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Genetic Quality Control of the Rat Strains at the National Bio Resource Project – Rat

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SYNOPSIS

The National Bio Resource Project-Rat (NBRP-Rat) comprises the largest bank of laboratory rat (*Rattus norvegicus*) strains in the world. Its main focus is to develop infrastructure that will facilitate the systematic collection, preservation, and provision of rat strains. To breed effectively more than 180 rat strains in living stock, we establish the genetic control system in which a systematic set of genetic diagnoses and genetic monitoring are included. Genetic monitoring is performed by using 20 polymorphic markers. Monitoring is carried out when a living animal stock is re-established by using cryopreserved embryos or sperm or when a rat strain is first introduced to the NBRP-Rat by a depositor. Additional monitoring is then carried out on each strain every two years. Genetic diagnosis is performed largely by employing the Amp-FTA method. Protocols which detail how to perform a genetic diagnosis of 11 transgenes and 24 mutations have been made. Among the mutations, nine can be detected by simple gel electrophoresis of the PCR products, 11 by restriction enzyme treatment of the PCR products, and four by direct PCR product sequencing. Using this genetic control system, the NBRP-Rat can guarantee the genetic quality of its rat strains.

Keywords: rat, National Bio Resource Project, strain, genotyping, genetic monitoring, transgenic rat, mutation, disease model, Hermansky-Pudlak syndrome 5, Amp-FTA method

Abstract

The National Bio Resource Project-Rat (NBRP-Rat) comprises the largest bank of laboratory rat (*Rattus norvegicus*) strains in the world. Its main focus is to develop infrastructure that will facilitate the systematic collection, preservation, and provision of rat strains. As of the end of September, 2010, more than 600 strains have been collected and preserved. Among them, 180 are maintained as living animal stocks. It is necessary to carry out a fast and reliable genetic diagnosis of mutations that each animal possesses to breed it effectively. In addition, a genetic monitoring system is needed to ensure its genetic quality. Here, we show the genetic control system of the NBRP-Rat in which a systematic set of genetic diagnoses and genetic monitoring is established. Genetic monitoring is performed by using 20 polymorphic markers. Monitoring is carried out when a living animal stock is re-established by using cryopreserved embryos or sperm or when a rat strain is first introduced to the NBRP-Rat by a depositor. Additional monitoring is then carried out on each strain every two years and each resulting genetic profile is compared with the original profile. Genetic diagnosis is performed largely by employing the Amp-FTA method. Protocols which detail how to perform a genetic diagnosis of 11 transgenes and 24 mutations have been made. Among the mutations, nine can be detected by simple gel electrophoresis of the PCR products, 11 by restriction enzyme treatment of the PCR products, and four by direct PCR product sequencing. As of the end of March, 2010, a total of 288 genetic profiles from 154 strains had been obtained and 16,812 samples had been subjected to genetic diagnosis. Furthermore, we reported on identifying a coat color mutation and a hairless mutation that is harbored by the mutant strains which are preserved at the NBRP-Rat. Using this genetic control system, the NBRP-Rat can guarantee the genetic quality of its rat strains. These rat strains are excellent model systems for biomedical research.

Introduction

The laboratory rat (*Rattus norvegicus*) is an indispensable tool for developing experimental medicines and drugs. It is used extensively as a model organism for studying normal processes and disease processes in humans. This is primarily due to our extensive knowledge of rat physiology and the large number of rat models that mimic human diseases. Rats are a key tool for drug development because they can be used to determine drug efficacy and toxicity prior to performing human clinical trials[1].

Various rat strains which are used in biomedical research are maintained by individual scientists. This is a time and labor intensive process. In addition, the ability of a scientist to maintain a strain depends on their available funding and also on local interests. Establishing a national rat resource center in Japan was therefore required to prevent these strains from disappearing. The National Bio Resource Project-Rat (NBRP-Rat) was initiated in 2002[2]. The main focus of it is on developing infrastructure that will facilitate the systematic collection, preservation, and provision of rat strains. As of the end of September, 2010, more than 600 strains have been collected and preserved. They include inbred, mutant, congenic, recombinant inbred, and transgenic strains. Among them, 180 are maintained as living animal stocks.

To ensure good colony management, it is essential to establish a genetic control system which enables fast and reliable genotyping. This allows us to perform effectively genetic monitoring and identification of mutations and transgenes. The genetic monitoring is employed to periodically check the genetic backgrounds of each rat strain by using a set of predetermined genetic markers. We can therefore detect genetic contamination due to breeding errors and deviations from the unique profile of each strain[3]. Alterations in the genetic background of a strain will change its characteristics substantially. Therefore this will compromise the reproducibility of experimental data between strains and within the same strain over time[4]. Thus, genetic background monitoring is essential to ensure the continued purity and uniformity of the rat strains.

To breed rat strains effectively, it is necessary to carry out fast and reliable genetic diagnoses of mutations or transgenes. Genomic DNA that is used as PCR templates for standard genetic diagnoses is extracted from tail biopsy samples by using an organic solvent such as phenol or chloroform. This is time-consuming even when an automated DNA extraction system is employed. In addition, the reagents that are used are harmful to human health. We recently developed a simple genotyping method that employs Ampdirect® and FTA® technologies to overcome these problems[5]. We call it the Amp-FTA method. The Amp-FTA method allows DNA from unpurified blood that has been immobilized on a FTA® card to be PCR amplified when used with Ampdirect® buffer. The immobilized DNA can also be stored at room temperature for as long as 17 years. PCR templates can be prepared simply by punching out discs from the FTA® card to which the immobilized DNA is bound. Additionally, the PCR products can be analyzed downstream by using techniques such as restriction enzyme digestion and direct sequencing. Thus, the Amp-FTA method enables us to perform easy, fast, reliable, and safe genetic diagnoses.

In this paper, we show the quality control system which we use to monitor and guarantee the genetic quality of the rat strains that are maintained at the NBRP-Rat. Moreover, we identify of two rat mutations that are harbored by the rat strains.

Results and Discussion

Genetic monitoring

A scheme that is used to depict how genetic monitoring of rat strains which are maintained in the NBRP-Rat is shown in Figure 1. When an animal stock is introduced into our breeding facility, we perform genetic monitoring of all animals of the stock. This can occur when we re-establish a living animal stock by using cryopreserved embryos or sperm, and when we perform microbiological cleaning of a stock by carrying out Caesarian

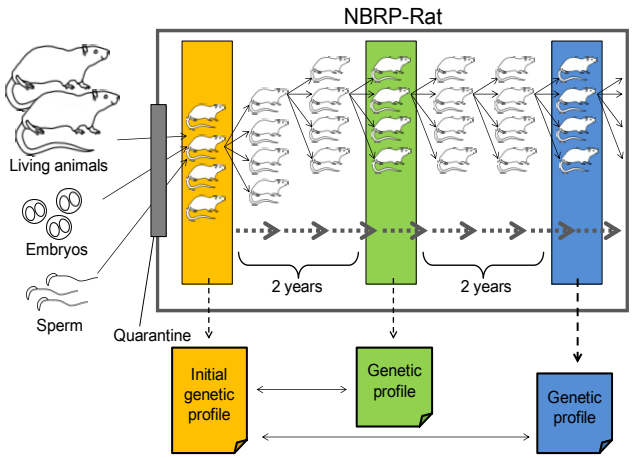

Figure 1. Genetic monitoring at the NBRP-Rat. Rat strains are deposited as living stocks, cryopreserved embryos, or sperm. After quarantining or microbiological cleaning, an initial living stock for each strain (yellow box) is established and then the initial genetic profile for each (highlighted in yellow) is produced. These rat strains are bred at the NBRP-Rat breeding facility. Additional genetic monitoring (green and blue boxes) is performed almost every two years. The additional genetic profiles that are obtained (highlighted in green and blue) are then compared with the initial profile.

sectioning. Additional monitoring is carried out on each strain every two years after the initial monitoring has been performed. All animals to be used for producing the next generation are monitored, usually 3 pairs of rats for each strain. We compare the genetic profile of each strain with the original profile we obtained by using the genomic DNA extracted from tissues that were provided by the depositor to us.

As of the end of March, 2010, 180 strains had been maintained as living animal stocks. Over the past eight years since initiating the NBRP-Rat, we have made a total of 288 genetic profiles from 154 strains, although the number of strains to be monitored was very limited in the first few years of the project. No genetic contamination has been found in any living stock. However, deviations from previous profiles have been identified in strains MV/Opu, MD/Tama, NE/Mave, and GAERS/Mave. These deviations probably resulted from residual heterozygous loci being randomly fixed. This is because the genetic background of each strain was not fixed when it was deposited and introduced into the NBRP-Rat.

Genetic contamination occurs mainly due to human errors such as mixing up the animals or cyropreserved gametes. Mixing up the animals could occur when we carry out the microbiological cleaning of rats by using Caesarian sectioning, in which the pups dissected from the uterus are fostered by the lactating rats. Thus, it is important to discriminate the pups from uterus from the nursing pups. We use a foster strain that has a different coat color from the pups dissected from the uterus. In addition, we keep permanent breeding records on any rat strains maintained at the NBRP-Rat. This allows us to perform retrospective analyses to identify when a contamination takes place.

Mutation and transgene genotyping

A scheme depicting how rat strains are genetically diagnosed is shown in Figure 2. As of the end of March, 2010, a total of 16,812 genotypes had been obtained. As soon as the rats have been weaned, we carry out genetic diagnoses of mutations or transgenes by using the Amp-FTA method[5]. At the breeding facility, a tip of tail (approximately 2.0 mm long) is cut from each rat. Several aliquots of blood are obtained from the clipping tail and smeared on to the FTA® card, which immobilizes the DNA. The blood-smeared FTA cards are then transferred to our genotyping laboratory along with a list of samples. Small discs that are 1.5 mm in diameter are punched out from the FTA cards and directly used as PCR templates in conjunction with Ampdirect® buffer. The PCR products are visualized by UV illumination after performing agarose gel electrophoresis. This procedure enables us to determine if a transgene(s) is present or if sequence-length mutations such as insertion and deletion are present. To date, nine mutations and eleven transgenes have been routinely genotyped by using this simple gel electrophoresis method (Table 1 and 2).

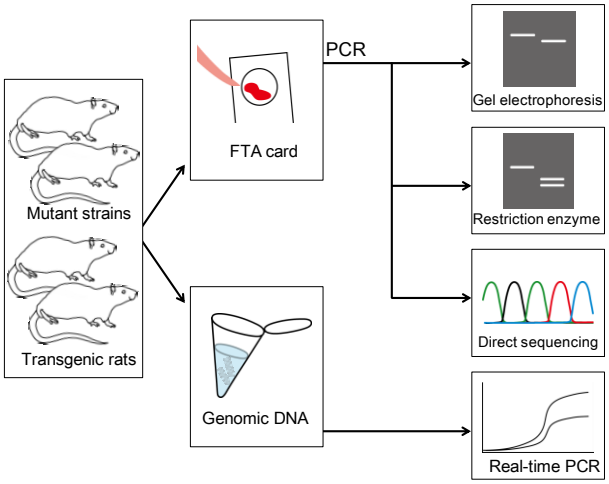

Figure 2. Genetic diagnosis of mutations or transgenes through the NBRP-Rat. Genetic diagnoses of mutations or transgenes that are possessed by the rat strains is mostly carried out by performing direct PCR amplification of the genomic DNA that is immobilized on to the FTA® card. The amplified PCR products are then subjected to downstream analyses, such as agarose gel electrophoresis, restriction enzyme digestion, and direct sequence, depending on the type of mutation. Recently, we introduced a method for quantitatively detecting a specific allele by using real-time PCR. For this, genomic DNA needs to be purified.

The single nucleotide polymorphism (SNP) is another type of mutation that is present in mutant strains. A SNP(s) can be detected by digesting the PCR products by using restriction enzymes if it has generated or disrupted a restriction site(s) in the products. If this is not possible then mutations can be detected by using direct sequencing. The PCR products that are obtained by the Amp-FTA method can be subjected directly to these types of downstream analyses. Eleven mutations were genotyped by using PCR product digestion and four mutations were identified by using direct sequencing (Table 1).

We recently introduced a quantitative measurement analysis system that can be used to analyze specific alleles by Cycleave® real-time PCR. This has allowed us to save time and reduce labor costs[6]. We tried to detect a nonsense mutation in the *Apc* gene that is present in the Kyoto *Apc* Delta (KAD) rat to evaluate the effectiveness of this system[7]. We detected clearly the mutant and wild-type alleles by using fluorescence-labeled probes. From this, we determined which rats were mutant homozygotes, heterozygotes, and wild-type homozygotes (Figure 3). Thus, the Cycleave® real-time PCR is a good alternative for detecting a SNP(s) that can be identified by using direct sequencing.

As described above, the genetic diagnoses that are performed through the NBRP-Rat are based mostly on the Amp-FTA method[5]. This fast, effective, and reliable method permits us to maintain a large number of rat strains at the NBRP-Rat while saving time and labor costs. Large numbers of genetically modified rats will be created by using rat embryonic stem cells or induced pluripotent stem cells in the near future[8,9]. It is reasonable to suppose that these newly-created rat strains will be deposited to the NBRP-Rat. This makes developing a fast and reliable genetic diagnosis system even more imperative. The Cycleave® real-time PCR method is most promising. It can be used to detect individual alleles during PCR without having to carry out subsequent PCR products analysis. The advantages of real-time PCR may be realized when the number of samples that has to be processed increases.

Identification of *am* and *khr* mutations

The American mink (*am*) mutation is a recessive coat color mutation. It was derived from a fancy rat colony and subsequently fixed in the fancy-derived strain KFRS3A[10]. Rats that are homozygous for *am* have light brown body hair (Figure 4A). The *am* has been mapped to rat Chr 1 and showed no recombination with *D1Mgh35*[10] and the dilute coat (tentatively named) found in the KFRS4/Kyo strain was mapped between *D1Rat27* and *D1Rat320* (Figure 4B). The Hermansky-Pudlak syndrome 5 (*Hps5*) gene that is located within the *am* locus is a strong candidate for *am*, because mice that are homozygous for the *Hps5* mutation *ruby-2* have hypopigmented eyes and hair[11]. We produced a larger PCR product for *am* by using the KFRS3A strain that is homozygous for this allele compared to the wild-type control when we amplified exons 7 and 8 of *Hps5* by using the primers 5'-TTGTGGGTGGGGTTATA TGG-3' and 5'-TCTTGAAAAGCAAACAACAAAA-3' (Figure 4C). Through the direct sequence analysis we found that a SINE sequence had been inserted into exon 7 (Figure 4D). The insertion also occurs in the rat strain KFRS4/Kyo (Figure 4C). KFRS3A and

Table 1. PCR primers used to detect mutations

Gene symbol	Type of mutation	Method*	Forward primer		Reverse primer		Strain harboring mutation
			name	Sequence (5' > 3')	name	Sequence (5' > 3')	
Apc^{m1Kyo}	SNP	2 (MnlI)	rApc-F3	ATCTGTTCAGGCAGGTGGAT	rApc-R3	TCACTCGAGGAAGGGATGAG	KAD/Kyo
Arsb	SNP	3	Arsb-ex3-F	CTTGATGCCATGCTGACTGT	Arsb-ex3-R	TCCCTTGGAAGCAACAAGAT	MRP/Iar
Atrm^{mv}	deletion	1	est13-117	TGCATCCTGTAAGCCAATTT	rAtrn-mv-12 est13-113	TGCAGGTGCAGGTAAGACAG GCAGGCCAGGTCTATGTGTA	MV/Opu
Atrn^{zi}	deletion	1	Atrn-F	GGAGTTGGCAACTGAAGAAC	Atrn-R	GAAAGGGCAGAGACAAAGTT	SER/Kyo, WTC.ZI-*Atrn^{zi}*/Kyo, ZI/Kyo
Cacna1a^{gry}	SNP	2 (PsiI)	rCacna1a-14-F	CGTCCTGAAGTCAATCATGAAG	rCacna1a-14-R	AGTCTCAGCTGCTCTGTGGTT	GRY/Idr, WTC.GRY-*Cacna1a^{gry}*/Kyo
Ednrb^{sl}	deletion	1	rSl-Del-F rSl-Wild-F	CCTCCTGGACTAGAGGTTCC AGGCATTAATGGGAATGTCG	rSl-Del-R rSl-Wild-R	ACGACTTAGAAAGCTACACT ACTCCAGTCTGATGCGTTCC	AR-*Ednrb^{Sl}*/Okkm, LE.AR-*Ednrb^{Sl}*/Okkm
Flcn	SNP	3	BHDRI	CCCTCTGCCACTTCTGCGA	BHDRJ	CTCGCACATGTCTGACTTCT	BHD/Dspe
Foxn1^{rnu}	SNP	3	Whn-ex8-F	CCTCTACACCCAATGCATCC	Whn-ex8-R	GCAGAGAGTATCGTGCATGGTC	BUF.Cg-*Foxn1^{rnu}*/Mna
Gja8	SNP	2 (AciI)	Cx50-7F	GCCAAGCCTTTTAGTCAG	Cx50-7R	TCACTAGGACAGTGGGTTTA	BN.UPL-(*D2Rat134-D2Rat2*)/Cas
Hr^{krh}	SNP	2 (Hpy188I)	rHr-05	GCAGGCAGCAGAATCTTTG	rHr-06	TCCTGTGGATGTCTCTGGTG	F344-*Hr^{krh}*/Kyo
Kcnq1^{dfk}	deletion	1	rKcnq1-75 rKcnq1-72	GAGCGGCTTGTCTTCTTCAACTT CACACCCTCTGGGAAGACAC	rKcnq1-76 rKcnq1-74	AAACAACACATATGGTAAACATGC GCTGAGGGACCATGAGGATA	WTC-*Kcnq1^{dfk}*/Kyo
Lepr^{fa}	SNP	2 (MspI)	rObn-8	TATGGAAGTCACAGATGATGG	rObn-11	CTTACGATTGTAGAATTCTCTAA	F344.Z-*Lepr^{fa}*/Tj, KZ-*Lepr^{fa}*/Tky
Lich	deletion	1	rLich-ex10-F	CTGGCAGACACCAGTGACAT	rLich-ex10-del_R rLich-ex10-Wild-R	TGGAATTTCGTGAACCAGAA CATCGCTGGTTGTGACTCTG	ALD/Hyo
Lmx1a^{qc}	deletion	1	Lmx1a-F	CCTTGAAGAAAATTAAACACATAG	Lmx1a-R	GTACCTCCTTACTCTAAGAAGTCAGC	F344.ACI-*Lmx1a^{qc}*/Kyo
Mrs2^{dmy}	SNP	2 (AciI)	rMrs2l-31	AAAGTTTGACAAAGAAGGAAACG	rMrs2l-32	GGGGATGGAGGGCTATGTAA	DMY/Kyo, WTC.DMY-*Mrs2^{dmy}*/Kyo
Myo5a^{dop}	deletion	1	MyoV-UIAI	ATCTGGCCTGGGCTACATAG	MyoV-LIBI	GGCTCCCGAAAGTTGTTCTG	DOP/Nem
Plp1^{md}	SNP	2 (AvaII)	rPLP<md>-F	AAGAAAAGGTGGAGGGCAAT	rPLP<md>-R	CCAAAGATCCTGCCTGACAGC	MD/Tama
Prkg2	deletion	1	rPrkg2-F	TGTATTTTCCCGTCCGACAC	rPrkg2-KMI-R rPrkg2-WT-R	CAGAGTACGCTAGGTTCCAAGG TCCTTCGATGCCACCGTAAT	KMI/Tky
Scn1a^{m1Kyo}	SNP	2 (BclI)	SCN1A-ex22-F	TGACTTTTCTTTCTCTCCGTTTG	SCN1A-ex22-R	TGGCTGCAATAATCACTTTGTT	F344-*Scn1a^{m1Kyo}*
Scn1a^{m2Kyo}	SNP	3	SCN1A-ex11-F	CTTGCACCTCATTGAAATGGT	SCN1A-ex11-R	AAAACCCCACCCATCTCTTC	F344-*Scn1a^{m2Kyo}*
Tg^{rdw}	SNP	2 (NlaIV)	rdw-F	CAATGCATCAGTTCTGGTGTTCTT	rdw-R	GACCCCCAGTCTGTAGTTAGCAGT	WIC-*Tg^{rdw}*/Kts
tm	deletion	1	TRM brk(-)-F TRM brk(+)-F	TTCAACAGAGGAATGGATACAGAAA CAATTAGGTAAGATTCCCAGCATTT	TRM brk(-)-R	CACTCCATGCTTCCTTTTCAACAGC	SER/Kyo, TRM/Kyo, TRMR/Kyo
Unc5h3^{cvd}	SNP	2 (BstEII)	rUnc5h3-15	CAGATCAGCTCTGCTTTCCA	rUnc5h3-16	ATTTTCAAATTGGGCAGTGG	F344.CVD-*Unc5c^{cvd}*/Kyo
Unc5h3^{hob}	SNP	2 (PmlI)	rUnc5h3-13	TGGGCGAGGATATCAGAAAC	rUnc5h3-14	TCAAGTAATGGCCACCACAC	HOB/Snk

*; PCR products were subjected to agarose gel electrophoresis (1), restriction enzyme treatment (2), or direct sequencing (3). The restriction enzymes that were employed are parenthesized.

Table 2. PCR primers used to detect transgenes

Transgene	Primer set	Forward primer (5' > 3')	Reverse primer (5' > 3')	Strain
CAG promoter	CAGGS-F&R	TAATCAATTACGGGGTCATTAGTTCATAGC	TCCCATAAGGTCATGTACTGGGCATAATGC	LEW-Tg(CAG-EGFP)1Ys, W-Tg(CAG-cre)81Jmsk
Cre	px1679/1701-F&px 2802/2780-R	GCTGGTTGTTGTGCTGTCTCATC	ACCATTGCCCCTGTTTCACTATC	W-Tg(CAG-cre)81Jmsk
EGFP	pr385-EGFP&pr38 6-EGFP	TGAACCGCATCGAGCTGAAGGG	TCCAGCAGGACCATGTGATCGC	LEW-Tg(CAG-EGFP)1Ys
EGFP	GnE-F2&GnE-ER1	TACTATGGTCTATGCTGCACT	ATCTGAAGAAGTCGTGCTGCT	W-Tg(*Gnrh1*-EGFP)Nphy
GFP	s100b-GFP-F&R	CTTGCCTAGGAAGCACAAGG	TCCAGCTCGACCAGGATG	W-Tg(*S100b*-EGFP)Scell
LacZ	LacZ-F&R	ATATGTGGCGGATGAGCGGCA	GCGCTCCACAGTTTCGGGTTT	DA-Tg(CAG-lacz)19Jmsk
LoxP site	LoxP-sense-F&Lox P-antisense-R	CAACGTGCTGGTTGTTGTGC	CTTCGGGCATGGCGGACTTG	W-Tg(CAG-DsRed2/GFP)15 Jmsk
Luciferase	Luc-F&R	AACATAAAGAAAGGCCCGGC	TGGAGAGCAACTGCATAAGGC	LEW-Tg(Gt(*Rosa*)26Sor-luc) 11Jmsk
modified LacZ	pMOD-LacZ-1&2	GATGGCAGCTACCTGGAAGA	CATCACAGGCTTCAGCTTCA	LEW-Tg(Gt(*Rosa*)26Sor-lac z)44Jmsk
pDsRed2-C1	DsRed2_F&R	TGTCCCCCCAGTTCCAGTAC	GTCCACGTAGTAGTAGCCGG	W-Tg(*Alb*-DsRed2)34Jmsk, W-Tg(CAG-DsRed2/GFP)15 Jmsk
YFP	pr385-EGFP&pr38 6-EGFP	TGAACCGCATCGAGCTGAAGGG	TCCAGCAGGACCATGTGATCGC	W-Tg(*Slc32a1*-YFP*)1Yyan, W-Tg(*Slc32a1*-YFP*)2Yyan

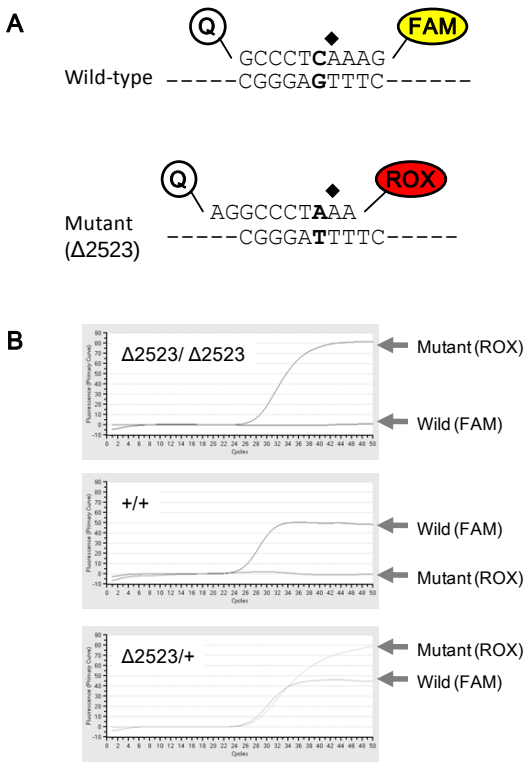

Figure 3. Detecting the KAD rat *Apc*-mutant allele. (A) Fluorescence-labeled probe hybridization with wild-type (upper) and mutant (lower) alleles. The 5' ends of the probes are labeled with a quencher (Q) and the 3' ends are labeled with fluorescent FAM or ROX. The C-to-A nonsense mutation sites that result in S2523X (Δ2523) are indicated in bold for the probe and complementary sequences. The RNA residues in the probe are indicated by the filled in square. When the probe hybridizes with the appropriate sequence, an RNA-DNA complex forms. Next, the RNaseH digests the complex. During PCR product denaturing, digested small molecules with fluorescence are released and detected. (B) Wild-type and mutant allele quantification by using Cycleave® real-time PCR. Mutant-homozygote genomic DNA (top), wild-type homozygote DNA (middle), and heterozygote DNA (bottom) is used. Note that mutant and wild-type alleles are distinguished clearly among all of the genotypes.

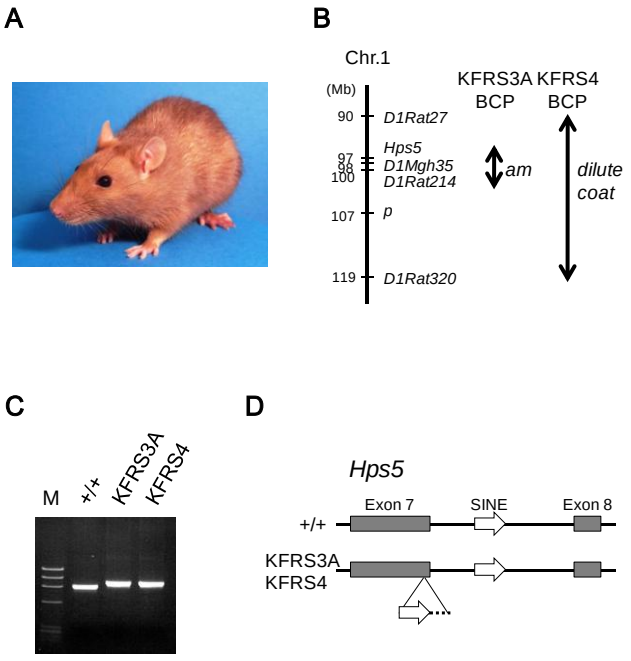

Figure 4. *am* mutation identification. (A) Dilute coat color of the KFRS3A/Kyo strain. KFRS3A is homozygous for the am mutation and its major coat color genotype loci are a/a, B/B, C/C, and D/D. (B) Physical map around am and position of am which was determined by using linkage analyses with two different crosses. am does not undergo recombination with D1Mgh35 and D1Rat214. The critical positions of am and dilute coat that were determined by using the different crosses are indicated with the double-headed arrows. (C) Electrophoresis of PCR products that were obtained by amplifying rat Hps5 exon7 and exon8. Larger products were obtained for KFRS3A and KFRS4 compared to the control. M; molecular marker ϕX174-HincII digest. (D) Genomic organization of Hps5 exon 7 and exon 8 for KFRS3A and KFRS4. A genomic sequence that mainly consisted of a SINE sequence, but was not completely determined because of the presence of a repetitive sequence, was inserted into Hps5 exon 7. This insertion is possessed by the KFRS3A and KFRS4 strains.

KFRS4 strains are derived from a fancy rat colony where the strict inbreeding was not carried out[10]. Thus, it is likely that both KFRS3A and KFRS4 strains may inherit the SINE insertion from the common ancestor kept in the fancy rat colony.

The Kaken hairless (*khr*) mutation is responsible for causing a recessive coat texture (Figure 5A). The mutation arose spontaneously from a colony of Gunn's rats, homozygous for jaundice (*j*) mutation, at Kaken pharmaceutical company in 1987. The *KHR*/Kyo stain was established and deposited to the NBRP-Rat. The *khr* was mapped to the telomeric part of Chr 7 to which the cluster of keratin (*Krt*) genes was also mapped (Figure 5B). Recently, Nanashima et al. identified an 80-kb deletion that is present in the *Krt* gene cluster of the Hirosaki hairless rat (HHR)[12]. We examined whether *KHR* possesses the same deletion as the HHR strain or not, since the HHR strain hairless phenotype is very similar to the *KHR* strain phenotype[13]. As shown in Figure 5C, the *KHR* strain genomic DNA produced the same PCR products as those for HHR when the primer sets for diagnosing the deletion were used[12]. This findings indicate that *KHR* and HHR strains share the 80-kb deletion that contains five basic *Krt* genes and the hairless phenotype of the *KHR* strain is caused by this deletion. HHR was derived from Sprague-Dawley rat colony maintained at Hirosaki Univeristy in 1984[12]. In those days, Sprague-Dawley rats carrying the *j* mutation was established[14]. Thus, it is speculated that the 80-kb deletion may originally occur in the Sprague-Dawley colony and it may be inherited to the HHR and *KHR*, respectively.

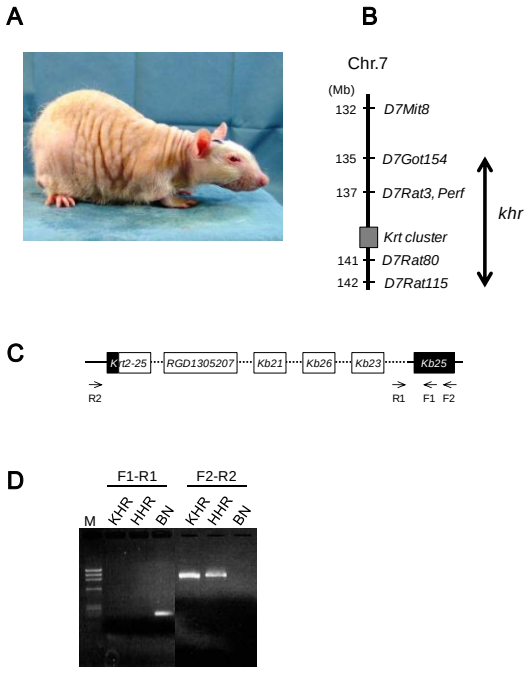

Figure 5. Fine mapping of *khr* on distal Chr7. (A) A rat that is homozygous for the krh mutation at 12 weeks of age. (B) Physical map around *khr*. *Khr* does not undergo recombination with D7Rat3, D7Rat80, and Perf in the 108 (BN/SsNSlc × *KHR*)F1 × *KHR* backcross progeny. The critical position of *khr* is indicated by the double-headed arrow. (C) Scheme for genomic structure of the 80-kb deletion that is identified in the HHR rat. Open boxes represent the deleted genes and dashed line represents deleted genomic sequence in the HHR rat[12]. Positions of primers, F1, F2, R1, and R2 are shown in arrows. (D) Electrophoresis of PCR products that were obtained by amplifying the breakpoints of an 80-kb deletion that was identified within the HHR strain[12]. A set of F1 and R1 primers was used to detect the wild-type allele, while a set of F2 and R2 primers was used to detect the mutant allele. Note that *KHR* possessed a pattern that was identical with HHR. M; molecular marker ϕX174-HincII digest.

Conclusion and Prospects

A rat strain genetic quality control system has been established at the NBRP-Rat. It can be used to guarantee the genetic quality of NBRP-Rat strains. Therefore this ensures that researchers can produce highly reproducible results in their experiments when they employ strains that are acquired from NBRP-Rat. Easier and faster genotyping methods will need to be employed at the NBRP-Rat concomitant with the increasing number of the rat strains maintained as living stocks. Additionally, cost-effective sample storage will be necessary to perform retrospective analyses of these strains. A promising way to resolve these issues is using the Amp-FTA method followed by performing Cycleave® real-time PCR.

Materials and Methods

PCR templates

Genomic DNA that is isolated from tail biopsies that are approximately 3 mm long by using an automatic DNA isolation system (PI-200, KURABO, Japan) is used for genetic monitoring. DNA from blood that has been immobilized on to the FTA® card is used to diagnose genetic mutations[5]. Briefly, once a rat has been weaned, a few aliquots of blood from the tail clipping are smeared on to an FTA® card. PCR templates are then prepared by punching out discs from the FTA® card which are 1.5 mm in diameter by using a conventional ear puncher.

PCR primers

The following twenty genetic markers are used for genetic monitoring; *D1Mgh19*, *D1Rat126*, *D2Rat202*, *D2Rat49*, *D3Rat80*, *D3Rat24*, *D4Rat101*, *D5Mgh23*, *D5Rat147*, *D6Rat105*, *D7Rat103*, *D9Mit3*, *D10Rat223*, *D11Rat63*, *D14Rat110*, *D14Rat75*, *D16Rat35*, *D18Mit1*, *D19Rat12*, and *DXRat16*. The sequences for each are available through the Rat Genome Database at http://rgd.mcw.edu/. These markers were selected based on polymorphic rates among rat strains, differences in the molecular sizes of PCR products, and mapping positions. PCR primers that are used to diagnose genetic mutations and transgenes are shown in Table 1 and 2.

PCR and direct sequencing

PCR analyses and PCR product direct sequencing was carried out as described previously[15]. Briefly, PCR was carried out in 15 μL volume reactions which contained 20 ng of genomic DNA.

Real-time PCR

A primer set and probes specific to the mutant and the wild-type alleles were synthesized to detect the *Apc* gene nonsense mutation in the KAD rat by real-time PCR[7]. Real-time PCR was carried out by using the Cycleave® core kit and the Thermal Cycler Dice® Real Time System II (Takara, Kyoto, Japan). The nucleotide sequences of the primer set that was used are as follows: rtApc-F&R; ATCCATCTGTTCAGGCAGGT and GTGTTCACGCTTCCAG GTTC. The sequence of the *Apc* mutant allele probe was aggccct aaa and its 3' end was labeled by using ROX. The sequence of the *Apc* wild-type allele probe was gccctcaaag and its 3' end was labeled by using FAM. The 5' ends of both of the probes were labeled by using the fluorescence quencher, Eclipse.

Genetic mapping of *khr* mutation

One hundred eight backcross progeny were produced from a (BN/SsNSlc × *KHR*) F1 × *KHR* cross. DNA pooling was performed to initially map *khr*[16]. DNA pools from 17 randomly selected *khr*/*khr* rats and 17 *khr*/+ rats were genotyped to identify 53 SSLP markers that were distributed across all of the chromosomes.

Genetic mapping of dilute coat color mutation

In the genetic mapping of the ear malformation mutation, dumbo (*dmbo*)[10], we found that the KFRS4/Kyo strain harbors a coat color

mutation. We tentatively called it dilute coat color. To map the mutation, 28 (BN × KFRS4) F1 × KFRS4 backcross progeny wee used.

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