Whole-genome sequencing of CRFK and PG-4 cells to infer the phenotype of the original donors

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Funding

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI grants (award numbers 24K01783 (T. I.) and 24K09248 (A. A.)).

Author contributions

Conceptualization: A.A., Data curation: T.K., A.K., R.H., and T.I., Formal analysis: G.T. and

R.G., Funding acquisition: A.A., Investigation: G.T., R.G., T.K., R.H., and A.A., Methodology: G.T., R.G., T.K., T.I., and A.A., Project administration: N.S. and A.A., Supervision: N.S. and A.A., Validation: R.G. and A.A., Visualization: T.G., N.S., and A.A., Writing - original draft: A.A., Writing - review & editing: T.G., T.K., T.I., and N.S.

Data availability

These raw read data of the short and long read sequencings were deposited in the DDBJ Sequence Read Archive (DRA) with BioProject accession PRJDB37530 (DRR748391-DRR748394, DRR786948, and DRR786949). All other data generated or analyzed during this study were included in this published article (and Supplementary Information files).

Conflicting interests

The authors have no conflicting interests to declare.

Abstract

Objectives Crandell-Rees Feline Kidney (CRFK; kidney-derived) cells and PG-4 cells (astrocyte-derived) have been in use and have been passaged for decades in laboratories worldwide; however, no detailed information on the genetic background of the donor individuals is available, particularly regarding phenotype characteristics such as coat and iris color.

Methods We performed whole-genome sequencing of CRFK and PG-4 cells. We analyzed the resulting data to infer the phenotype of the individual from which the cells were derived, specifically for the coat color, coat length, coat pattern, and iris color.

Results Our data suggested that CRFK cells originated from a cat with long chocolate-brown fur lacking stripes and with non-blue irises; PG-4 cells originated from a cat with long bicolored white and dark brown fur without stripes, and with non-blue irises. Analysis of publicly available RNA-seq data confirmed that genes associated with coat phenotype and iris color are expressed in the skin and eyes, as well as in various other organs.

Conclusions and relevance Variants of the genes affecting coat phenotype and iris color may influence physiological functions throughout the body. These results shed light on the previously unknown genetic background of commonly used feline cultured cells and the phenotype of the donor individuals. These insights may facilitate a more accurate interpretation of data derived from feline cultured cells and inform guidelines to establish cell lines with various genotypes (for example, disease-associated variants) through genome editing, which will help elucidate genetic factors affecting resistance to infectious diseases and cancer, the risk of internal organ afflictions, and genetic diseases such as deafness in cats with white fur and blue irises.

Keywords: genomics; coat pattern; iris color; RNA sequencing;

Introduction

Domestic cats (*Felis catus*) are widely kept as companion animals around the world, and the phenotypic diversity of their coat color, coat pattern, coat length, and iris color has been a topic of interest to breeders and scientists alike. Recent advances in genomics have markedly improved our understanding of the genetic basis controlling these diverse phenotypes, and the respective genotype-phenotype relationships are being increasingly elucidated.[1] Examples include the *TYR* gene, which is the color locus associated with albinism and a color point phenotype;[2–4] the *TYRP1* gene, conveying brown coat color;[2,5] the *MC1R* genes, associated with in red coat color;[6] the *KIT* gene, determining white coat and white spotting;[7–11] the *ARHGAP36* gene, involved in orange coat color;[12,13] the *MLPH* gene, causing dilute pigmentation;[14] the *ASIP* gene, controlling the expression of the agouti pattern;[15] the *LVRN* gene, determining the type of tabby pattern;[16] the *Dkk4* gene, associated with the ticked tabby pattern;[17] the *FGF5* gene, involved in long-fur variants;[18,19] the *KRT71* and *LPAR6* genes, involved in hairless and rexing (curly hair) phenotypes;[20–22] and the *PAX3* gene, which is associated with blue irises.[23,24] Furthermore, technological advancements such as long-read sequencing facilitated the construction of a more continuous and accurate draft genome of the domestic cat,[25–27] and a plethora of genetic polymorphisms associated with disease have been identified.[17,25,28–32]

Immortalized cell lines are an indispensable tool in current biomedical and veterinary science. Crandell-Rees Feline Kidney (CRFK) cells, derived from the kidneys of a domestic cat,[33–34] have been used for numerous virological studies, including feline calicivirus,[35–37] feline immunodeficiency virus,[38–41] feline coronavirus,[42–45] and various feline endogenous retroviruses such as RD114,[46-48] as well for vaccine development.[49–51] PG-4 cells, derived from feline fetal astrocytes, have also played an important role in retrovirus research.[52–53] These cell lines have been passaged for decades in laboratories worldwide and have facilitated numerous scientific breakthroughs. However, despite their widespread use, no detailed information on the genetic background of the donor individuals, particularly with regard to their phenotype, such as coat and iris color, is available. Genomic information on such cell lines is crucial for a deeper understanding of their biological properties, and elucidating the phenotypes of their donors may provide new insights into the influence of specific genotypes on cell properties.

In this study, we performed whole-genome sequencing of CRFK and PG-4 cell lines. The sequencing data were used for in-depth analysis of the nucleotide sequences of genes known to be associated with coat and iris phenotypes (i.e., *TYR*, *TYRP1*, *MC1R*, *KIT*, *ARHGAP36*,

MLPH, *ASIP*, *LVRN*, *DKK4*, *FGF5*, *KRT71*, *LPAR6*, and *PAX3*; Table 1). Based on the genotyping results for each gene, we inferred the previously unknown phenotypes of the donor individuals. Furthermore, analysis of publicly available RNA-seq data confirmed that these genes were expressed in skin and eyes as well as in various other tissues throughout the body. Our results provide fundamental insights into the genetic background of widely used feline cell lines, offering valuable information that will be beneficial for interpreting the results of future research using these cell lines, such as informing guidelines to establish cell lines with various genotypes, including disease-associated genotypes, through genome editing.

Materials and methods

Cell lines and culture conditions

CRFK cells (JCRB9035, RRID: CVCL_2426) and a feline sarcoma-positive leukemia-negative (S+L−) astrocyte cells termed PG-4 (S+L-) (JCRB9125, RRID: CVCL_3322) were obtained from the JCRB Cell Bank (National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan). CRFK cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco), penicillin (100 IU/mL), streptomycin (100 ng/mL) (Sigma-Aldrich, St. Louis, MO, USA), and 1% non-essential amino acids (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 37 °C and 5% CO_2. PG-4 cells were cultured in McCoy's 5A Modified Medium (Gibco) supplemented with 10% heat-inactivated FBS (Gibco), penicillin (100 IU/mL), and streptomycin (100 ng/mL) (Sigma-Aldrich) at 37 °C and 5% CO_2. For cell passaging, cells were washed using BASIC DPBS (no calcium or magnesium; Gibco) and then treated with TrypLE™ Express Enzyme (1X; Gibco) for 5 min at 37 °C and 5% CO_2 conditions.

Paired-end short-read whole-genome sequencing

Genomic DNA was extracted from logarithmically growing cells (approximately 1×10^6 cells) using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity (A260/A280 ratio) of the extracted DNA were measured using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). Genomic DNA extracted from CRFK cells and PG-4 cells was used for library preparation and paired-end sequencing (150 bp read length) at Novogene (Beijing, China) using a Rapid Plus DNA Lib Prep Kit for Illumina V2 (Illumina) and an Illumina NovaSeq X Plus platform

130 (Illumina). Library quality control was performed by Novogene using qPCR and fragment size
131 analysis according to Novogene's standard protocols. The raw sequence data were obtained
132 as .fastq files.

133 **Mapping of paired-end reads and quantification**

134 Adapter sequences were trimmed, and low-quality reads were removed from the original .fastq
135 files using fastp software (ver. 0.21.0) with default parameters. Reads in processed .fastq files
136 were mapped to the reference genome sequence of *Felis catus*, F.catus_Fca126_mat1.0 (RefSeq
137 assembly GCF_018350175.1) or AnAms1.0 (Genbank assembly GCA_013340865.2)[27] using
138 bwa mem software (ver. 0.7.19) with the setting "-R
139 "@RG¥tID:sample1¥tSM:sample1¥tLB:lib1¥tPL:ILLUMINA"" and default parameters
140 otherwise. Mapping results were obtained as .sam files. Text data in .sam files were transformed
141 to binary data in sorted .bam files using Samtools (ver. 1.7) with default parameters. Index files
142 were created for the obtained .bam files in the form of .bam.bai files.

143 **Small variations in coat morphology-related genes**

144 The variations of coat morphology-related phenotypes of the donor cats from which CRFK and
145 PG-4 cell lines originated were inferred based on the comparisons between the wild-type
146 sequences and mapped sequences on variant positions of each coat morphology-related gene
147 (Table 1). Coat phenotype-related polymorphisms in *TYR*, *TYRP1*, *MC1R*, *KIT*, *MLPH*, *ASIP*,
148 *LVRN*, *Dkk4*, *FGF5*, *KRT71*, and *LPAR6* genes, which relate to phenotypes such as the color,
149 brown, red, white glove, dilution, agouti, tabby, ticked, long-fur, hairless, and rexing (Table 1),
150 were examined using alignments of paired-end sequence reads with wild-type sequences.

151 **Large deletions in intron 1 of ARHGAP36 and the intergenic region between *KIT* and**
152 ***KDR* genes**

153 The deletion of a part of intron 1 of the *ARHGAP36* gene (X: 109,186,183_109,191,258 in
154 AnAms1.0; Table 1) was reported to produce orange coat color,[12,13] and that of the intergenic
155 region between the *KIT* and *KDR* genes (B1: 161142880_161237957 in F.catus_Fca126_mat1.0,
156 Table 1) produces salmiak coat color.[11] The occurrence of these deletions in CRFK and PG-4
157 was estimated by comparing the mapped read number distributions between these regions and
158 their neighboring regions with the same region length. It should be noted that
159 F.catus_Fca126_mat1.0 has a deletion of the genomic region corresponding to X:
160 109186183_109191258 in AnAms1.0 (Table 1). Thus, AnAms1.0 (or felCat9[25]) was used as the

161 reference genome for analysis of the orange locus.

162 **Insertions of FERV1-LTR and RD114-LTR into *PAX3***

163 The insertions of homologous sequences of the long terminal repeat (LTR) of the feline
164 endogenous retrovirus 1 (FERV1), termed FERV1-LTR, into a specific region in intron 4 of
165 *PAX3* (C1: g.205833101_205833102 in F.catus_Fca126_mat1.0, Table 1) or the LTR of RD114
166 retrovirus, termed RD114-LTR, into another specific region in the same intron (C1:
167 g.205834854_205834855 in F.catus_Fca126_mat1.0, Table 1) are associated with blue iris color
168 in cats.[23,24] The occurrence of such insertions was estimated by extracting the genome reads that
169 were mapped in both FERV1-LTR and intron 4 of *PAX3* or both RD114-LTR sequences and
170 intron 4 of *PAX3*. The genome sequence of RD114-LTR was obtained from the NCBI database
171 (https://www.ncbi.nlm.nih.gov/nuccore/NC_009889.1/).

172 **Long-read whole-genome sequencing**

173 Genomic DNA of CRFK cells and PG-4 cells was extracted for long-read genome sequencing
174 using the NucleoBond HMW DNA kit (TaKaRa, Tokyo, Japan), and libraries for the Oxford
175 Nanopore Technologies (ONT, UK) sequencer were constructed using a ligation library
176 preparation kit (SQK-LSK114, ONT). All procedures were performed according to the
177 manufacturer's instructions, and the quality and molecular weight of the genomic DNA were
178 measured using the Qubit fluorometric quantification system (Thermo Fisher Scientific).
179 Sequencing was conducted using a PromethION sequencer (PromethION 2 Solo, ONT) and
180 R10.4.1 flow cells. POD5 files were basecalled to .fastq files using MinKNOW software
181 v25.05.14.

182 **Insertions of FERV1 homolog into *KIT* examined by long-read sequencing**

183 The insertion of FERV1 into a specific region of intron 1 of the *KIT* gene (B1:
184 161388687_161388688 in F.catus_Fca126_mat1.0, Table 1) and that of FERV1-LTR make the
185 coat color of cats white-spotted and white throughout, respectively.[8] The occurrence of such
186 insertions in CRFK and PG-4 cells was estimated by mapping the reads of long-read sequencing
187 to the genome sequence named KIT-FERV1 sequence using minimap2 (ver. 2.30), where the
188 KIT-FERV1 sequence is a part of the *KIT* intron 1 sequence with the insertion of the FERV1
189 sequence, as shown in Supplementary Figure 1 of the study by David et al.[8]

190 **Transcriptome data analysis of coat phenotype- and iris color-related genes in various**
191 **tissues of domestic cats**

Mapped RNA-seq data on the F.catus_Fca126_mat1.0 were obtained in .bam and .bam.bai formats for various tissues from the Ensembl public database,[54] specifically, the file transfer protocol (ftp) site (https://ftp.ensembl.org/pub/data_files/felis_catus/F.catus_Fca126_mat1.0/rnaseq/). The annotation file of the F.catus_Fca126_mat1.0 in gene transfer format (GTF) was obtained from the NCBI database (Bethesda, MD, USA). In the file, the chromosome numbers were converted from NC_058368.1, NC_058369.1, etc. to A1, A2, etc.

Using FeatureCounts with the options—M-O—fraction,[55] read counts data for each gene described in the GTF file were obtained from the .bam and .bam.bai formatted files. This count data could also be obtained from a file "F.catus_Fca126_mat1.0.ENA_gene_exp_mat_by_FeatureCounts.csv" deposited in F.catlas (https://sites.google.com/view/fcatlas/). From these counts, the transcript level of each gene was calculated by dividing the read count by the total exon length. The total transcript level was defined as the sum of all the transcript levels of the genes. Based on this result, the transcripts per million (TPM) of each gene were calculated as 1,000,000*(transcript level)/(total transcript level).

Results

Short- and long-read whole-genome sequencing

For CRFK and PG-4 cells, the total output of raw sequence data of 150 bp paired-end sequencing was 60.6 Gb and 60.1 Gb, and the [average] $\pm$ [standard deviation] of mapping depth of the reads for F.catus_Fca126_mat1.0 were 23.59 ± 9.79 and 23.45 ± 8.17, respectively; the total output of raw sequence data of long-read sequencing was 9.6 Gb and 11.8 Gb, N50 values were 23.5 kb and 38.2 kb, and the [average] $\pm$ [standard deviation] of mapping depth of reads for F.catus_Fca126_mat1.0 were 3.91 ± 3.11 and 4.79 ± 3.34, respectively. The average and standard deviations specified above were estimated through the entire genome region without the regions with singular sequences where the read coverage exhibited >100.

Predicted phenotype of the CRFK cell donor

The mapping of paired-end reads from the whole-genome sequences of the CRFK cell revealed that most coat morphology-related genes were conserved (Table 1). However, the following variations were identified in mapped genome reads from CRFK cells: a homozygous mutation

223 of c.123_124del of the *ASIP* gene (the *nonagouti* allele; Fig. 1), a homozygous mutation of
224 c.475A>C of the *FGF5* gene (the *long coat* allele; Fig. 2), a homozygous mutation of
225 c.2522G>A of the *LVRN* gene (the *blotched-tabby* allele; Fig. 3), a homozygous mutation of
226 c.8C>G of the *TYRP1* gene (the *chocolate-blown* allele; Fig. 4), and a heterozygous mutation of
227 c.83delT of the *MLPH* gene (the *dilute* allele; Fig. 5). We found no 5 kbp deletion at intron 1 of
228 the *ARHGAP36* gene and no 95 kbp deletion at the intergenic region between the *KIT* and *KDR*
229 genes. We also found no genome reads that could be mapped in both the *PAX3* intron 4 and
230 FERV1-LTR or both the *PAX3* intron 4 gene and RD114-LTR sequences. Additionally, four
231 reads from the long-read sequencing mapped to the KIT-FERV1 sequence, but these reads
232 contained no insertions of FERV1 and FERV1-LTR (Fig. 6).

233 The abovementioned homozygous mutation of the *TYRP1* gene is known to exhibit chocolate-
234 brown coat color.[2] Additionally, the homozygous mutation of the *FGF5* gene was known to
235 exhibit long fur.[18] However, the mutation of the *LVRN* did not influence coat morphology in this
236 case because of the mutation of the *ASIP* gene that suppresses the stripe pattern on the cat's skin
237 coat, and the mutant genotype of the *MLPH* gene was recessive.[1] Therefore, the cat from which
238 the CRFK cells originated is expected to have had long, chocolate brown, unstriped fur and non-
239 blue eyes (Fig. 7).

240 **Predicted phenotype of the PG-4 cell donor**

241 Mapped paired-end reads from the whole-genome sequences of the PG-4 cell confirmed that
242 most coat morphology-related genes were conserved (Table 1) but identified following
243 variations: a homozygous mutation of c.123_124del of the *ASIP* gene (Fig. 1), a compound
244 heterozygous mutation of c.474delT and c.475A>C of the *FGF5* gene (Fig. 2) where one allele
245 exhibits c.474delT and the other allele exhibits c.475A>C, the heterozygous mutation of
246 c.2522G>A of *LVRN* gene (Fig. 3), a heterozygous mutation of c.904G>A of the *TYR* gene (the
247 *Siamese-color* allele; Fig. 8), and a homozygous mutation of c.416C>A of the *LVRN* gene (Fig.
248 9). We found no large deletions at intron 1 of the *ARHGAP36* gene and the intergenic regions
249 between the *KIT* and *KDR* genes, as was also the case for CRFK. We also found no genome
250 reads that could be mapped in both the *PAX3* intron 4 and FERV1-LTR or both the *PAX3* intron
251 4 gene and RD114-LTR sequences.

252 We found that six long-read sequences mapped to the KIT-FERV1 sequence, and four of them
253 contained the insertions of the more than 7 kbp FERV1 homolog sequence (Fig. 6). This
254 suggested the PG-4 genome contains the heterozygous insertion of the entire FERV1 homolog

sequence.

Note that the mutant genotype of the *TYR* gene was recessive, and the two heterozygous mutations of the *LVRN* do not influence coat morphology in this case because of the mutation of the *ASIP* gene, the same as in the case of CRFK.[1] However, the effect of *FERV1* is known to be dominant.[8] Additionally, the compound heterozygous mutation of the *FGF5* gene produces long fur.[18] Therefore, the cat from which the PG-4 cells originated is expected to have had white and dark brown, unstriped, long fur and non-blue irises (Fig. 7).

Expression features of coat phenotype- and iris color-related genes in various tissues

The analysis of publicly available RNA-seq data from various organs of domestic cats in the Ensembl database (https://ftp.ensembl.org/pub/data_files/felis_catus/F.catus_Fca126_mat1.0/rnaseq/) revealed that coat phenotype-related genes are expressed in various organs at levels (TPM) comparable to or greater than those in the skin (Fig. 4, Table S2). For example, *TYR*, *TYRP1*, and *MLPH* were highly expressed in the ear tip, retina, and optic nerve, and *MC1R*, *KIT*, *DKK4*, and *FGF5* were highly expressed in the brain. *KIT*, *DKK4*, and *LPAR6* tend to be expressed throughout the body, with prominent expression in the reproductive organs. Additionally, *ARHGAP36* was highly expressed in the spinal cord, and *ASIP*, along with *MLPH*, was highly expressed in the lungs. The iris color-related gene *PAX3* was also highly expressed in the cerebellum, in addition to visual organs, and, along with *DKK4*, in the kidney and embryonal tissue.

Discussion

We performed whole-genome sequencing of CRFK, a cultured feline kidney cell line, and PG-4, a feline fetal astrocyte-derived cell line, and analyzed the genome data to determine the coat color, hair length, coat pattern, and iris color of the donor individuals. The results suggested that the CRFK cell line originated from a cat with long dark brown hair without stripes and non-blue irises, and the PG-4 cell line came from a cat with long fur, dark brown and white bicolor coat without stripes, and non-blue irises.

Coat morphology and iris color are phenotypes that can be readily identified and used to predict from genotypes. However, the expressions of these genes are not limited to the skin and eyes; rather, they are expressed in various tissues throughout the body (Fig. 10). Therefore, it is possible that differences in these genotypes may contribute to disease susceptibility in specific

organs. The effects of these genetic variations are likely weaker than those of the rearing environment and may not be significant, especially in young, healthy individuals; however, as domestic cats have become longer-lived in recent years, individual-specific risks may become more apparent and the underlying genotypes may exert increasing effects as the cats age. Therefore, elucidating the relationships between genotypes underlying readily recognizable phenotypes and disease risk is crucial from the perspectives of both medical care and welfare, such as prevention and symptom suppression/alleviation.

Results from research on cultured cells to elucidate disease mechanisms and develop therapeutic drugs must be interpreted with caution, i.e., not only on the experimental results obtained using established, immortalized cells but also with regard to their genotype and expression profiles of the genes harboring polymorphisms in various tissues and organs. However, so far, such information has not been available, even for certain frequently used cell lines such as CRFK. The present study provides such information and facilitates a more comprehensive interpretation of research on feline cultured cell lines.

Furthermore, with the availability of detailed genomic information, genome editing can be applied to these cell lines to establish various derivative lines that differ only in specific aspects, e.g., regarding coat morphology or other traits, and compare their properties. This is expected to advance research on the regulatory mechanisms of coat color, for example, the validation of the role of the novel *KIT* isoform caused by FERV1-LTR insertion in the formation of white coat,[56] as well as the relationships between the visually identifiable phenotypes examined in the present study and disease risk and treatment methods. Simultaneously, such detailed genomic information can inform guidelines for establishing cell lines with various disease-associated genotypes through genome editing. Establishing such derivative cell lines can help elucidate genetic factors associated with the resistance to infectious diseases and cancer, the risk of internal organ afflictions, as well as genetic diseases such as deafness in cats with white fur and blue irises.

It should be noted that the karyotype of CRFK differs from that of the normal domestic cat genome.[33] It is thus possible that the karyotype of PG-4 cells, which has not been reported, differs from the normal domestic cat genome. However, using only whole-genome analysis of the present sequence read data, approximately 60 Gb paired-end sequencing data and 10 Gb long-read data, it was difficult to detect such large structural variations. Similarly, it was difficult to identify the accurate sequences and locations of various long repeat sequences,

endogenous retroviral sequences, and their homologs because of the deficiency of the read coverage, particularly in the long-read coverage. However, these sequences may also be associated with various diseases, as exemplified by the involvement of FERV1-LTR in deafness in cats[8,23,24] and the positive and negative roles of enFeLV-LTR and enFeLV-env in resistance to exogenous feline leukemia virus.[57-59] The larger volume of ongoing long-read sequencing is expected to resolve these issues in the future.

Ethical approval

This work did not involve the use of animals and, therefore, ethical approval was not specifically required for publication in *JFMS*.

Informed consent

This work did not involve the use of animals (including cadavers) and therefore informed consent was not required. No animals or people are identifiable within this publication, and therefore, additional informed consent for publication was not required.

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497 **Table 1: Phenotypic traits of coat and iris conferred by DNA variants, and genotypes of**
498 **CRFK and PG-4 cells.** List of genes and phenotypes focused on in this study, mutation position
499 on CDS coordinate and on genomic coordinate provided by Fca126_mat1.0 and AnAms1.0, and
500 a summary of genotypes of CRFK and PG-4 cells obtained by the present study.

Locus Gene	Phenotype (Alleles)	Mutation in CDS (or related noncoding region): F.catus_Fca126_mat1.0 Genome: F.catus_Fca126_mat1.0 Genome: AnAms1.0	Genotype in CRFK	Genotype in PG-4
Color TYR[2–4]	Burmese (c^b)	c.679G>T D1: g.44022819C>A D1: g.46081417C>A	+ / +	+ / +
	Siamese (c^s)	c.904G>A D1: g.44013031C>T D1: g.46071661C>T	+ / +	+ / c^s
	Albino (c^a)	c.939del D1: g.44012996del D1: g.46071626del	+ / +	+ / +
	Mocha (c^m)	c.820_936delinsAATCTC D1: g.44012836_44012998delinsGAGATT D1: g.46071466_46071628delinsGAGATT	+ / +	+ / +
Brown TYRP1[2,5]	Chocolate (b^{ch})	c.1261+5G>A D4: g.38142265G>A D4: g.39391308G>A	+ / +	+ / +
	Chocolate (b^c)	c.8C>G D4: g.38129873C>G D4: g.39378919C>G	b^c / b^c	+ / +
	Cinnamon (b^i)	c.298C>T D4: g.38130163C>T D4: g.39379209C>T	+ / +	+ / +

Amber MC1R[6]	Red (e)	c.250G>A E2: g.61570294G>A E2: g.62623146G>A	+ / +	+ / +
White KIT[7–11]	White (w^w)	(Intron 1: FERV1-LTR insertion) B1: g.161388687_161388688insFERV1-LTR B1: g.163130991_163130992insFERV1-LTR	+ / +	+ / +
	White spotted (w^s)	(Intron 1: FERV1 insertion) B1: g.161388687_161388688insFERV1 B1: g.163130991_163130992insFERV1	+ / +	+ / w^s
	White glove (w^g)	c.1035_1036delinsCA B1: g.161337385_161337386delinsTG B1: g.163079561_163079562delinsTG	+ / +	+ / +
	Salmiak (w^a)	(KIT-KDR Intergenic region: 95 kb deletion) B1: g.161142880_161237957del B1: g.162885414_162980052del	+ / +	+ / +
Orange Arhgap36[12,13]	Orange (o)	(Intron 1: 5 kb deletion) (Lacking) X: g.109186183_109191258del	+ / +	+ / +
Dilution MLPH[14]	Dilute (d)	c.83del C1: g.218197448del C1: g.219535339del	+ / d	+ / +
Agouti ASIP[15]	Nonagouti (a)	c.123_124del A3: g.24831083-24831084del A3: g.25283750_25283751del	a / a	a / a
Tabby LVRN[16]	Blotched tabby (t^b)	c.176C>A A1: g.94477275C>A A1: g.97133509C>A	+ / +	+ / +
	Blotched tabby (t^b)	c.416C>A A1: g.94477515C>A A1: g.97133749C>A	+ / +	+ / t^b

	Blotched tabby (t^b)	c.682G>A A1: g.94477781G>A A1: g.97134015G>A	+ / +	+ / +
	Blotched tabby (t^b)	c.2522G>A A1: g.94536796G>A A1: g.97192983G>A	t^b / t^b	+ / t^b
Ticked Dkk4[17]	Ticked (ti)	c.188G>A B1: g.40429492G>A B1: g.41805975G>A	+ / +	+ / +
	Ticked (ti)	c.53C>T B1: g.40428846C>T B1: g.41805329C>T	+ / +	+ / +
Longhair FGF5[18,19]	Long fur (l)	c.356_357insT B1: g.139634675_139634676insA B1: g.141368532_141368533insA	+ / +	+ / +
	Long fur (l)	c.406C > T B1: g.139645946G>A B1: g.141379803G>A	+ / +	+ / +
	Long fur (l)	c.474del B1: g.139634261del B1: g.141368118del	+ / +	+ / l
	Long fur (l)	c.475A > C B1: g.139634260T>G B1: g.141368117T>G	l / l	l / +
	Long fur (l)	c.577G>A B1: g.139634158C>T B1: g.141368015C>T	+ / +	+ / +
Hairless *Rexing* KRT71[20,21]	Hairless (hr)	c.816+1G>A B4: g.78941699C>T B4: g.80471148C>T	+ / +	+ / +

	Rexing (re)	c.1108-4_1184del, c.1184_1185insAGTTGGAG, and c.1196_1197insT B4: g.78939390_78939470del, B4: g.78939389_78939390insCTCCAACT, and B4: g.78939377_78939378insA B4: g.80468919_80468919del, B4: g.80468918_80468919insCTCCAACT, and B4: g.80468826_80468827insA	+ / +	+ / +
	Rexing (re)	c.445-1G>C B4: g.78943283C>G B4: g.80472733C>G	+ / +	+ / +
Rexing LPAR6[22]	Rexing (re)	c.250_253del A1: g.22865220_22865223del A1: g.23372953_23372956del	+ / +	+ / +
Blue iris PAX3[23,24]	Blue iris (bi)	(Inrton4: FERV1-LTR homolog insertion) C1: g.205833101_205833102insN[395] C1: g.207180017_207180018insN[395]	+ / +	+ / +
	Blue iris (bi)	(Intron4: RD114-LTR homolog insertion) C1: g.205834854_205834855insN[433] C1: g.207181773_207181774insN[433]	+ / +	+ / +

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Figure and captions

(a) CRFK

AICP: c. 123_124
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WT                                                  gacaggaacctgaggagcaactcctc ca tgaacatgttggatctctcttctgtctctatt
ASIP:c.123_124del                                   gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2172:18161:21220/2       -------------------------tc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2154:39418:15051/2       -----------------caactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1121:32465:10021/1          -----------------caactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1121:32483:10021/1          -----------------caactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1127:37254:18192/2          -----------------caactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1265:52002:1288/1           ------------aggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1265:51992:1304/1           ------------aggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1265:52002:1320/1           ------------aggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1295:7352:9540/1            ------aacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1194:49024:17375/1          --caggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2108:45770:16990/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2133:13704:6640/1        gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2267:4218:14234/1        gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:188:22Y5KLLT3:4:2214:3709:14955/2        gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2203:11245:13241/2       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2201:26177:28062/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2163:46842:29295/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
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_R_LH00504:186:22YKYJLT3:1:1204:40518:15132/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:2217:36292:25658/2          gacaggaacctgaggagcaactcctc -- tgaanatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:2278:15156:6752/2           gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2213:10200:20403/2       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:1236:28202:21637/2       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2292:26991:14282/1       tacaggaacctgatgagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2292:26982:14298/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:2209:37069:23319/1       gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:2213:10200:20403/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:1287:7981:14411/2        gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1159:22312:16814/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:1159:22322:16830/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:2201:26177:28062/2          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
LH00504:186:22YKYJLT3:1:2161:8591:24216/1           gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
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LH00504:186:22YKYJLT3:1:2172:34295:5278/2           gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctatt
_R_LH00504:186:22YKYJLT3:1:1295:25567:9251/2        gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctc----
LH00504:186:22YKYJLT3:1:2172:18161:21220/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtc------
LH00504:186:22YKYJLT3:1:1167:44753:20547/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctg-------
LH00504:186:22YKYJLT3:1:1167:44762:20563/1          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctg-------
LH00504:186:22YKYJLT3:1:1217:23422:26652/2          gacaggaacctgaggagcaactcctc -- tgaacatgttggatctctcttc----------
LH00504:186:22YKYJLT3:1:1161:1980:25706/2           gacaggaacctgaggagcaactcctc -- tgaacatgttggatctct--------------
LH00504:186:22YKYJLT3:1:1161:1999:25706/2           gacaggaacctgaggagcaactcctc -- tgaacatgttggatctct--------------
_R_LH00504:186:22YKYJLT3:1:1188:27657:19570/2       gacaggaacctgaggagcaactcctc -- tgaacatgttg---------------------
_R_LH00504:186:22YKYJLT3:1:2224:40370:18689/2       gacaggaacctgaggagcaactcctc -- tgaacatgttg---------------------
LH00504:186:22YKYJLT3:1:1126:14277:18080/1          gacaggaacctgaggagcaactcctc -- tgaacatgttg---------------------
LH00504:186:22YKYJLT3:1:1126:14287:18096/1          gacaggaacctgaggagcaactcctc -- tgaacatgttg---------------------
_R_LH00504:186:22YKYJLT3:1:1238:14203:2859/1        gacaggaacctgaggagcaactcctc -- tgaacatg------------------------
LH00504:186:22YKYJLT3:1:2191:26982:26892/2          gacaggaacctgaggagcaactcctc -- tgaa----------------------------
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(b) PG-4

AICP: c. 123_124
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WT                                                  ggaacctgaggagcaactcctc ca tgaacatgttggatctctcttctgtctctattgtag
ASIP:c.123_124del                                   ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2187:30865:8082/2        -------------caactcctc -- tgaacatgttggatctctcttctttctctattgtag
LH00504:186:22YKYJLT3:1:1206:24541:28270/2          ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:1206:24550:28286/2          ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:2130:12271:4541/2           ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:2225:28054:21957/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:1260:28646:11062/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:2116:20935:13914/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:1293:27878:3515/2           ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:1293:27897:3515/2           ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:2105:45298:24408/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:2184:27749:23447/2          ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:1214:43393:25274/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:2214:41914:25530/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:1160:9340:13850/1           ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
LH00504:186:22YKYJLT3:1:1107:18706:24024/2          ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:1148:38743:22213/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:1148:38761:22213/1       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctctattgtag
_R_LH00504:186:22YKYJLT3:1:1235:30393:18769/2       ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttctgtctct-------
_R_LH00504:186:22YKYJLT3:1:1184:40398:2586/2        ggaacctgaggagcaactcctc -- tgaacatgttggatctctcttct-------------
_R_LH00504:186:22YKYJLT3:1:1261:6991:5839/1         ggaacctgaggagcaactcctc -- tgaacatgttggatctct------------------
_R_LH00504:186:22YKYJLT3:1:1261:7010:5839/1         ggaacctgaggagcaactcctc -- tgaacatgttggatctct------------------
LH00504:186:22YKYJLT3:1:1242:15969:22582/1          ggaacctgaggagcaactcctc -- tgaacatgtt-------------------------
_R_LH00504:186:22YKYJLT3:1:1168:39270:8002/2        ggaacctgaggagcaactcctc -- tgaacatg----------------------------
_R_LH00504:186:22YKYJLT3:1:2267:29071:12119/1       ggaacctgaggagcaactcctc -- tgaacatg----------------------------
```

506 **Figure 1: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells**
507 **with wild-type (WT) and mutant sequences of ASIP genes.** Alignment results of genome
508 sequencing reads of around ASIP: c.123_124; CRFK (a) and PG-4 (b) cells. Left string indicates
509 the name of sequenced read, and red colored symbols indicate coat phenotype-related mutations

510

(a) CRFK

```
                                                                  FGF5: c. 474, c. 475
                                                                                ↓
WT                                                   tccccccaccaggccaaatt ta ccgatgactgcaagttcagggagcgattccaagaaaac
FGF5:c.474del                                        tccccccaccaggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaac
FGF5:c.475A>C                                        tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:1136:23098:8018/2         -------------------- tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:1192:25992:16846/1        ----------------aatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:1187:1481:21444/1         ---------caggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:1220:37134:16734/1        ----cccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2219:7629:10021/2            --cccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:2212:14989:24120/1        tccccccaccaggccaaatt tc ccgatgactacaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2139:51206:15580/1           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1247:37291:5630/1            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:2246:43264:25113/2        tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:2246:43255:25129/2        tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2185:31669:12744/2           tccccccaccaggccaaatt t- ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1132:13233:8867/2            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1106:21286:25834/1           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1172:1749:11559/2            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1172:1740:11575/2            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1181:35396:19906/1           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2274:13473:12616/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2274:18586:13209/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1267:31882:27341/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
_R_LH00504:186:22YKYJLT3:1:1204:11014:26171/1        tccccccaccgggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1192:25992:16846/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1161:30662:6960/2            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:1161:30671:6976/2            tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaac
LH00504:186:22YKYJLT3:1:2212:14989:24120/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccacgaaaac
LH00504:186:22YKYJLT3:1:2157:26510:25049/2           tccccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaaa
LH00504:186:22YKYJLT3:1:2144:20306:10389/2           tccccccacccggccaaatt tc ccgatgac------------------------------
```

(b) PG-4

```
                                                                  FGF5: c. 474, c. 475
                                                                                ↓
WT                                                   cccccaccaggccaaatt ta ccgatgactgcaagttcagggagcgattccaagaaaaca-
FGF5:c.474del                                        cccccaccaggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaaca-
FGF5:c.475A>C                                        cccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaaca-
Read name and sequence
_R_LH00504:188:22Y5KLLT3:4:2128:16034:3147/2         -------------aaatt -a ccgatgactgcaagttcagggagcgattccaagaaaacag
_R_LH00504:186:22YKYJLT3:1:1209:31290:3628/1         --------aggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2102:7361:11959/1            -------caggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaacag
_R_LH00504:186:22YKYJLT3:1:1153:39436:4957/2         --cccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2154:16238:26411/2           ccccaccaggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2217:22645:11783/2           ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
_R_LH00504:186:22YKYJLT3:1:2203:41359:11014/2        ccccaccaggccaaact -a ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:188:22Y5KLLT3:4:1147:27379:6303/2            ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2249:9710:19490/2            ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:1153:39436:4957/1            ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2149:37162:21492/2           ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:1270:2452:27453/1            ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2276:50356:6095/2            ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2154:27490:12200/2           ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2136:34998:5022/1            ccccaccaggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaacag
_R_LH00504:186:22YKYJLT3:1:1233:2174:8963/2          ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
_R_LH00504:186:22YKYJLT3:1:1233:2165:8979/2          ccccaccaggccaaatt tc ccgatgactgcaagttcagggagcgattccaagaaaacag
LH00504:186:22YKYJLT3:1:2287:47203:29279/2           ccccaccaggccaaatt -a ccgatgactgcaagttcagggagcgattccaagaaaaca-
_R_LH00504:186:22YKYJLT3:1:1202:22719:14218/1        ccccaccaggccaaatt tc ccgatgactgcaagttc-----------------------
LH00504:186:22YKYJLT3:1:1222:6446:7233/1             ccccaccaggccaaatt -a ccgatgactgc------------------------------
```

Figure 2: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of FGF5 genes. Alignment results of genome sequencing reads around FGF5: c.474 and c.475; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the same as in Figure 1

Figure 3: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of LVRN genes. Alignment results of genome sequencing reads around LVRN: c.2522; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the same as in Figure 1

(a) CRFK

```
                                                                                TYRP1: c. 8
                                                                                      ↓
WT                                                   ttcactcttgcttcgagcagaatgaaag c ccataaattcctctctctgggatacatcgtc
TYRP1:c.8C>G                                         ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2209:8083:25546/1         ttcactcttgcttcgagcagaatgaaag g c------------------------------
LH00504:186:22YKYJLT3:1:1250:21073:19345/1           ttcactcttgcttcgagcagaatgaaag g ccataaa------------------------
LH00504:186:22YKYJLT3:1:2252:43532:16990/1           ttcactcttgcttcgagcagaatgaaag g ccataaat-----------------------
_R_LH00504:186:22YKYJLT3:1:1155:33657:14619/1        ttcactcttgcttcgagcagaatgaaag g ccataaattcctct-----------------
_R_LH00504:186:22YKYJLT3:1:2197:43588:20964/1        ttcactcttgcttcgagcagaatgaaag g ccataaattcctct-----------------
LH00504:186:22YKYJLT3:1:1226:48886:14603/2           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctg------------
LH00504:186:22YKYJLT3:1:1162:43467:18192/1           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1112:5780:10309/2            ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1177:35719:21781/2           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1198:20528:6640/1            ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:188:22Y5KLLT3:4:1283:44549:19105/2        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1193:37217:17423/2           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:2185:7315:11398/2            ttcactcttgcttcgagcagaatgaaag g ccataaattcctctatatgggatacatcgtc
LH00504:186:22YKYJLT3:1:2223:50273:22614/1           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:188:22Y5KLLT3:4:2188:36006:20098/1        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1177:35719:21781/1        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1198:20528:6640/2         ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1254:19548:27501/1        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1157:40102:20916/2           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:2153:37587:26844/2        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1292:13686:27116/2        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1292:13695:27132/2        ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1253:6659:26924/2            ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:1106:33907:24248/2           ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:2284:42635:9764/1         ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:2284:42654:9764/1         ttcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:2252:43532:16990/2        -tcactcttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:2161:44521:18352/1           ------cttgcttcgagcagaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:2182:27472:9027/2            -----------------cagaatgaaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1226:48886:14603/1        -------------------gaatgaaag g ccataaattcctctctctgggatacatcgtc
LH00504:186:22YKYJLT3:1:2145:26519:19458/2           ------------------------aaag g ccataaattcctctctctgggatacatcgtc
_R_LH00504:186:22YKYJLT3:1:1157:40102:20916/1        --------------------------ag g ccataaattcctctctctgggatacatcgtc
```

(b) PG-4

```
                                                                                TYRP1: c. 8
                                                                                      ↓
WT                                                   cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctg----
TYRP1:c.8C>G                                         cgagcagaatgaaag g ccataaattcctctctctgggatacatcgtcttgcccctg----
Read name and sequence
LH00504:186:22YKYJLT3:1:1179:39002:16349/1           cgagcagaatgaaag c ccataaattcctct------------------------------
_R_LH00504:186:22YKYJLT3:1:2286:22775:10565/1        cgagcagaatgaaag c ccataaattcctct------------------------------
_R_LH00504:186:22YKYJLT3:1:1254:4957:6063/1          cgagcagaatgaaag c ccataaattcctctc-----------------------------
LH00504:186:22YKYJLT3:1:2286:36172:8691/2            cgagcagaatgaaag c ccataaattcctctctctgggat---------------------
_R_LH00504:186:22YKYJLT3:1:1129:1814:29071/1         cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccct-----
_R_LH00504:186:22YKYJLT3:1:1149:38844:27228/1        cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
LH00504:186:22YKYJLT3:1:1139:34647:15404/2           cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
LH00504:186:22YKYJLT3:1:2182:49727:29391/1           cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
LH00504:186:22YKYJLT3:1:2132:17014:23848/2           cgagcagaatgaaag c caataaattcctatctctgtgatacatcgtcttgcccctgctgt
LH00504:186:22YKYJLT3:1:2158:18873:17935/1           cgagcagaatgaaag c ccataaattcccctctctgggatacatcgtcttgcccctgctgt
_R_LH00504:186:22YKYJLT3:1:2286:36172:8691/1         cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
LH00504:186:22YKYJLT3:1:2149:47711:5678/1            cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
_R_LH00504:186:22YKYJLT3:1:1268:45363:9524/1         cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
_R_LH00504:186:22YKYJLT3:1:1235:31420:11607/1        cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
_R_LH00504:186:22YKYJLT3:1:1179:39002:16349/2        cgagcagaatgaaag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
_R_LH00504:186:22YKYJLT3:1:1139:34647:15404/1        -------------ag c ccataaattcctctctctgggatacatcgtcttgcccctgctgt
```

Figure 4: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of TYRP1 genes. Alignment results of genome sequencing reads around TYRP1: c.8; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the same as in Figure 1

(a) CRFK

```
                                                                    MLPH: c. 83
                                                                          ↓
WT                                                  ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
MLPH:c.83delT                                       ggaagtggttcagcgggactttgatc - gagaaggaaagaagaggaaaggctggggtgagt
Read name and sequence
LH00504:186:22YKYJLT3:1:1119:21721:28350/1          ggaagtggttcagcgggactttgatc - gaga-----------------------------
_R_LH00504:186:22YKYJLT3:1:1141:12151:25706/1       ggaagtggttcagcgggactttgatc - gagaaggaaagaagag-----------------
_R_LH00504:186:22YKYJLT3:1:1245:25308:6848/2        ggaagtggttcagcgggactttgatc t gagaaggaaagaagag-----------------
LH00504:186:22YKYJLT3:1:2151:42663:11286/1          ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctgggg-----
LH00504:186:22YKYJLT3:1:1286:27666:28590/1          ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
_R_LH00504:186:22YKYJLT3:1:1248:27925:24809/1       ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
_R_LH00504:186:22YKYJLT3:1:1248:27943:24809/1       ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
_R_LH00504:186:22YKYJLT3:1:2253:43874:10790/2       ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:2253:38780:4108/1           ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1203:29580:15212/1          ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:188:22Y5KLLT3:4:1167:40546:7521/2           ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1296:23977:20884/1          ggaagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
_R_LH00504:186:22YKYJLT3:1:1119:21721:28350/2       ggaagtggttcagcgggactttgatc - gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1161:36930:6896/1           --aagtggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1247:45206:4220/2           -------gttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1228:45141:5871/1           -------------------tttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:1228:45159:5871/1           -------------------tttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:2292:40065:20018/1          -------------------tttgatc t gagaaggaaagaagaggaaaggctggggtgagt
LH00504:186:22YKYJLT3:1:2206:45391:22999/2          ----------------------tgatc - gagaaggaaagaagaggaaaggctggggtgagt
```

(b) PG-4

```
                                                                    MLPH: c. 83
                                                                          ↓
WT                                                  ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
MLPH:c.83delT                                       ggttcagcgggactttgatc - gagaaggaaagaagaggaaaggctggggtgagtgtatga
Read name and sequence
LH00504:186:22YKYJLT3:1:2163:37874:6448/1           ggttcagcgggactttgatc t gagaaggaa------------------------------
LH00504:186:22YKYJLT3:1:2163:37892:6448/1           ggttcagcgggactttgatc t gagaaggaa------------------------------
LH00504:186:22YKYJLT3:1:2163:37883:6464/1           ggttcagcgggactttgatc t gagaaggaa------------------------------
_R_LH00504:186:22YKYJLT3:1:1108:33796:17871/2       ggttcagcgggactttgatc t gagaaggaaagaa--------------------------
_R_LH00504:186:22YKYJLT3:1:1163:10810:14603/1       ggttcagcgggactttgatc t gagaaggaaagaagagg----------------------
_R_LH00504:186:22YKYJLT3:1:1135:23450:12888/1       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctg--------------
LH00504:186:22YKYJLT3:1:1170:24938:14475/2          ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatg-
_R_LH00504:186:22YKYJLT3:1:2293:32298:19057/2       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2293:32317:19057/2       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1284:17837:6303/1        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1284:17846:6319/1        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2176:37282:19810/1       ggttcagcgggactttgatt t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2208:4578:11270/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1170:24938:14475/1       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2170:44845:7153/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2259:18651:1304/1        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1255:37004:17952/2          ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1255:36995:17968/2          ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:2201:47073:5374/1           ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2270:11346:13225/1       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:2297:29506:25113/2          ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:2223:18605:11158/1          ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1223:47120:11350/1       ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1204:41868:7505/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1204:41877:7521/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2283:16746:10213/2       ggttcagcgggactttgatc t gagtaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:1115:3811:27981/1        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2163:37874:6448/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2163:37892:6448/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggcttgggtgagtgtatga
_R_LH00504:186:22YKYJLT3:1:2163:37883:6464/2        ggttcagcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1142:4560:6175/1            ------gcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1142:4569:6191/1            ------gcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:2248:38280:27373/1          ------gcgggactttgatc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1229:33593:24985/2          -------------------tc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1229:33602:25001/2          -------------------tc t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:186:22YKYJLT3:1:1268:16376:6816/2           -------------------c t gagaaggaaagaagaggacaggctggggtgcgtgtatga
_R_LH00504:186:22YKYJLT3:1:2201:47073:5374/2        -------------------c t gagaaggaaagaagaggaaaggctggggtgagtgtatga
LH00504:188:22Y5KLLT3:4:1197:37531:18512/2          -------------------- t gagaaggaaagaagaggaaaggctggggtgagtgtatga
```

Figure 5: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of MLPH genes. Alignment results of genome sequencing reads around MLPH: c.83; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the same as in Figure 1

```
                                                                  B1: g.161388687
                                                                          ↓
WT                                          ggggcgggggt-gggagatggagtggaacttt----------------------------
White spotted                               ggggcgggggt-gggagatggagtggaactttttgttatgccc--aaaattcgtgatcccc
Cell: Read name and sequence
CRFK:b7c5b596-3c70-45bb-b00c-988c08291521   ggggcgggggt-gggagatggagtggaacttt----------------------------
CRFK:4f28cb2e-0b77-4276-927b-22cf1cd859a9   ggggcgggggt-gggagatggagtggaacttt----------------------------
CRFK:1a121a2d-cbd1-4954-953a-c59b730e8896   ggggcgggggt-gggagatggagtggaacttt----------------------------
CRFK:5cc67035-40b4-4180-8075-baa2bb4be3e7   ggggcgggggtggggagatggagtggaacttt----------------------------
PG-4:1d375068-d36d-463b-a23b-b79a65e7211e   ggggcgggggt-gggagatggagtggggttttact-----------------------
PG-4:22777b42-f4c6-4e0d-994b-27b1798695bb   ggggcgggggt-gggagatggagtggaactttttgttatgccc--aaaattcgtgatcccc
PG-4:befbbc54-737b-4de1-802e-a01ed3fea542   ggggcgggggt-gggagatggagtggaactttttgttatgccc--aaaattcgtgatcccc
PG-4:9baf3e20-d0e4-4767-94ac-af0d2ef545da   ggggcgggggt-gggagatggagtggaacttt----------------------------
PG-4:8bc2a176-7ea8-4825-a2f8-488be22f49bf   ggggcgggt---gggagatggagtggaactttttgttatgccccaaaaaatcgtgatcccc
PG-4:7ed64960-07a4-4574-8739-89a5ecb31ad5   ggggcgggggt-gggagatggagtggaactttttgttatgccc--aaaattcgtgatcccc

WT                                          ------------------------------------------------------------
White spotted                               aaagaccaccagggagccgagtccgatgcaaaagcaaagagcctttattcgagctagctc
Cell: Read name and sequence
CRFK:b7c5b596-3c70-45bb-b00c-988c08291521   ------------------------------------------------------------
CRFK:4f28cb2e-0b77-4276-927b-22cf1cd859a9   ------------------------------------------------------------
CRFK:1a121a2d-cbd1-4954-953a-c59b730e8896   ------------------------------------------------------------
CRFK:5cc67035-40b4-4180-8075-baa2bb4be3e7   ------------------------------------------------------------
PG-4:1d375068-d36d-463b-a23b-b79a65e7211e   ------------------------------------------------------------
PG-4:22777b42-f4c6-4e0d-994b-27b1798695bb   aaagaccaccagggagccgagtccgatgcaaaagcaaagagcctttattcgagctagctc
PG-4:befbbc54-737b-4de1-802e-a01ed3fea542   aaagaccaccagggagccgagtccgatgcaaaagcaaagagcctttattcgagctagctc
PG-4:9baf3e20-d0e4-4767-94ac-af0d2ef545da   ------------------------------------------------------------
PG-4:8bc2a176-7ea8-4825-a2f8-488be22f49bf   aaagaccaccagggagccgagtccgatgcaaaagcaaagagcctttattc-agctagctc
PG-4:7ed64960-07a4-4574-8739-89a5ecb31ad5   aaagaccaccagggagccgagtccgatgcaaaagcaaagagcctttattcgagctagctc
                                                                                                        |
                                                                                                        |
                                            |--------------------- 7020 bp ---------------------------|
                                            |
                                            ↓                                          B1: g.161388688
                                                                                                   ↓
WT                                          ------------------------------------------------------------ctg
White spotted                               aggtgaaggtcacagaacaagatggcgacggctggcgtaggcccgccctttcactttctg
Cell: Read name and sequence
CRFK:b7c5b596-3c70-45bb-b00c-988c08291521   ------------------------------------------------------------ctg
CRFK:4f28cb2e-0b77-4276-927b-22cf1cd859a9   ------------------------------------------------------------ctg
CRFK:1a121a2d-cbd1-4954-953a-c59b730e8896   ------------------------------------------------------------ctg
CRFK:5cc67035-40b4-4180-8075-baa2bb4be3e7   ------------------------------------------------------------ctg
PG-4:1d375068-d36d-463b-a23b-b79a65e7211e   ------------------------------------------------------------
PG-4:22777b42-f4c6-4e0d-994b-27b1798695bb   aggtgaaggtcacagaacaagatggcgacggctggcgtaggcccgccctttcactttctg
PG-4:befbbc54-737b-4de1-802e-a01ed3fea542   aggtgaaggtcacagaacaagatggcgacggctggcgtaggcccgccctttcactttctg
PG-4:9baf3e20-d0e4-4767-94ac-af0d2ef545da   ------------------------------------------------------------ctg
PG-4:8bc2a176-7ea8-4825-a2f8-488be22f49bf   aggtgaaggtcacagaacaagatggcgacggctggcgtaggcctgccctttcac----tt
PG-4:7ed64960-07a4-4574-8739-89a5ecb31ad5   aggtgaaggtcacagaacaagatggcgacggctggcgtaggcccgccctttcactttctg

WT                                          caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
White spotted                               caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
Cell: Read name and sequence
CRFK:b7c5b596-3c70-45bb-b00c-988c08291521   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
CRFK:4f28cb2e-0b77-4276-927b-22cf1cd859a9   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
CRFK:1a121a2d-cbd1-4954-953a-c59b730e8896   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
CRFK:5cc67035-40b4-4180-8075-baa2bb4be3e7   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
PG-4:1d375068-d36d-463b-a23b-b79a65e7211e   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
PG-4:22777b42-f4c6-4e0d-994b-27b1798695bb   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
PG-4:befbbc54-737b-4de1-802e-a01ed3fea542   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
PG-4:9baf3e20-d0e4-4767-94ac-af0d2ef545da   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-ggttttatctgcgcggg
PG-4:8bc2a176-7ea8-4825-a2f8-488be22f49bf   tgaaatcttaattcttggatctgaaagctgtagtgaaaatccaagttttatctgcgcggg
PG-4:7ed64960-07a4-4574-8739-89a5ecb31ad5   caaaatctta---cttggatcctaaagctgtagtgaaaatcc-agttttatctgcgcggg
```

Figure 6: Parts of upper and lower streams of alignment results of long-read sequencing reads of CRFK and PG-4 cells with WT and KIT-FERV1 sequences of KIT intron 1. Alignment results of long-read genome sequencing reads from CRFK and PG-4 cells to the KIT-FERV1 that contains sequence around the region B1: g.161388687_161388688 (F.catus_Fca126_mat1.0). Left string indicates [the name of cell (CRFK or PG-4): name of sequenced read], and red colored sequences indicate FERV1 and aligned sequences with it. Four long reads from CRFK could align, but no reads contained the FERV1 homologue, while 6

542 reads from PG-4 could align, and four of the six reads contained the FERV1 homologue. This
543 figure shows only a part of the results at the upper and lower streams of the KIT-FERV1
544 sequence. See Table S1 for the alignment result with the full-length KIT-FERV1 sequence

545

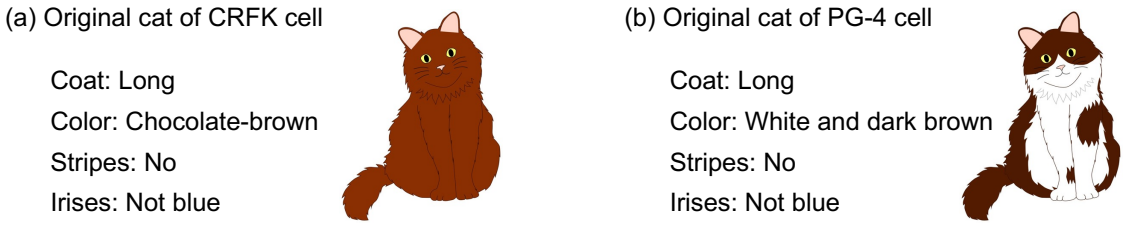

Figure 7: Example illustrations of inferred original cats of CRFK and PG-4 cells. An example illustration of the inferred phenotypes of coat morphologies and iris colors of the original cats of the CRFK cell (a) and the PG-4 cell (b). CRFK cells are expected to originate from a cat with long, chocolate-brown fur, lacking stripes, and with non-blue irises. PG-4 cells were expected to be derived from a cat with long, white and dark brown bicolored fur without stripes and non-blue irises

(a) CRFK

```
                                                                TYR: c. 904
                                                                     ↓
WT                                                   gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
TYR:c.904G>A                                         gaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctcct
Read name and sequence
LH00504:188:22Y5KLLT3:4:1226:28840:5502/1            ---------tgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
LH00504:188:22Y5KLLT3:4:1226:28859:5502/1            ---------tgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1210:36357:4461/2         ----attactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
LH00504:186:22YKYJLT3:1:1218:6464:20916/2            gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1172:11411:27725/2        gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
LH00504:188:22Y5KLLT3:4:2148:33066:10709/2           gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
LH00504:188:22Y5KLLT3:4:2148:33084:10709/2           gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1104:4846:24424/1         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:2136:21878:12536/1        gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1168:8286:24905/2         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1209:10403:16013/2        gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1293:24448:7729/2         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1174:13251:6944/1         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
LH00504:188:22Y5KLLT3:4:1288:22969:9940/1            gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:188:22Y5KLLT3:4:1241:2526:10149/2         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctcct
_R_LH00504:186:22YKYJLT3:1:1111:51798:14234/1        gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc--
_R_LH00504:186:22YKYJLT3:1:2275:52066:2778/1         gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaatgctcccctc--
LH00504:186:22YKYJLT3:1:2147:33861:15388/1           gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctc-------
LH00504:186:22YKYJLT3:1:2147:33851:15404/1           gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctc-------
LH00504:186:22YKYJLT3:1:2147:33861:15420/1           gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctc-------
LH00504:186:22YKYJLT3:1:2160:12188:4749/2            gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaag-----------
_R_LH00504:188:22Y5KLLT3:4:1106:16496:14555/2        gaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccca-------------
LH00504:186:22YKYJLT3:1:1172:11411:27725/1           gaccattactgcgcaatccc g gaaaccatgacaaagc-----------------------
_R_LH00504:186:22YKYJLT3:1:2264:44170:2330/1         gaccattactgcgcaatccc g gaaaccatg------------------------------
```

(b) PG-4

```
                                                                TYR: c. 904
                                                                     ↓
WT                                                   gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
TYR:c.904G>A                                         gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2281:44041:6656/2         --------------------ccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:1233:50467:22502/2           ---------------caatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2293:19270:2570/2            ---------------caatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:2163:33371:27357/2        --------tactgcgcaatccc a gaaaccatgacaaagccaggaccccaatgctcccctc
LH00504:186:22YKYJLT3:1:2285:45474:26123/1           -----cattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2213:23893:19490/2           ----ccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1170:41230:16013/1        -ggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1182:36847:21364/1        -ggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2121:6474:23720/2            gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2121:6464:23736/2            gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2254:5706:1721/2             gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1152:17458:1705/1         gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1152:40250:13481/1        gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1275:39057:19682/1        gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2292:8129:5278/1             gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2292:8147:5278/1             gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:2107:26806:17166/1           gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1235:42866:29488/2        gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:1152:17458:1705/2            gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:186:22YKYJLT3:1:1275:39057:19682/2           gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1183:32039:23319/2        gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:2266:34018:11014/2        gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:2266:34027:11030/2        gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:2138:39556:9940/2         gggaccattactgcgcaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
LH00504:188:22Y5KLLT3:4:1280:10126:11943/2           gggaccattactgcgcaatccc g gaaactatgacaaagccaggaccccaaggctcccctc
LH00504:188:22Y5KLLT3:4:1280:10144:11943/2           gggaccattactgcgcaatccc g gaaactatgacaaagccaggaccccaaggctcccctc
LH00504:188:22Y5KLLT3:4:2101:7740:14347/2            gggaccattactgcgcaatccc g gaaactatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:2266:44762:11142/2        gggaccattactgcacaatccc a gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1262:51576:13593/2        gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggctcccctc
_R_LH00504:186:22YKYJLT3:1:1127:34378:1288/1         gggaccattactgcgcaatccc g gaaaccatgacaaagccaggaccccaaggc-------
LH00504:186:22YKYJLT3:1:2257:38521:27244/2           gggaccattactgcgcaatccc a gaaaccatgacaaagccaggacccc------------
_R_LH00504:186:22YKYJLT3:1:1116:52085:25562/2        gggaccattactgcgcaatccc a gaaaccatgacaaagcc--------------------
LH00504:186:22YKYJLT3:1:1182:36847:21364/2           gggaccattactgcgcaatccc g gaaaccatgacaaagc---------------------
_R_LH00504:186:22YKYJLT3:1:1267:17042:18128/2        gggaccattactgcgcaatccc a gaaaccat-----------------------------
_R_LH00504:186:22YKYJLT3:1:1267:17033:18144/2        gggaccattactgcgcaatccc a gaaaccat-----------------------------
_R_LH00504:186:22YKYJLT3:1:1267:17051:18144/2        gggaccattactgcgcaatccc a gaaaccat-----------------------------
LH00504:186:22YKYJLT3:1:1262:51576:13593/1           gggaccattactgcgcaatccc g gaaacca------------------------------
```

Figure 8: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of TYR genes. Alignment results of genome sequencing reads around TYR: c.904; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the

same as in Figure 1

(a) CRFK

```
                                                                    LVRN: c. 416
                                                                          ↓
WT                                                aacatcaccgtgcgctgca c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LVRN:c.416C>A                                     aacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcctcttcctg
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2286:21147:21492/2     aacatcaccgtgcgctgcg c cgcggccacc------------------------------
LH00504:186:22YKYJLT3:1:1112:19936:29391/1        aacatcaccgtgcgctgcg c cgcggccaccgc-----------------------------
_R_LH00504:186:22YKYJLT3:1:1112:34064:26379/1     aacatcaccgtgcgctgcg c cgcggccaccgcgcg-------------------------
_R_LH00504:186:22YKYJLT3:1:1105:42737:8530/2      aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgct-------------------
LH00504:186:22YKYJLT3:1:2147:8175:6319/1          aacatcaccgtgcgctgcg c cgcggccaccgcgcggctactgctgcaca-----------
LH00504:186:22YKYJLT3:1:1164:22664:7874/2         aacatcaccgtgcgctgcg c tgcggccaccgcgcggctgctgctgcacagcctctt----
LH00504:186:22YKYJLT3:1:1164:22682:7874/2         aacatcaccgtgcgctgcg c tgcggccaccgcgcggctgctgctgcacagcctctt----
LH00504:186:22YKYJLT3:1:1164:22673:7890/2         aacatcaccgtgcgctgcg c tgcggccaccgcgcggctgctgctgcacagcctctt----
LH00504:186:22YKYJLT3:1:2142:12021:8787/1         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:2229:3728:15949/2         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1164:39667:12216/1        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1224:45853:18448/2        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:1149:4708:20659/1      aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2211:13279:10037/1     aacatcaccgtgcgctgcg c cgcggccactgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1208:26926:12632/1        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1128:18124:1192/1         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1211:41775:4172/2         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1211:41785:4156/2         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:1159:15017:20868/1     aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:2181:41443:14298/1        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2147:8175:6319/2       aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1127:28674:12905/1        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1290:19224:26587/1        aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:1112:19936:29391/2     aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2204:18309:27373/1     aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:1156:33620:10870/2     aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2142:12021:8787/2      aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2151:30181:7217/2      aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2151:30172:7233/2      aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1277:19058:3644/1         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:1277:19048:3660/1         aacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:2228:49570:9155/1         ---atcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
LH00504:186:22YKYJLT3:1:2228:49579:9171/1         ---atcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcctcttcctg
_R_LH00504:186:22YKYJLT3:1:2107:36126:18192/1     ---------gtgcgctgcg c cgcggccaccgcgctgctgctgctgcacagcctcttcctg
```

(b) PG-4

```
                                                                    LVRN: c. 416
                                                                          ↓
WT                                                ccgcgtgaacatcaccgtgcgctgca c cgcggccaccgcgcggctgctgctgcacagcct
LVRN:c.416C>A                                     ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
Read name and sequence
_R_LH00504:186:22YKYJLT3:1:2260:20047:3403/2      ccgcgtgaacattaccgtgcgctgca a cgc------------------------------
_R_LH00504:186:22YKYJLT3:1:2260:20038:3419/2      ccgcgtgaacatcaccgtgcgctgca a cgc------------------------------
LH00504:188:22Y5KLLT3:4:1249:36727:13241/2        ccgcgtgaacatcaccgtgcgctgcg c cgcggcaaccgcg--------------------
LH00504:188:22Y5KLLT3:4:2288:4134:6528/2          ccgcgtgaacatcaacgtgcgctgcg c cgcggccaccgcg--------------------
_R_LH00504:186:22YKYJLT3:1:2140:3062:8546/2       ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1209:27148:7922/1         ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:186:22YKYJLT3:1:1125:39288:9572/2      ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1286:31198:24649/2        ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1286:31207:24665/2        ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:186:22YKYJLT3:1:1242:24235:18833/2     ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1136:25511:21428/1        ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1218:38844:17327/2        ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1197:44864:23111/2        ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1223:41849:9460/2         ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:186:22YKYJLT3:1:2176:15415:14282/1     ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:186:22YKYJLT3:1:2176:15424:14298/1     ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:2116:38077:4685/2         ccgcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:2109:26177:3772/2         ccgcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:1139:11115:7858/1         --gcgtgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:188:22Y5KLLT3:4:1249:36727:13241/1     --gcgtgaacatcaccgggcgctgcg t cgcggccaccgcgcggctgctgctgcacagcct
_R_LH00504:188:22Y5KLLT3:4:2288:4134:6528/1       --gcgtgaacatcaccgtgcgctgcg c cgcggccaccgcgcggctgctgcggcacagcct
_R_LH00504:186:22YKYJLT3:1:1256:16386:4365/2      -----tgaacatcaccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
LH00504:186:22YKYJLT3:1:2292:31124:27052/1        ------------caccgtgcgctgca a cgcggccaccgcgcggctgctgctgcacagcct
```

Figure 9: Alignment results of whole genome sequencing reads of CRFK and PG-4 cells with WT and mutant sequences of LVRN genes. Alignment results of genome sequencing reads around LVRN: c. 416; CRFK (a) and PG-4 (b) cells. Left strings and symbol colors are the same as in Figure 1

	TYR	TYRP1	MC1R	KIT	ARHGAP36	MLPH	ASIP	LVRN	Dkk4	FGF5	KRT71	LPAR6	PAX3
skin	0.97949123	4.979143826	0.907122448	3.586949825	0.595540759	1.890367316	0.617256057	0.790593088	2.788113859	1.322693065	10.420824	4.264054274	1.420870477
skin (orange color)	1.287074139	4.681136779	1.514178733	4.728150746	0.92351867	1.992335312	0.560564858	0.730570842	2.385149963	2.506351052	11.57185462	4.780163285	1.519920078
skin (white color)	0.106508109	0	0.153761119	4.416760098	0.281844346	1.38368236	0.375739542	0.623645798	1.360833766	1.628767281	10.27795109	4.513211603	0.498482548
fibroblast adult	0.201311442	0.021819551	0.730664277	0.704854625	0.003685788	0.186873412	0.100232329	0.124979228	1.500135676	0	0.019069387	1.488602888	0.019355019
muscle	0.056483647	0	0.092118876	1.414603576	0.067172023	0.052994709	0.077034761	0.278525184	2.672768585	0.040640712	0	2.118117862	0.303226425
tooth	0.266573907	2.524633257	0.217491846	4.150102427	0.297112095	1.773640024	0.567123047	0.264163973	2.596387987	0.317583819	1.358416774	6.087545257	0.549355469
brain	0.074138775	0.244408995	6.154690407	6.125305599	0.488360957	0.106920683	0.445307287	0.482900784	2.997739706	1.214526236	0.082337234	2.495234234	0.920727113
hippocampus brain	0.104049158	0.986809378	7.141695431	6.633483047	1.263545739	0.381895847	0.462627145	0.267753817	3.196347533	2.064892	0.362603007	3.018231011	0
occipital brain	0.071192089	0.039376982	7.448888471	3.963105509	0.312113692	0.193440716	0.170021122	0.101684789	4.119749695	1.540684343	0.011456374	1.605354354	0
parietal lobe brain	0	0	7.186145561	4.875402302	0.660400161	0.64629313	0.441065494	0.042402384	3.887808995	1.329709621	0.132720662	2.664134262	0
temperal lobe brain	0	0	7.599838074	5.105537251	0.42981211	0.928785547	0.501174959	0.149430158	4.035299228	0.314304896	0.031937969	2.246188765	0
cerebellum brain	0.022500547	0.331065581	5.309532593	7.45398614	0.251584181	0.41274569	0.149029272	0.025240316	4.380271309	2.432348488	0.042684587	1.665399115	3.984523055
spinal cord	0	0.374623015	6.555970102	3.815470814	4.054688656	0	0.529844749	0.020240874	3.532849165	0.36837159	0	2.899959482	1.204872472
ear cartilage	0.103888854	0	1.723159734	5.477792674	0.473531193	1.938874602	0.644717239	2.07648309	2.615927848	0.123064897	0.133560503	3.788115166	0.40246664
ear tip	2.697859441	5.199579694	2.58708282	4.675513343	1.711850594	3.075466823	0.814532665	0.396620186	1.503211535	1.193018933	11.47542623	4.398175564	1.791957956
optic nerve head glial	4.130895559	8.012365116	1.665723362	3.485028822	0.400480608	1.551928804	0.247193444	0.058079205	4.050267469	0.184663749	0.013562176	4.736100557	2.687500024
retina	4.87612993	8.045708036	4.178402823	4.258174233	1.512735409	4.36109265	0.314914546	0.039583889	3.898728626	0.436863555	0.047022265	2.713403406	4.612167496
iridiocorneal angle	0	0	0	3.398316494	1.340508557	0	0.832811799	0.294549849	3.890824087	0.021837031	0.167713357	4.251573057	0
thymus	0	0	0	2.877720336	0	0.053402831	0.193039961	0.157927938	2.951650685	0	0	5.682280737	0.275646391
lung	0.46317526	1.08688907	1.00873704	4.4408657	0	3.890849264	2.986203115	0.073203084	2.716721885	0	0.036791184	3.877821731	0.02499963
blood	0	0	0	0.006915628	0.004805397	0.001327058	0	0.001050701	0	0	0	0.507191811	0
pbmc adult	0.074872749	0.063952512	0.502852639	1.648542894	0.074860346	0.082377222	0.074963936	0.028480146	1.806553716	0.036201871	0.260477102	2.751137087	0.043494327
pbmc kitten	0.028385287	0	0.12101391	0.275256019	0.026084545	0.034435013	0.006286765	0.002297281	1.584521711	0.020367796	0	2.268045487	0.018412154
peripheral blood	0	0	0.515689299	0.170624486	0.000698519	0.006921187	0.009584428	0.061271917	2.006770673	0	0	1.438811972	0.003684287
heart	0.029440623	0.063653346	0.446935034	3.011956496	0.021684551	0.60214092	0.712725987	0.406792622	2.349859914	0	0.109389212	3.178257348	0.056555256
liver	0.002312635	0	0.020241341	1.302396352	0.000849826	1.797793796	0.01719273	0.102107549	0.818617394	0.001655185	0	3.134084928	0.00448134
pancreas	0	0.013125298	0	0.496673638	0	1.264438441	0.021037088	0.006750463	0.281244484	0	0.017165925	1.504350567	0
spleen	0.012814297	0.095348535	0.25874521	2.535923479	0.028089502	0.571690571	0.093113907	0.028619065	1.858826078	0.089280815	0.024382448	2.679015895	0.016545063
kidney	0.096950442	0.362604563	3.114634737	0.733737527	0.043426715	0.056746401	0.215789184	0.42457845	2.804342234	0.117904886	0.028226921	1.230818933	2.817516663
small instestine	0.02306041	0.022430093	0.919040713	3.996618633	0.024456245	2.577793702	0.093414428	0.128730281	1.772402464	0.199878752	0.009835045	4.093872667	0.005000054
uterus	0	0	0.205531703	4.085978971	0	1.742410888	0.315615181	4.607945819	2.968203859	0.035454435	0	4.719142051	0.032066243
ovarian cortex	0.04283244	0.276187361	0.582670387	3.53684483	0.843720594	0.982349469	0.448130552	1.012993997	2.856184178	0.172768503	0.096062791	3.784122401	2.382116768
ovarian follicles	0.00495286	0.230281623	0	1.531380368	0.345167523	1.761071281	0.266114162	0.171579633	0.76826987	1.00405354	0.336193392	2.984532996	3.591937717
ovarian granulosa cell	0.008991358	0	1.641464675	1.34165827	0.003308901	0.007291401	0.008893111	1.374887713	1.83266614	0.06930108	0	1.809366183	0.017385505
sorted germ cells	0.642845699	0.171782823	1.686028601	4.851533678	0.507402211	1.575586521	1.613303246	0.984520286	2.500689812	0.319754165	0.724100131	3.873636288	0.73733179
testes	0.299475317	0	0.465669398	3.948613829	0.451191728	2.258861052	0.93877363	0.454226974	2.136381202	0.185946121	0.413609009	1.801861257	0.968554674
testis	0.24053022	0.125749477	1.0236267	3.966700341	0.83726628	1.840459266	0.733582575	0.640244481	2.440294652	0.169048244	0.723056047	2.635496549	0.961670128
seminiferous tubules	0.384927802	0.035315508	1.033753649	5.559293771	0.280038998	1.528919203	0.768256782	0.664462007	2.059442182	0.107003835	0.753137427	2.890890396	0.720638172
whole testes	0.242898475	0.107476514	0.504191868	3.382755818	0.445615224	2.131378771	0.599954091	0.501315507	2.123284656	0.053722971	0.727927623	3.216319425	0.293530387
embryo fetus	0.191399234	0.084838436	1.583090233	3.563052317	0.6927721	0.201677545	0.789584671	0.1446442	2.490336787	0.429363083	0.141768181	4.068385141	2.890874164
head embryo	0.277447948	0.107457441	1.063088406	4.490549175	1.944556238	0.246320218	0.978889696	0.151267039	3.035272045	1.711925434	0	4.256039654	4.241113543
body embryo	0.29294407	0.127453247	3.118263135	4.178248004	2.802053829	0.227815419	1.051822179	0.217242241	3.044565518	1.572135718	0.009663073	4.408907278	3.509347983

Figure 10: Expression levels of coat phenotype-related and iris color-related genes in various tissues. The expression level of each gene in each tissue is quantified as $\log_2$(transcripts per million+1). The length of the red bar for gene A for each tissue is proportional to the [expression levels of gene A in each tissue] / [the maximum expression level of gene A among all focused tissues]. Most coat phenotype-related genes were found to exhibit similar or higher expressions in various tissues other than skin, and the iris color-related gene (PAX3) was also found to exhibit similar or higher expressions in a variety of tissues other than the opt-functional organs

575 **Table S1: Alignment results of long-read sequencing reads of CRFK and PG-4 cells with**
576 **WT and KIT-FERV1 sequences of KIT intron 1.**

577 (File Table_S1)

578

579 **Table S2: Gene expression levels (transcripts per million) in various tissues estimated from**
580 **domestic cats (Felis catus) in the Ensembl database**
581 **(https://ftp.ensembl.org/pub/data_files/felis_catus/F.catus_Fca126_mat1.0/rnaseq/)**

582 (File Table_S2)

583