Cloning of Rabbit *HPRT* Gene Using the Recombineering System

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Abstract Hypoxanthine phosphoribosyltransferase (HPRT) plays an important role in the metabolic salvage of purines, and been used as an alternative pathway for mutant selection in many studies. To facilitate its application in rabbits, we have cloned the cDNA and genomic DNA of the rabbit *HPRT* gene using an approach that combines bioinformatics and recombineering methods. The cDNA is comprised of 1449 bp containing a coding sequence for a protein of 218 amino acids. The deduced amino acid sequence of the rabbit *HPRT* gene shares 98%, 97%, 98% and 94% identity with human, mouse, pig and cattle *HPRT* genes, respectively. Reverse transcription-polymerase chain reaction analysis showed that this gene is ubiquitously expressed in tissues of adult rabbit. The rabbit *HPRT* gene spans approximately 48 kb in length and consists of nine exons. The cloning of the rabbit *HPRT* gene shows the usefulness of the recombineering system in cloning genes of large size. This system may facilitate the subcloning of DNA from bacterial artificial chromosomes for cloning genes of large size or filling big gaps in genomic sequencing.

Keywords hypoxanthine phosphoribosyltransferase; BAC; gap repairing vector; genomic organization; homologous recombination

Hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8), an enzyme that catalyzes the conversion of hypoxanthine and guanine to their respective 5′-mononucleotides, is essential for the metabolic salvage of purines in mammalian cells. A deficiency of the enzyme causes the clinical disorders of Lesch-Nyhan syndrome and gouty arthritis in human males [1]. In mammalian cells, the x-linked *HPRT* gene has been extensively used in mutation studies because of its functional haploidy. It is used to design powerful selections for isolating cells lacking enzyme activity. The orthologs of the *HPRT* gene have been cloned in mouse [2] and human [3], and the gene's exon-intron organization is conserved in these mammalian species. Gene targeting at the *HPRT* locus has successfully corrected a mutant *HPRT* gene in mouse embryonic stem (ES) cells [4]. Importantly, the *HPRT* locus has been used as an optimal surrogate site for integrating a copy of a transgene into the genome by a precise homologous recombination event [5,6].

To obtain an animal model for Lesch-Nyhan syndrome, two groups independently reported success in generating *HPRT*-deficient male mice. But they did not find any spontaneous behavioral abnormalities characteristic of Lesch-Nyhan syndrome in these mice [7,8]. In 1996, Engle *et al*. obtained HPRT/adenine phosphoribosyltransferase (APRT) doubly deficient mice, but they did not observe any behavioral abnormalities related to Lesch-Nyhan syndrome in humans [9]. Until now, there has been no animal model for Lesch-Nyhan syndrome.

Rabbit ES cells represent an excellent *in vitro* system to study gene expression and regulation in stem cell self-renewal and differentiation. They are also a potential resource for producing transgenic rabbits by somatic cell

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nuclear transplant and gene targeting. Transgenic rabbits provide a great advantage compared to transgenic mice because, as a relatively large mammalian model, they has provided unprecedented opportunities to study human disease mechanisms and alternative ways to produce therapeutic proteins to these diseases [10,11]. Rabbit ES cells have been isolated [12,13] and can proliferate for a prolonged period *in vitro* while remaining pluripotent. They readily integrate and express exogenous genes and can be used as nuclear donors to generate cloned rabbits [12, 14].

To facilitate gene targeting in rabbit ES cells and the production of transgenic rabbits, we cloned and analyzed the rabbit *HPRT* gene and its cDNA.

Materials and Methods

Bacterial strain

Recombinogenic strains EL350 that carry a defective λ prophage with inducible *Red* recombination proteins were kindly provided by Dr. Neal COPELAND (National Cancer Institute-Frederick, Frederick, USA) [15].

Rabbit bacterial artificial chromosome (BAC) library screening

Rabbit BACs were obtained from the Children's Hospital Oakland Research Institute (Oakland, USA). The BAC clones containing the rabbit *HPRT* gene were isolated by screening an LBNL-1 rabbit BAC library with genomic-specific overgo probes followed by polymerase chain reaction (PCR) identification. The overgo probes (Hprt-Ova, 5′-ATTGTAGCCCTCTGTGTGCTCAAG-3′; and Hprt-Ovb, 5′-AGAACTTATAGCCCCCCTTGAGCA-3′) and PCR primers (OCHprt7, 5′-CCCTCGAAGTGTTG-GATACAGG-3′; and OCHprt8, 5′-GTCAAGGGCATA-TCCTACAACAAAC-3′) were designed based on the partial cDNA sequence of the rabbit *HPRT* gene (GenBank accession No. AF020294).

In silico cloning of rabbit *HPRT* cDNA

A BLASTN (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&MEGA-BLAST=on&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on) search of the rabbit expressed sequence tags database (dbEST) using a partial cDNA sequence of the rabbit *HPRT* gene as a query identified three EST sequences (GenBank accession Nos. EB373457, EB373458 and EB378005). These EST sequences were retrieved, assembled into a contig sequence, and used to guide the isolation of the gene and its cDNA. DNase I-treated total RNA from the adult liver tissue was reverse-transcribed using M-MLV reverse transcriptase (Promega, Madison USA) and a putative gene-specific primer (HPRT-RT, 5′-TGGTAATTT-ATTTGATTGCA-3′). A fragment containing the complete coding region was then amplified using the sense primer [5′-GAGCGAGCCTCTCGGCTTTCC-3′, located in the putative 5′ untranslated region (UTR)] and the anti-sense primer (5′-ATTCAATCACTTCTGTTCTTTCCTG-3′, located in the putative 3′ UTR). All of these primers were designed according to the assembled contig sequence.

Expression analysis using reverse transcription (RT)-PCR

Total RNA from adult tissues, including heart, liver, spleen, kidney, brain and muscle, was isolated and reverse-transcribed using random hexadeoxyribonucleotide primers (TaKaRa, Dalian, China). *HPRT* mRNA was analyzed by RT-PCR using primers 5′-GTAATCGGTGG-AGATGATCTCTCA-3′ and 5′-GTCAAGGGCATATCC-TACAACAAAC-3′. Water was used as the negative control.

Cloning the entire *HPRT* gene

The assembled contig sequence was used as a query to search the rabbit whole genome shotgun sequences database using the BLASTN tool. As shown in **Fig. 1(A)**, five sequences were identified (GenBank accession Nos. AAGW01580029, AAGW01067486, AAGW01700953, AAGW01715926 and AAGW01580025). The approach to clone the complete genomic DNA of the *HPRT* gene is shown in **Fig. 1(B)**. Four overlapping DNA fragments spanning the entire gene were subcloned by homologous recombination, sequenced and assembled.

Construction of gap repairing vectors

An *Hpa*I restriction site was introduced into the downstream region of the Ap resistance cassette in the pKS-plasmid by PCR amplification using primers 5′-AGTTAACATTTCCCCGAAAAGTGCCAC-3′ (*Hpa*I restriction site underlined) and 5′-ACTCCGCTCATGA-GACAATAACCCTG-3′ (GenBank accession No. X52329). The modified vector (mpKS) maintains all the characteristics of pKS-plasmid. The vector was linearized with *Hpa*I or *Eco*RV, and the blunt ends were modified by a T-tailing procedure [16].

A three-step procedure was carried out to synthesize the linear gap repairing vector (**Fig. 2**). First, four up-

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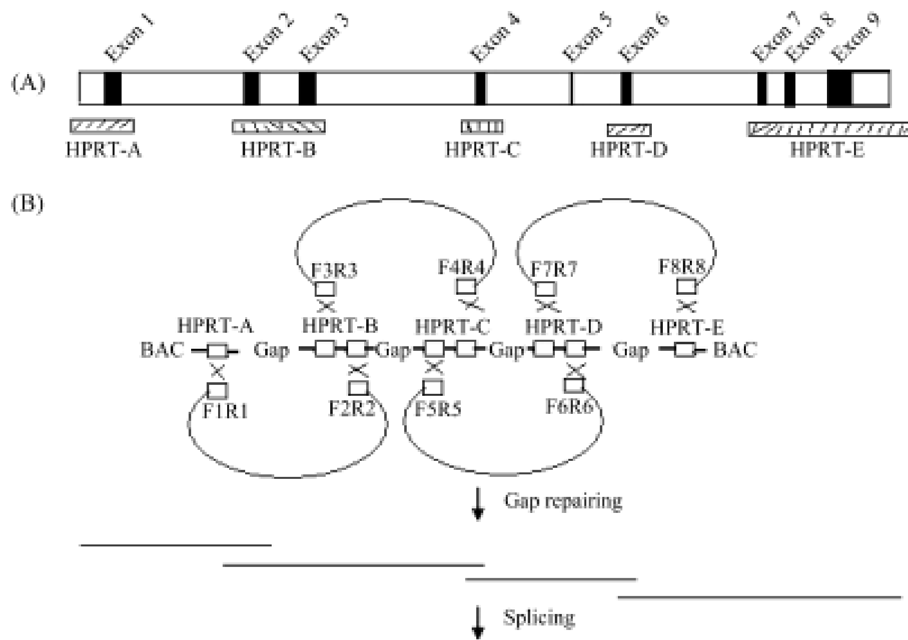

Fig. 1 Cloning of the rabbit *HPRT* gene

(A) Schematic structure and genomic organization of the rabbit *HPRT* gene. Nine exons (black boxes) and five identified partial genomic DNA sequences (shaded boxes; *HPRT*-A, *HPRT*-B, *HPRT*-C, *HPRT*-D and *HPRT*-E) are shown according to their relative positions. (B) The strategy for cloning the complete genomic DNA of the *HPRT* gene. Four overlapping DNA fragments were subcloned by homologous recombination between the *HPRT*-containing bacterial artificial chromosome (BAC) and four linear gap repairing vectors designed according to the four identified partial genomic DNA sequences. The four overlapping DNA fragments were sequenced and assembled into the complete genomic DNA sequence of the *HPRT* gene.

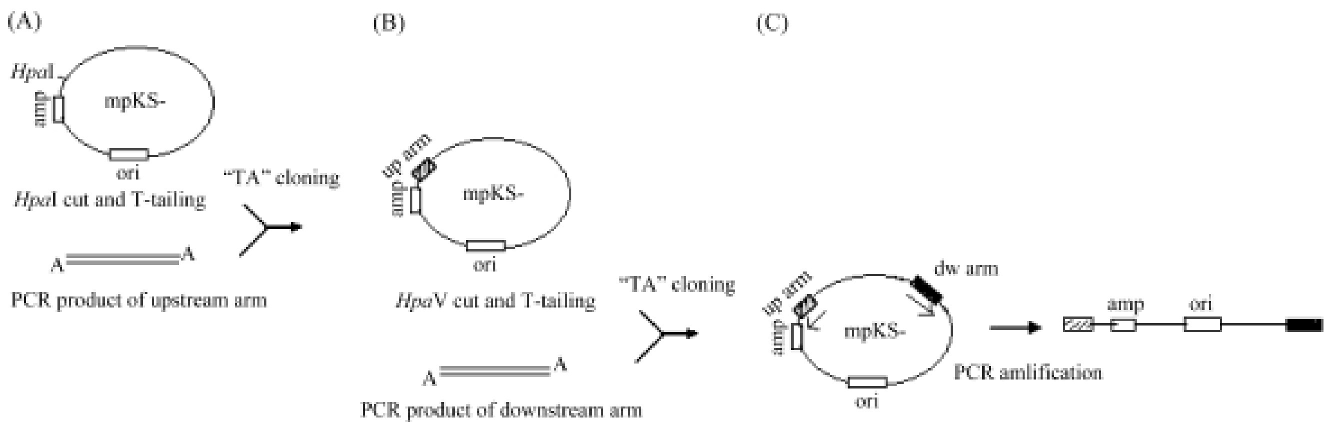

Fig. 2 Polymerase chain reaction (PCR) synthesis of the linear gap repairing vector

(A) Four upstream homology arms (up arm; F1R1, F3R3, F5R5 and F7R7) were amplified from bacterial artificial chromosome (BAC) DNA and inserted into the *Hpa*I site of mpKS plasmid in the desired direction by TA cloning. (B) Four downstream homology arms (dw arm; F2R2, F4R4, F6R6 and F8R8) were amplified from BAC DNA and inserted into the *Eco*R V site of four recombinant plasmids (mpKSF1R1, mpKSF3R3, mpKSF5R5 and mpKSF7R7) in the desired direction by TA cloning. (C) PCR amplification of four linear gap repairing vectors from four recombinant plasmids (mpKSF1R1F2R2, mpKSF3R3F4R4, mpKSF5R5F6R6 and mpKSF7R7F8R8) using specific primers indicated by arrows.

stream homology arms (F1R1, F3R3, F5R5 and F7R7) were amplified from BAC DNA and inserted into the *Hpa*I site of mpKS plasmid by TA cloning. Second, four downstream homology arms (F2R2, F4R4, F6R6 and F8R8) were amplified from BAC DNA and inserted into the *Eco*RV site of four recombinant plasmids (mpKSF1R1,

Table 1 Primers for synthesis of four linear gap repairing vectors and identification of recombinant subclones

Primer sequence	Application	Size (bp)	GenBank accession No.
F1, 5′-CTTGGAGTCTGATTTGCACAAGG-3′	F1R1	358	AAGW01580029
R1, 5′-GGAAGAAACGGAGACCACCTG-3′	F1R1	358	AAGW01580029
F2, 5′-GCAGCAGCCAAGACTTGAACCAG-3′	F2R2	361	AAGW01067486
R2, 5′-GCTAATCCTCCACCTTGCAGCAC-3′	F2R2	361	AAGW01067486
F3, 5′-TGTAGGACTGAAAGGCTTGCTCG-3′	F3R3	471	AAGW01067486
R3, 5′-GGGAAAGCACTGGAAGATGGTC-3′	F3R3	471	AAGW01067486
F4, 5′-GGAAGTGATTGTGACTGAAGATGG-3′	F4R4	352	AAGW01700953
R4, 5′-GGTTGAGAGATCATCTCCACCG-3′	F4R4	352	AAGW01700953
F5, 5′-TGCACTATTGCAATGGTTGAGC-3′	F5R5	272	AAGW01700953
R5, 5′-ACTCATCTTCAGCAATGTCTGGG-3′	F5R5	272	AAGW01700953
F6, 5′-TCTCTCAAGGTTTGGAGCTCCTG-3′	F6R6	504	AAGW01715926
R6, 5′-CCAGTTTCCTGCTAATGCACACC-3′	F6R6	504	AAGW01715926
F7, 5′-GTAAGTAGGAACGTTCTCGGAGATC-3′	F7R7	264	AAGW01715926
R7, 5′-CCCTTCCTACCACATCCTTATCC-3′	F7R7	264	AAGW01715926
F8, 5′-ACCATTCCAGTAGCCTCTGACCC-3′	F8R8	735	AAGW01580025
R8, 5′-GGTTGGCATTGTGGTGAAGTGAG-3′	F8R8	735	AAGW01580025
F9, 5′-GGTTGTAGGAGATAGGGAAGCAGC-3′	NA	NA	AAGW01580025
M13R, 5′-CAGGAAACAGCTATGAC-3′	NA	NA	X52329

F, forward; M13, M13 phage; R, reverse. NA, not applicable

mpKSF3R3, mpKSF5R5 and mpKSF7R7) by TA cloning, respectively. Finally, the four recombinant plasmids (mpKSF1R1F2R2, mpKSF3R3F4R4, mpKSF5R5F6R6 and mpKSF7R7F8R8) were used to prepare the four linear gap repairing vectors by PCR using the primers shown in **Table 1**.

Transformation of BAC into EL350 recombinogenic strains

A single colony of *Escherichia coli* DH10B containing 304M19 BAC was grown overnight in 5 ml of Luria broth (LB) with chloramphenicol (12.5 μg/ml). BAC DNA was isolated according to the BAC miniprep protocol using the Plasmid Mini kit (Qiagen, Valencia, USA). A single colony of EL350 cells was inoculated in 5 ml LB at 32 ºC overnight with shaking. The cells were collected by centrifuging at 3000 *g* (0 ºC) for 5 min the next day. The pellets were resuspended in 900 μl of ice-cold water, transferred to a 1.5 ml Eppendorf tube on ice, centrifuged at 20,000 *g* (4 ºC) for 20 s, and the supernatant was discarded. The washing process was repeated three times, the cells were resuspended in 50 μl of ice-cold water, and mixed with 1 μl (50–100 ng) of freshly prepared BAC DNA. The DNA-bacteria mixture was transferred into a 0.1 cm cuvette (Bio-Rad, Hercules, USA) and electroporated at 1.8 kV, 25 μF and 200 Ω using a Gene Pulser II electroporator (Bio-Rad, Hercules, USA). One microliter of LB was added to the electroporated bacteria, incubated at 32 ºC for 1 h with shaking, spun down, spread onto a plate containing chloramphenicol (12.5 μg/ml), and incubated for 24 h at 32 ºC.

Homologous recombination

A single colony of EL350 containing BAC was inoculated into 5 ml LB with chloramphenicol and incubated at 32 ºC overnight with shaking. The next day, 1 ml of the culture was transferred to 20 ml LB with chloramphenicol and incubated at 32 ºC for approximately 2 h (A_{600}=0.5) with shaking. The culture (10 ml) was transferred to a 50 ml Falcon tube and shaken in a 42 ºC water bath for 15 min. The tube was immediately put into wet ice, shaken for 2–3 min to make sure that the temperature dropped as quickly as possible, then left in ice for 6 min. Cells were spun at 3000 *g* (0 ºC) for 5 min. The pellet was resuspended in 900 μl of ice-cold water followed by three washes with ice-cold water, as described above. Finally, the cells were resuspended in 50 μl ice-cold water, mixed with 2 μl (200–400 ng) of purified PCR fragments of the four linear gap repairing vectors (mpKSF1R1F2R2, mpKSF3R3F4R4, mpKSF5R5F6R6 and mpKSF7R7F8R8), and electroporated as described above. After electroporation, cells were resuspended in 1 ml LB, incubated at 32 ºC

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with gentle shaking for 1 h, spread onto a plate with ampicillin (60 μg/ml), and incubated at 32 ºC for 18–20 h. The recombinant BAC subclones from four linear gap repairing vectors were identified by PCR using sense primers F3, F5, F7, F9 and antisense primer M13R (**Table 1**).

Results

cDNA cloning of *HPRT*

Three overlapping EST sequences (GenBank accession Nos. EB373457, EB373458 and EB378005) were identified by searching the rabbit dbEST database using a partial cDNA sequence (GenBank accession No. AF020294) of the rabbit *HPRT* gene as a query. A 1449 bp contig sequence was assembled and identified by amplifying a 1179 bp cDNA fragment from the liver cDNA pool using a pair of specific primers designed according to the contig (**Fig. 3**).

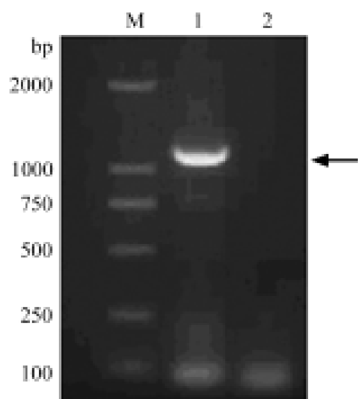

Fig. 3 Reverse transcription-polymerase chain reaction (RT-PCR) identification of the assembled cDNA sequence of the rabbit *HPRT* gene

A 1179 bp specific fragment was amplified from the cDNA of rabbit liver using a pair of specific primers designed according to the assembled *HPRT* cDNA sequence. M, DNA marker; 1, RT-PCR product of *HPRT* cDNA fragment indicated by arrow; 2, negative control without adding reverse transcriptase.

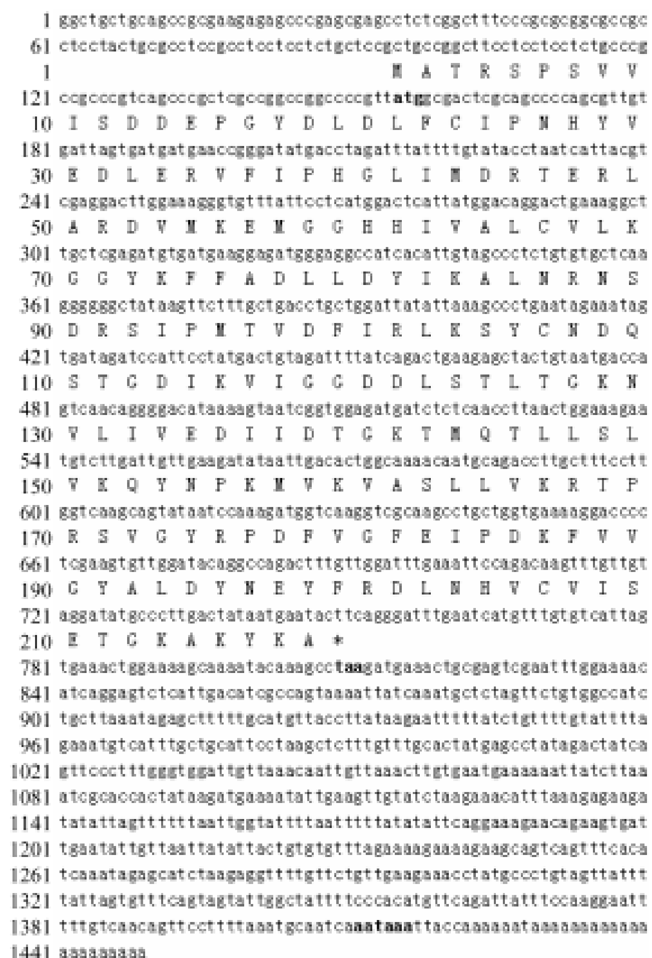

Fig. 4 cDNA and predicted amino acid sequences of the rabbit *HPRT* gene

The nucleotide sequence is shown underneath the amino acid sequence and numbered separately. The ATG start codon, TAA stop codon and polyadenylation signal are shown in bold. This sequence has been submitted to the GenBank database under the accession No. EF062857. * asterisk means TAA stop codon.

The cDNA contains a 154 bp 5′-UTR, a 657 bp open reading frame encoding a protein of 218 amino acids, and a 638 bp 3′-UTR. The nucleotide sequence has been submitted to the GenBank databases under the accession No. EF062857 (**Fig. 4**). The coding area showed 98%, 97%, 98% and 94% identity in the amino acid sequence with human, mouse, pig and cattle *HPRT* genes, respectively (**Fig. 5**), suggesting that this is the rabbit homolog of the *HPRT* gene.

mRNA expression in adult tissues

Expression of the *HPRT* gene in the adult rabbit heart, liver, spleen, kidney, brain, and muscle was examined using RT-PCR. A 237 bp PCR product was amplified from cDNAs of adult tissues examined (**Fig. 6**). RT-PCR analysis showed that the rabbit *HPRT* gene is expressed ubiquitously in adult tissues.

Cloning the *HPRT* gene

Five genomic DNA fragments (*HPRT*-A, *HPRT*-B,

Fig. 5 Alignment of deduced amino acid sequences of rabbit, mouse, human and cattle HPRT proteins
GenBank accession numbers for rabbit, human, mouse, pig and cattle *HPRT* are EF062857, NM_000194, NM_013556, NM_001032376 and NM_001034035, respectively. An asterisk (*) indicates that the residues or nucleotides are identical in all sequences in the alignment. Sequence alignment was carried out using the ClustalW method.

Fig. 6 Reverse transcription-polymerase chain reaction analysis of *HPRT* expression in adult rabbit tissues
A 237 bp fragment of rabbit *HPRT* cDNA was amplified from adult tissues including heart, liver, spleen, kidney, brain and muscle. M, DNA marker. Water, negative control.

HPRT-C, *HPRT*-D and *HPRT*-E) of the putative *HPRT* gene were obtained through a homology search of the rabbit whole genome shotgun sequences database using the *HPRT* cDNA sequence as a query. *HPRT*-A contains exon 1 and partial intron 1. *HPRT*-B contains partial intron 1, intron 2, partial intron 3, exon 2 and exon 3. *HPRT*-C contains partial intron 3, partial intron 4 and exon 4. *HPRT*-D contains partial intron 5, partial intron 6 and exon 6. *HPRT*-E contains partial intron 6, intron 7, intron 8, exon 7 and exon 9. The relative positions of the five fragments in the *HPRT* gene are shown in **Fig. 1(A)**.

As well as the sequenced area, the sequences of four big gap regions are still missing. The genomic DNA of these big gap regions was subcloned from the BAC clone 304M19 using a recombineering-based method. The 304M19 clone contains the complete coding region of the *HPRT* gene. The subclones were PCR screened and those with PCR products of the expected sizes were selected and sequenced. Four fragments, approximately 12, 13, 10.5 and 14.5 kb in length, were obtained, which covered all the missing gaps in the *HPRT* gene [**Fig. 1(B)**]. These sequences were assembled into a contig sequence spanning the complete coding region of the rabbit *HPRT* gene.

Genomic organization of the rabbit *HPRT* gene

The assembled sequence of the rabbit *HPRT* gene has been submitted to the GenBank database under the accession No. EF219063. Alignment of the *HPRT* cDNA with the genomic sequence revealed that the entire gene is 47.9 kb in length and split into nine exons. Exon-intron junctions were deduced by comparing the *HPRT* cDNA

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Table 2 Exon-intron organization of the rabbit *HPRT* gene

Exon No.	Exon size (bp)	Sequence of exon-intron junction		Intron size (bp)
		5′-Splice donor	3′-Splice acceptor	
1	181	TGTG**gt**gagtagct	tctttttc**ag**ATTA	8959
2	107	ACAG**gt**tagtaata	gtttctgt**ag**GACT	1536
3	184	CTGT**gt**aagtatat	ttttaacc**ag**AATG	12,867
4	66	AAAG**gt**atgtatct	tttcttct**ag**AATG	5121
5	18	TGAA**gt**aagttcta	ttttttaa**ag**GATA	3706
6	83	CAAG**gt**aagtatca	taattaac**ag**CCTG	8888
7	47	GACT**gt**aagtgaat	ttctttct**ag**TTGT	173
8	77	GAAT**gt**aagtaatt	tattttct**ag**CATG	723
9	665			

Exon and intron sequences are shown in capital and lowercase letters, respectively. The gt and ag splice sites of the donor and acceptor sequences of each intron are shown in bold. The genomic sequence of the rabbit *HPRT* gene has been deposited in the GenBank database with accession No. EF219063.

with the genomic sequence following consensual splicing signal rules (GT/AG) [17]. The boundary of each exon and its flanking intron sequences are shown in **Table 2**. The exon-intron organization is similar to that previously determined for the *HPRT* orthologs of mouse [2,3]. The sizes of the seven internal exons are identical to those of the mouse and human *HPRT* gene. The 5′end of the gene contains extremely GC-rich sequences and two GC hexanucleotide motifs (5′-GGCGGG-3′), but lacks the prototypical 5′ transcriptional regulatory sequence elements. These structural features of the gene are highly conserved.

Discussion

Whole genome shotgun sequencing and ESTs have produced a tremendous amount of sequencing information for genes of many species, now available in the public domain. However, sequences generated by these approaches are often fragmented, small in size (<20 kb), and separated by big gaps of unknown sequences. For complicated large genes, filling in these big gaps could be difficult. The human *PDE11A* gene is in a genomic DNA region of over 300 kb and contains 23 exons. To obtain its exon-intron organization, long-distance PCR and the screening of the human genomic DNA phage library and BAC library were carried out [18]. For some genes with big introns and small exons, it is impossible to obtain complete sequences by the PCR method alone.

In order to obtain sequences for the entire *HPRT* gene, we tried to clone the big gap regions using long-distance PCR. However, complicated template structures in the genome posed problems that were difficult to overcome. In addition, nucleotide substitutions arising from misincorporation by *Taq* DNA polymerase potentially reduced the quality of the cloned sequences. The attempt to directly sequence BAC clones by a shotgun sequencing strategy was time-consuming, laborious and expensive, and generated many superfluous sequencing reactions.

During the past few years, it has become possible to manipulate BAC clones by recombineering in *E. coli*. Efficient homologous recombination, mediated by the *Red* proteins of λ phage in *E. coli*, permits insertion of linear fragments into the BAC constructs, as well as the subcloning of DNA fragments from them [19–21]. The gap repairing process that enables homologous recombination between the linear gap repairing vector and the genomic DNA in the BAC clones makes it very convenient to subclone large-sized DNA from the BAC constructs into high-copy plasmid vectors.

We have used sequences provided by databases to design arms to subclone large DNA sequences from the BAC clones through homologous recombination. The approach, which combines bioinformatics with recombineering, has greatly improved the efficiency of subcloning and sequencing of the rabbit *HPRT* gene. Our work showed that this approach is very efficient, and should be applicable to similar works on large-sized genomic DNA.

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References

1 Caskey CT, Kruh, GD. The HPRT locus. Cell 1979, 16: 1–9
2 Melton, DW, Konecki DS, Brennand J, Caskey CT. Structure, expression, and mutation of the hypoxanthine phosphoribosyltransferase gene. Proc Natl Acad Sci USA 1984, 81: 2147–2151
3 Kim SH, Moores JC, David D, Respess JG, Jolly DJ, Friedmann T. The organization of the human HPRT gene. Nucleic Acids Res 1986, 14: 3103–3118
4 Doetschman T, Gregg RG, Maeda N, Hooper M, Melton DW, Thompson S, Smithies O. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature 1987, 330: 576–578
5 Guillot PV, Liu L, Kuivenhoven JA, Guan J, Rosenberg RD, Aird WC. Targeting of human eNOS promoter to the Hprt locus of mice leads to tissue-restricted transgene expression. Physiol Genomics 2000, 2: 77–83
6 Heaney JD, Rettew AN, Bronson SK. Tissue-specific expression of a BAC transgene targeted to the *Hprt* locus in mouse embryonic stem cells. Genomics 2004, 83: 1072–1082
7 Hooper M, Hardy K, Handyside A, Hunter S, Monk M. HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. Nature 1987, 326: 292–295
8 Kuehn MR, Bradley A, Robertson EJ, Evans MJ. A potential animal model for Lesch-Nyhan syndrome through introduction of HPRT mutations into mice. Nature 1987, 326: 295–298
9 Engle SJ, Womer DE, Davies PM, Boivin G, Sahota A, Simmonds HA, Stambrook PJ *et al*. HPRT-APRT-deficient mice are not a model for Lesch-Nyhan syndrome. Hum Mol Genet 1996, 5: 1607–1610
10 Bosze Z, Hiripi L, Carnwath JW, Niemann H. The transgenic rabbit as model for human diseases and as a source of biologically active recombinant proteins. Transgenic Res 2003, 12: 541–553
11 Fan J, Watanabe T. Transgenic rabbits as therapeutic protein bioreactors and human disease models. Pharmacol Ther 2003, 99: 261–282
12 Fang ZF, Gai H, Huang YZ, Li SG, Chen XJ, Shi JJ, Wu L *et al*. Rabbit embryonic stem cell lines derived from fertilized, parthenogenetic or somatic cell nuclear transfer embryos. Exp Cell Res 2006, 312: 3669–3682
13 Wang S, Tang X, Niu Y, Chen H, Li B, Li T, Zhang X *et al*. Generation and characterization of rabbit embryonic stem cells. Stem Cells 2007, 25: 481–489
14 Li S, Chen X, Fang Z, Shi J, Sheng HZ. Rabbits generated from fibroblasts through nuclear transfer. Reproduction 2006, 131: 1085–1090
15 Lee EC, Yu D, de Velasco JM, Tessarollo L, Swing DA, Court DL, Jenkins NA *et al*. A highly efficient *Escherichia coli*-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics 2001, 73: 56–65
16 Holton TA, Graham MW. A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors. Nucleic Acids Res 1991, 19: 1156
17 Mount SM. A catalogue of splice junction sequences. Nucleic Acids Res 1982, 10: 459–472
18 Yuasa K, Kanoh Y, Okumura K, Omori K. Genomic organization of the human phosphodiesterase PDE11A gene. Evolutionary relatedness with other PDEs containing GAF domains. Eur J Biochem 2001, 268: 168–178
19 Zhang Y, Buchholz F, Muyrers JP，Stewart AF. A new logic for DNA engineering using recombination in *Escherichia coli*. Nat Genet 1998, 20: 123–128
20 Muyrers JP, Zhang Y, Testa G, Stewart AF. Rapid modification of bacterial artificial chromosomes by ET-recombination. Nucleic Acids Res 1999, 27: 1555–1557
21 Yu D, Ellis HM, Lee E, Jenkins NA, Copeland NG, Court DL. An efficient recombination system for chromosome engineering in *Escherichia coli*. Proc Natl Acad Sci USA 2000, 97: 5978–5983

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