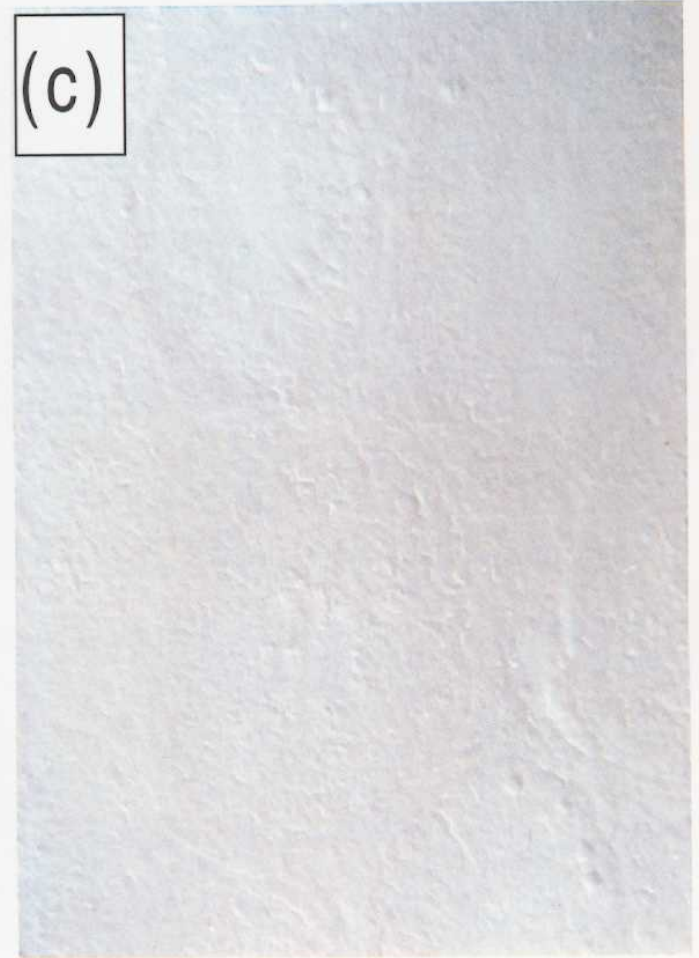
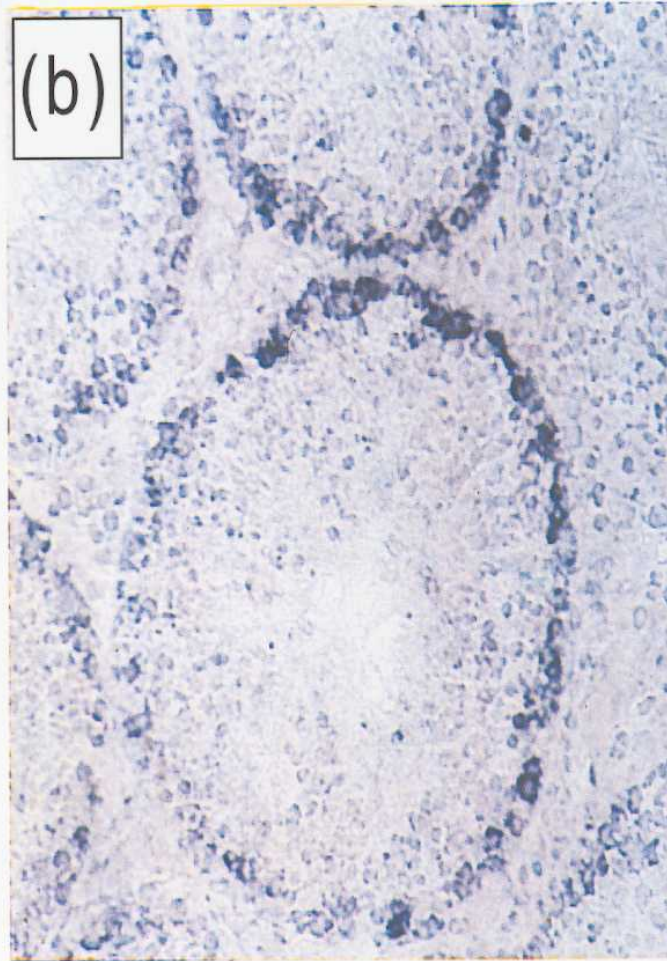




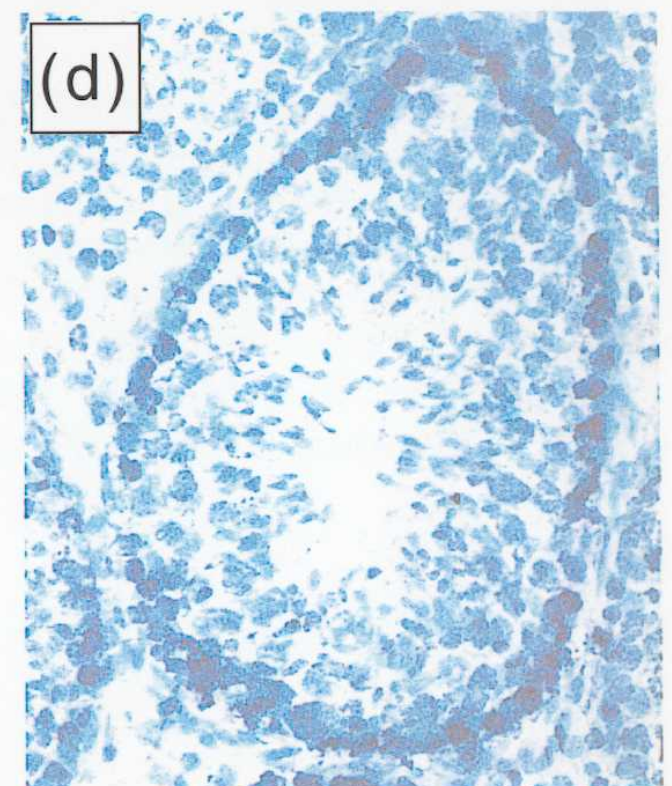
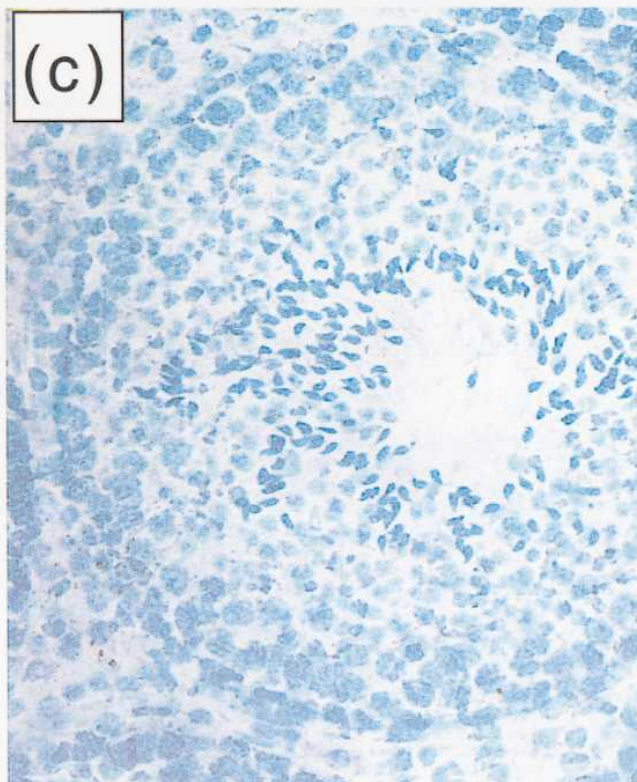
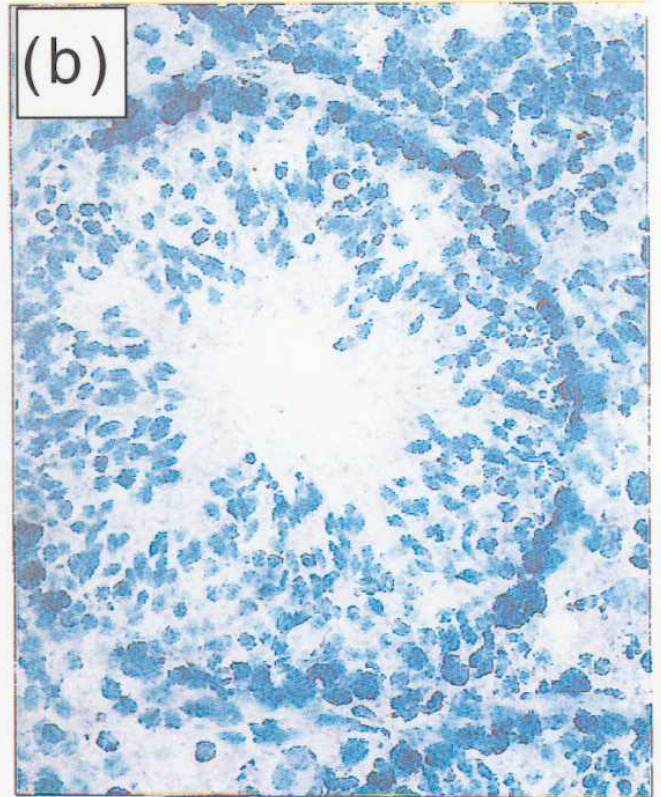
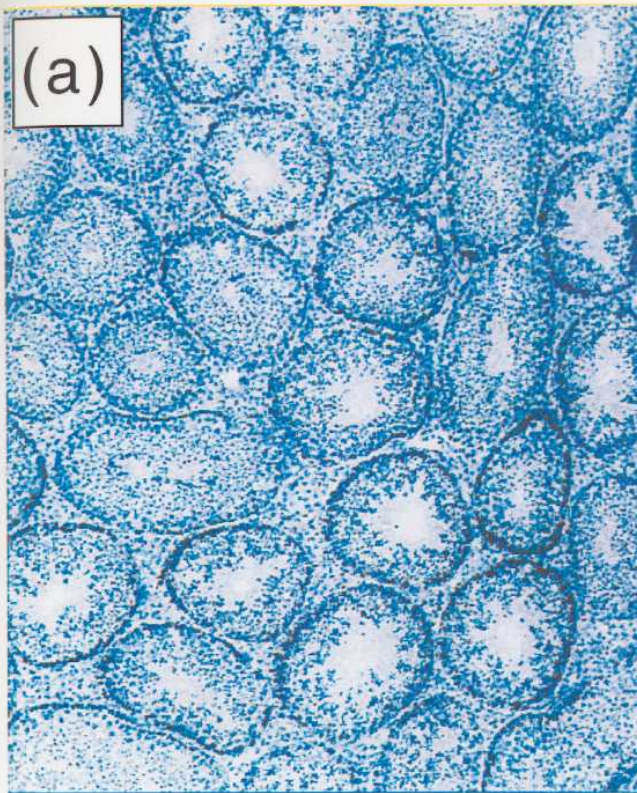
**Fig. 7. Assignment of the mMAN2B2 gene to chromosome5 by the fluorescence *in situ* hybridization.** (a) A representative chromosome spread displaying fluorescence signals. The arrows indicate the position of the mMAN2B2 gene on chromosome5. (b) The assignment of the mMAN2B2 gene to 5ChrB.



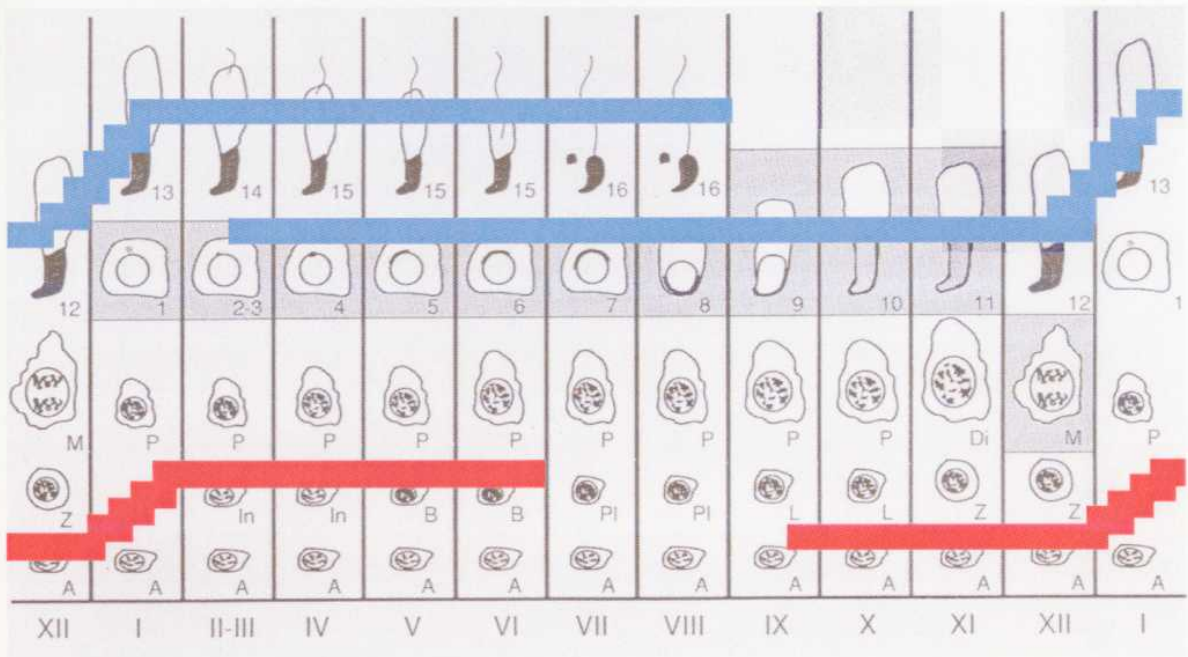
**Fig. 8. Distribution of the mMAN2B2 mRNA in various mouse tissues.** The total RNAs( 5 $\mu$ g each ) prepared from mouse lung, skeletal muscle, kidney, spleen, liver, heart, brain, testis, or epididymis were electrophoresed (a) and transferred to nylon membrane and were probed with DIG-labeled oligonucleotides synthesized according to the sequence of the mouse MAN2B2 homologue cDNA (b).



**Fig. 9.** *In situ* localization of the mMAN2B2 mRNA in mouse testis. Frozen sections of mouse testis were hybridized *in situ* with DIG-labeled anti-sense (a) and (b) or sense (c) probe. Magnification : (a) and (c), x 31.25; (b), x 62.5.

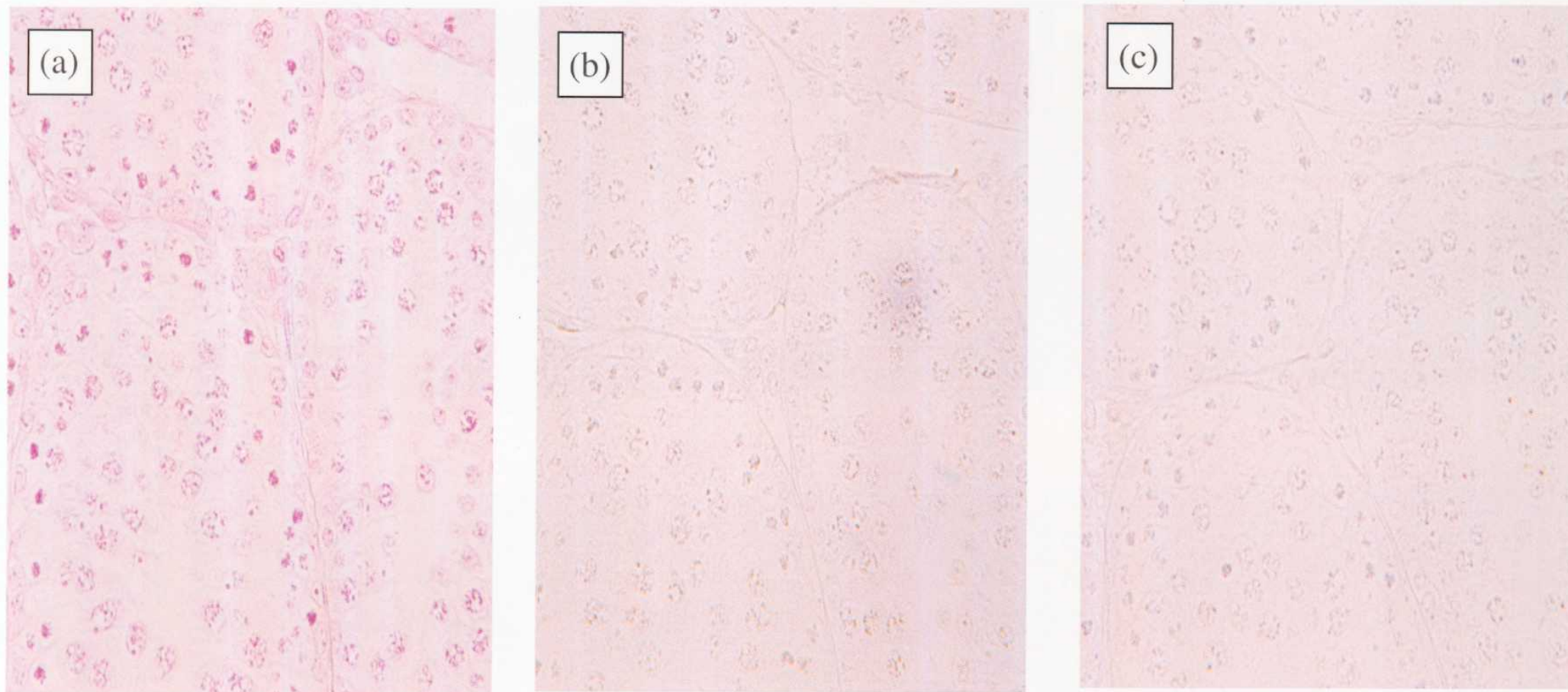


**Fig. 10. Determination of stages of the seminiferous epithelium expressing the mMAN2B2 mRNA.** The sections used for *in situ* hybridization were counterstained by methyl green. The stages of the seminiferous epithelium in (b), (c), and (d) are X~XI, VII~VIII, and I~VI, respectively. Magnification : (a), x 20; (b)~(d), x 200.

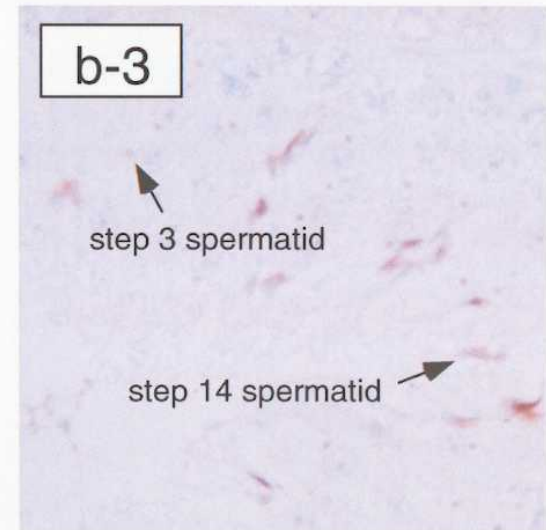
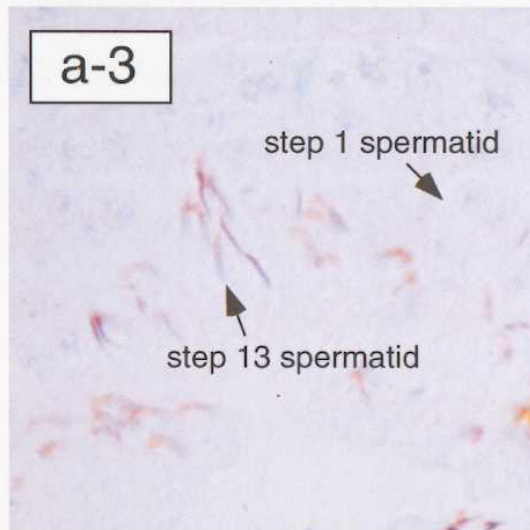
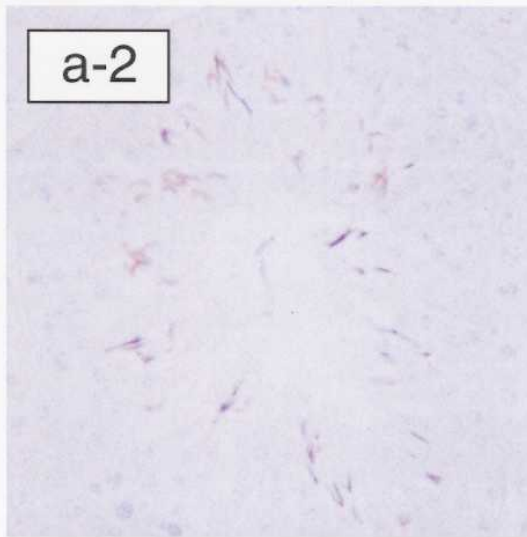
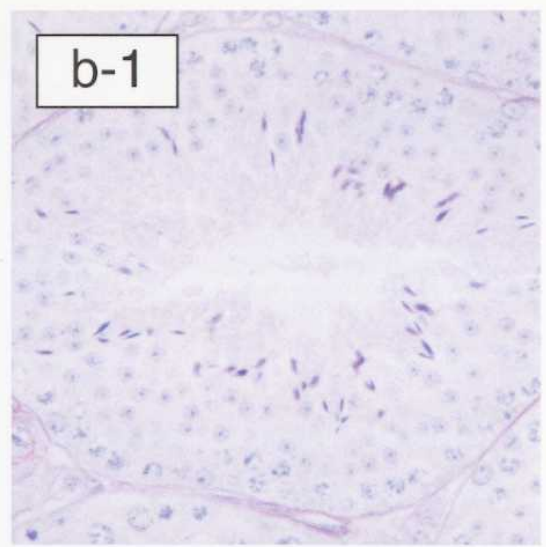
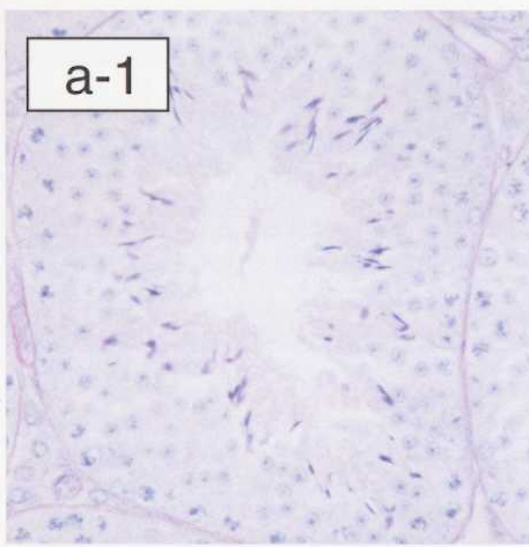


**Fig. 11. The cycle of spermatogenesis in mouse.**

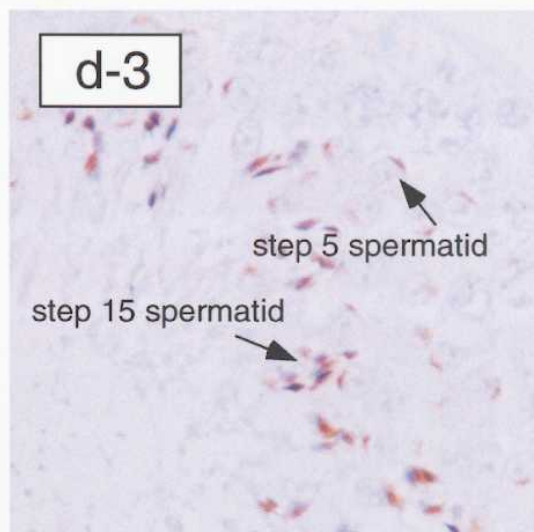
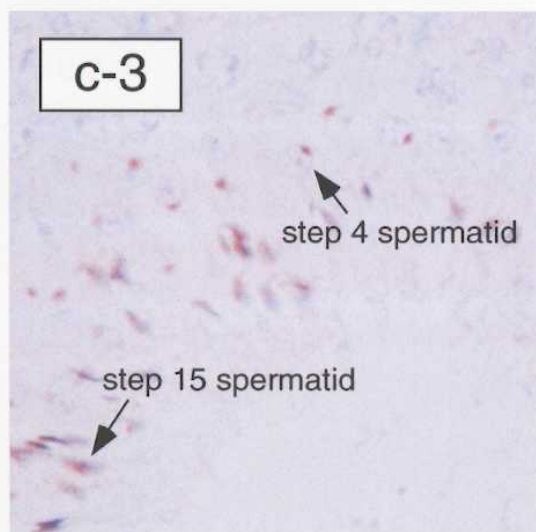
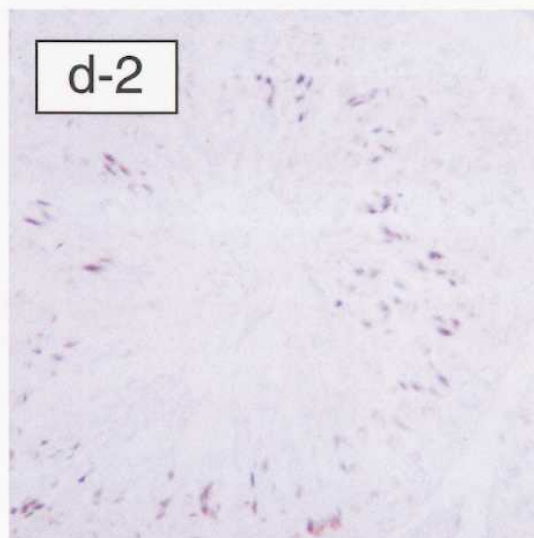
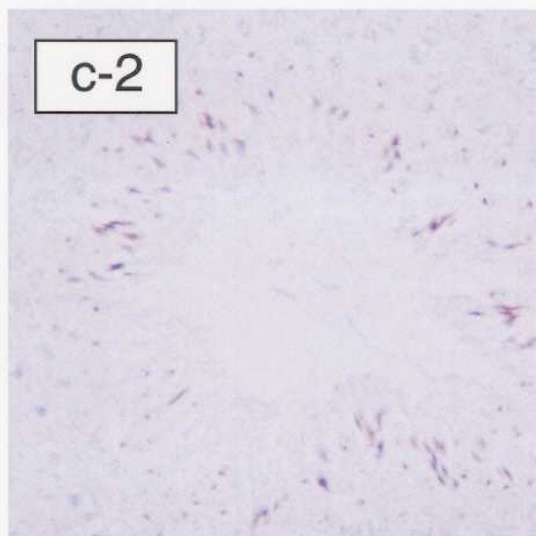
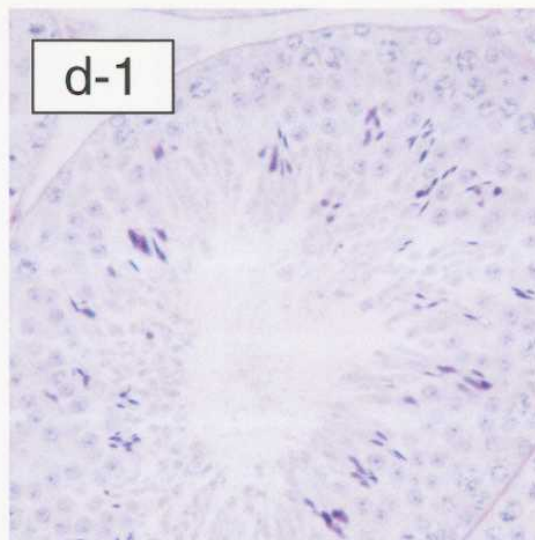
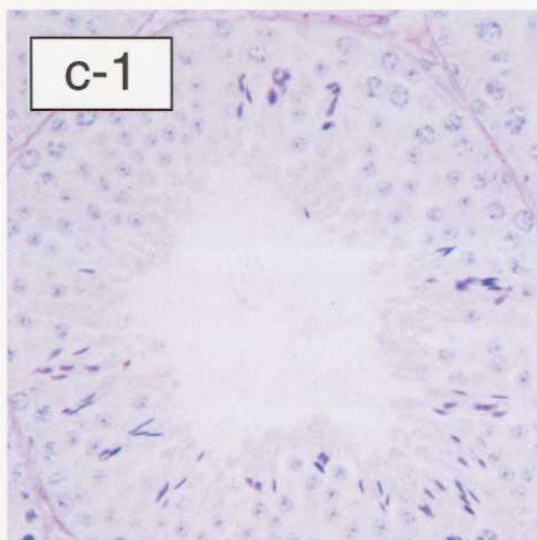
The cycle of spermatogenesis is divided into 12 stages. A: Type A spermatogonia, In: Intermediate spermatogonia, B: Type B spermatogonia, Pl: Preleptotene primary spermatocytes, L: Leptotene primary spermatocytes, Z: Zygotene primary spermatocytes, P: Pachytene primary spermatocytes, Di: Diplotene primary spermatocytes, M: Meiosis, 1~16: steps of the development spermatids (Takahashi;1994). The mMAN2B2 mRNA is present in the cells indicated by ■, stages lateIX~VI. The mMAN2B2 protein is present in the cells indicated by ■

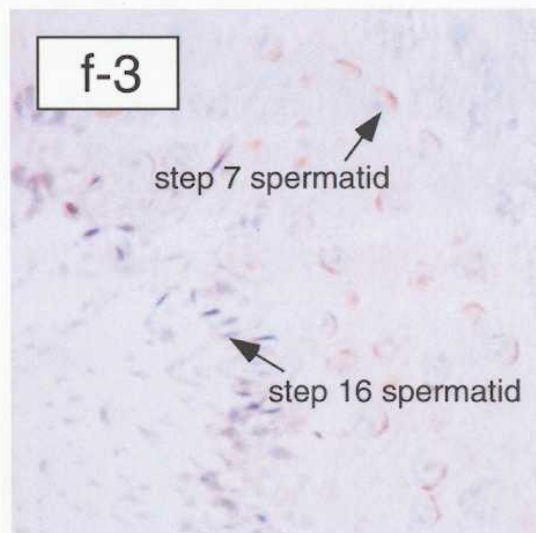
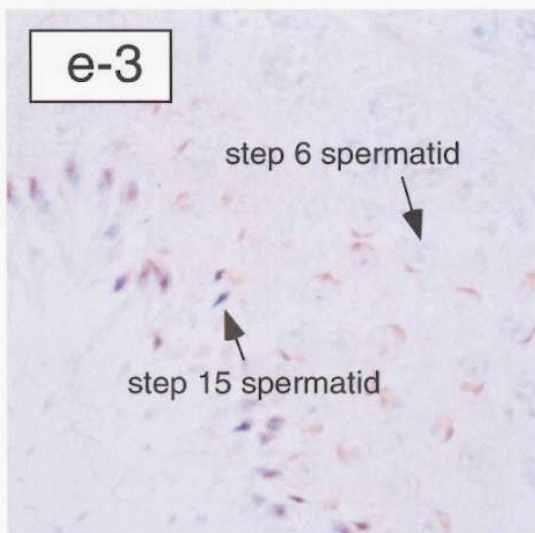
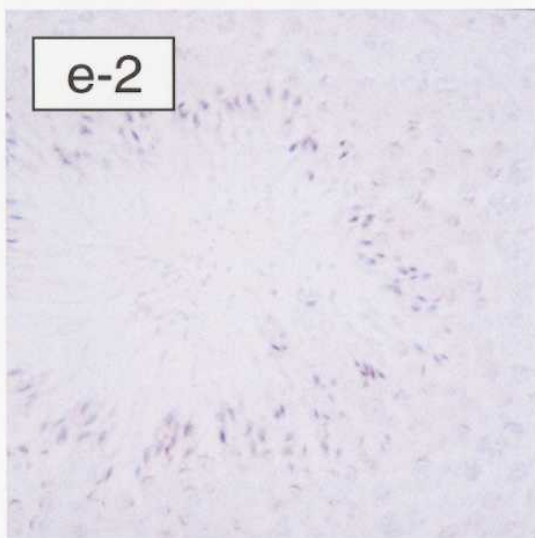
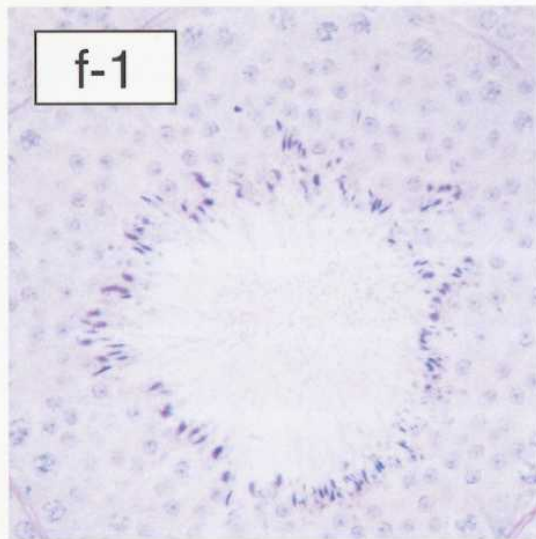


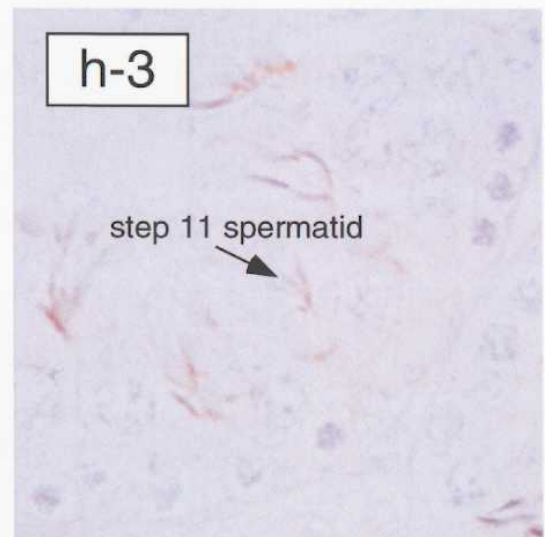
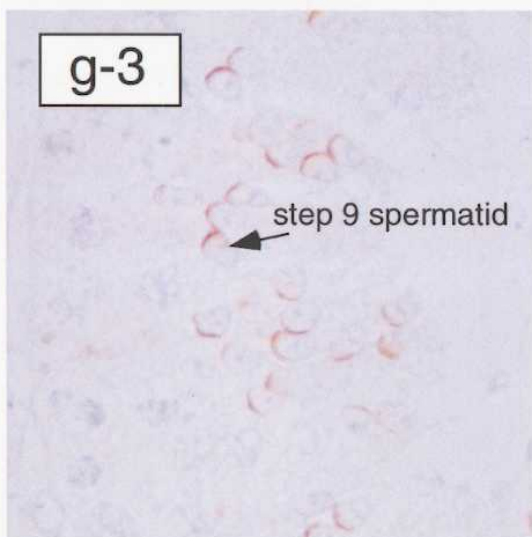
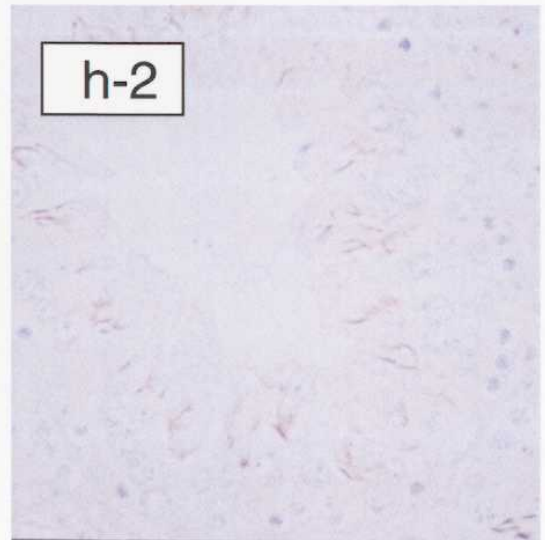
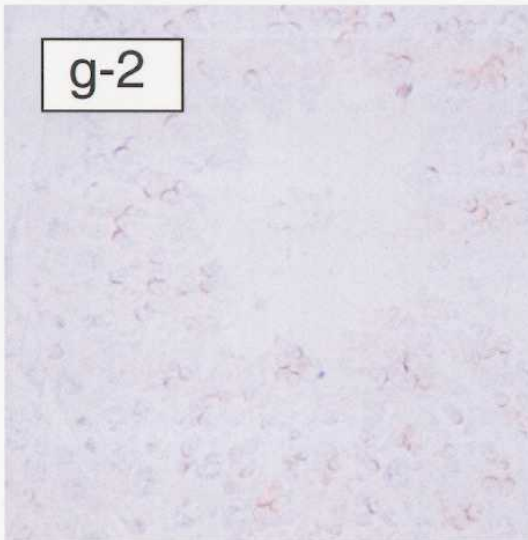
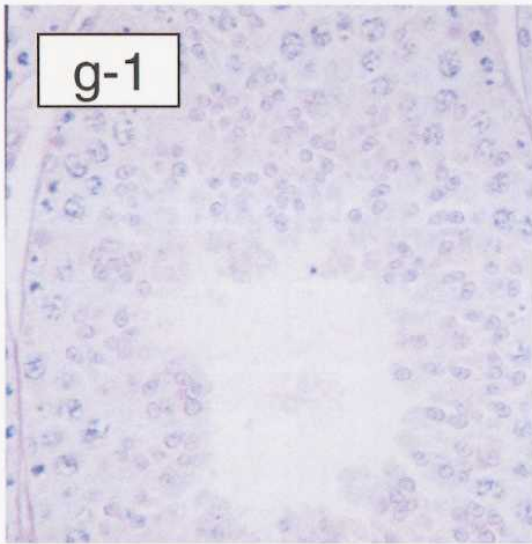
**Fig. 12. Immunohistochemical localization of the mMAN2B2 into Day 20 mouse testis.** The sections were stained with hematoxylin and eosin (a), The sections were incubated with monospecific antibody against mMAN2B2 (b) or pre-immune serum (c). Magnification ; (a), (b) and (c),  $\times 200$ .

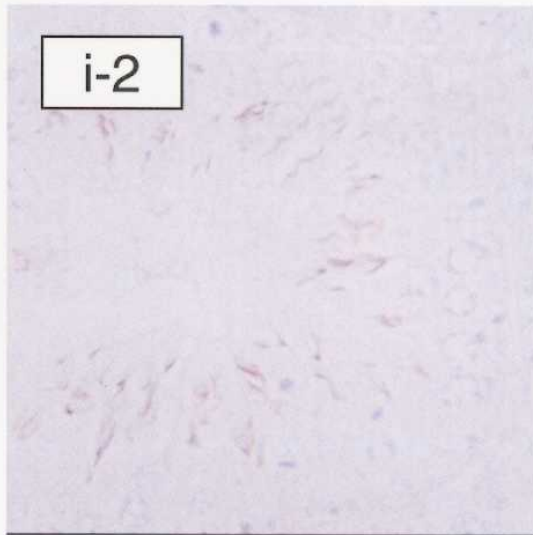
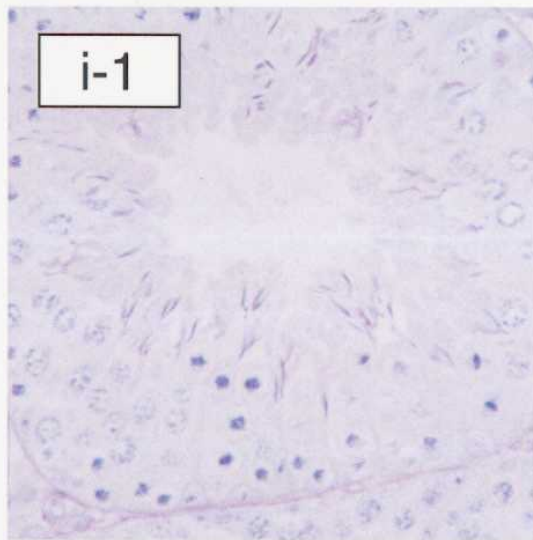


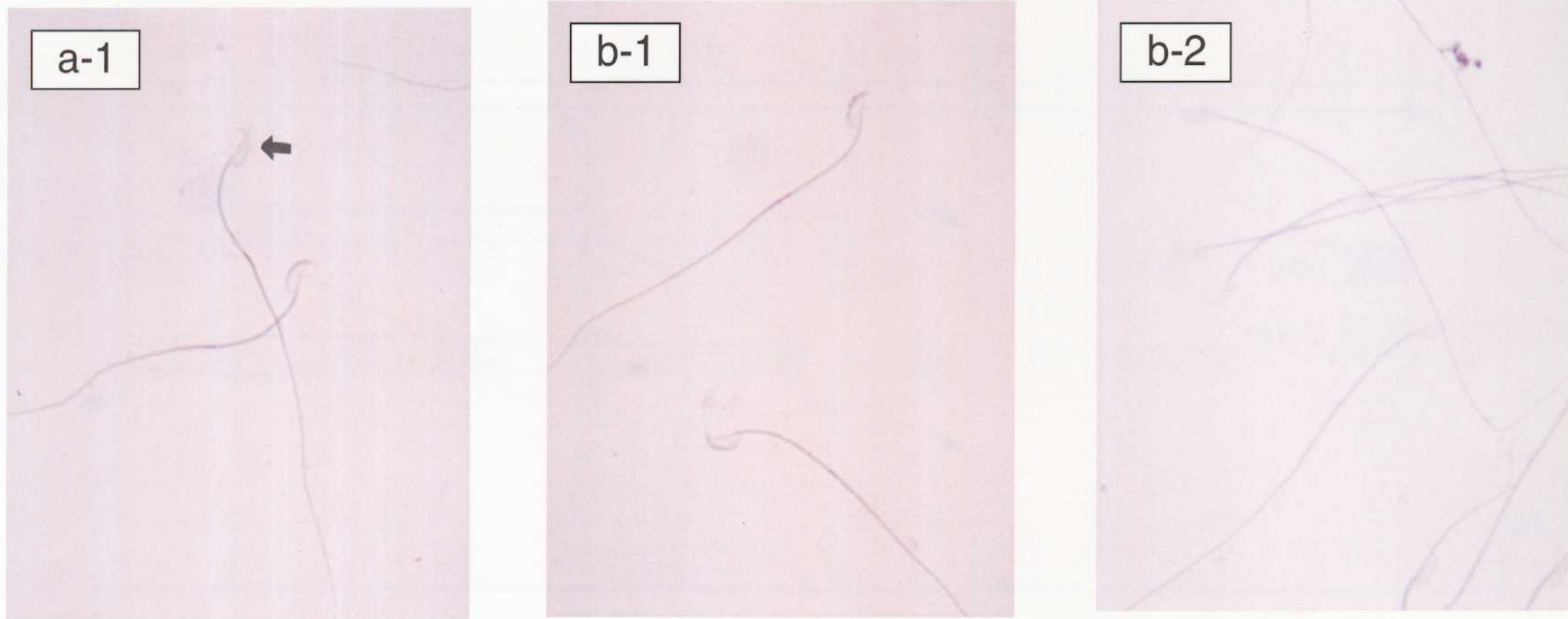
**Fig. 13. Immunohistochemical localization of mMAN2B2 in the adult mouse testis.** -1; Determination of the mouse spermatogenesis by PAS stain. -2; and -3; DAB-peroxidase immuno-staining of testis counterstained with hematoxylin. (a), (b), (c), (d), (e), (f), (g), (h) and (i) are represented stage I, II ~ III, IV, V, VI, VII, IX, XI and XII, respectively. Magnification : (1) and (2),  $\times 200$  ; (3),  $\times 400$ .



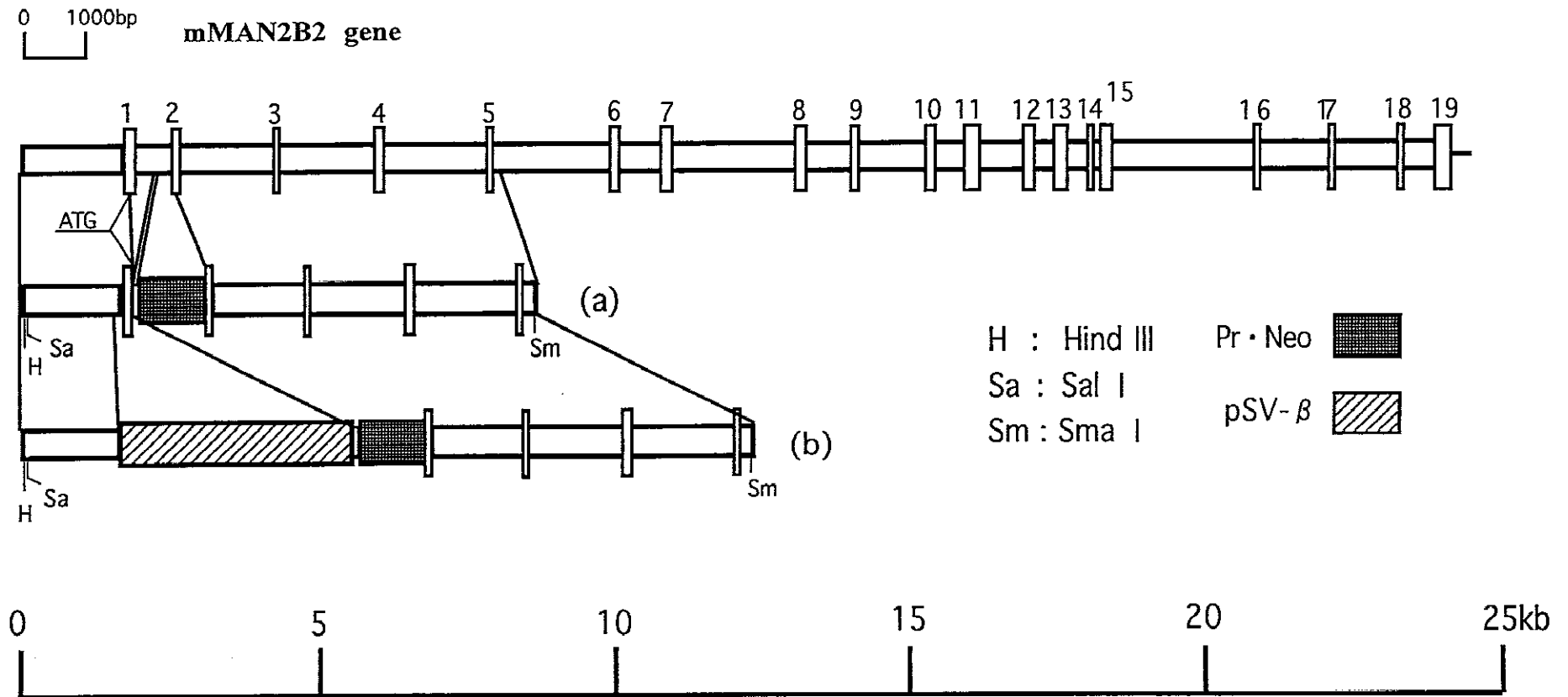








**Fig. 14. Immunocytochemical localization of the mMAN2B2 on mature sperm.** Capacitated cauda epididymal sperm were incubated in the presence (a) or the absence (b) of calcium ionophore A23187 as an inducer of the acrosome reaction. Then the sperm were incubated with the monospecific anti mMAN2B2 antibody (1) or pre-immune serum (2). The acrosome reacted sperm was indicated by the arrow. Magnification ; (a), (b) and (c),  $\times 400$ .



**Fig. 15. Design of two targeting vectors for producing the mMAN2B2 knock out mouse.**

(a) The region from the distal half of exon1 to the proximal half of exon2 is replaced by Neo gene.

(b) The region from the exon1 to the proximal half of exon2 is replaced by pSV- $\beta$  and Neo gene.

Table. 1. Effect of the anti mMAN2B2 anti body on the fertilization rate.

Treatment	Number of test replicates	Number of formation of the two cell embryo / number of eggs examined	Average of fertilization rate $\pm$ SD #	Relative fertilization rate *
The absence of antibody (0 $\mu$ g/ml ; control)	5	408/465	86.9 $\pm$ 7.4 % a	—
The presence of anti mMAN2B2 antibody (100 $\mu$ g/ml)	6	405/620	65.9 $\pm$ 6.0 % b	75.9 %

# a VS b :  $p < 0.001$

\* Relative fertilization rate (%) = (% of fertilization in the presence of anti mMAN2B2 antibody / % of fertilization in the absence of antibody (control) )  $\times$  100