# The preliminary study about taste-active compound-related genes and the on-site real-time PCR assay in Japanese Black beef

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**Objectives:** The concentration of inosine 5′-monophosphate (IMP) in beef is an important factor contributing to beef palatability. A previous study suggested that single nucleotide polymorphisms (SNPs) in the *ecto-5′-nucleotidase* (*NT5E*) gene strongly affect the concentration of IMP under postmortem conditions by regulating NT5E enzymatic activity in beef. In addition, our preliminary study showed SNPs in the *calpastatin* (*CAST*) gene which regulates postmortem proteolysis affected the concentration of some amino acids in beef on postmortem day 20 (unpublished data). Information on these genotypes can be utilized not only for the genetic improvement of cattle in meat production farms but also for the management of postmortem aging in the distribution stages of beef. The genotyping of these SNPs is commonly performed using PCR-RFLP method. However the laboratory assays require complicated procedures and are time consuming. Here, we first established the genotyping of the *NT5E* gene using a conventional real-time PCR instrument. Subsequently, we examined a mobile real-time PCR device that enables the rapid genotyping of the *NT5E* gene on-site.

**Materials and Methods:** Three Japanese Black steers containing each *NT5E* genotype (*QQ*, *Qq*, *qq*), raised in the Livestock Institute of Yamagata Integrated Agricultural Research Center, were used in this study. The *Q* allele has a positive effect, and the *q* allele has a negative effect on the concentration of IMP under postmortem conditions. Blood samples were collected from the jugular vein. Genomic DNA was extracted using the spin-column method with the DNeasy® Blood & Tissue Kit, a commonly used method for DNA extraction and purification. The PCR primers and probes for the *NT5E* gene (rs42508588 SNP) were designed for the allelic discrimination assay by real-time PCR. PCR primers producing a 66 bp amplicon were as follows: forward primer, 5′-GTC GTG TGC CCA GTT ATG AG-3′; reverse primer, 5′-GGA AGC TTG GGA GGA TCA C-3′. The LNA probe synthesized with ROX was specific for the *A* allele (*Q* allele) and was as follows: 5′-(ROX)-TTGTAC**T**CCTTATCC-(BHQ)-3′. The nucleotides complementary to the identified SNP are indicated in bold. The 3′ end of the LNA probe is a non-fluorescent Black Hole Quencher™ (BHQ) that eliminates the background fluorescence. The LNA probe synthesized with the FAM is specific for the *T* allele (*q* allele) and as follows; 5′-(FAM)-TTGTAC**A**CCTTATCC-(BHQ)-3′. The genomic DNA and reaction mixtures prepared using the KAPA3G Plant PCR Kit were applied to the conventional real-time PCR instrument, Light cycler® 96 System, and set as: 45 cycles of 95°C for 5 sec, and 62°C for 30 sec. Furthermore, a mobile real-time PCR device, PicoGene PCR1100, was set as: 45 cycles of 95°C for 3.5 sec, and 60°C for 10 sec. The cycle threshold (Ct), which indicates the rise time of the amplification of each fluorescent reporter dye, was automatically recorded in the device.

**Results and Discussion:** According to a conventional real-time PCR instrument, Light cycler® 96 System, the total run time was approximately 55 min. After PCR, three *NT5E* genotypes (*QQ*, *Qq*, *qq*) were correctly identified. The PCR1100 enabled rapid amplification of each allele at approximately 19.4 sec per cycle, and the total run time was 13 min 36 sec. Thus, PCR1100 is superior to conventional real-time PCR equipment in terms of analysis time per sample. In addition, the cost of PCR1100 was about one-tenth that of the conventional real-time PCR equipment. However, it is inferior in that it cannot analyze multiple samples simultaneously; therefore, further equipment improvements are needed. Information on the *NT5E* genotype can be utilized not only for the genetic improvement of Japanese black beef but also for the aging management of the buyer's beef because the *NT5E* genotype strongly affects the degradation rate of IMP. The assay described here can be used as a simple and rapid method in the slaughter- houses, and the information obtained will allow the buyers to know the optimal aging period, and the sales destinations according to consumer preferences. This device is portable and does not require a power supply, which facilitates its use not only in specific laboratories but also in meat production farms and distribution stages of beef. Finally, the assay in this study can be applied to the other genes, such as *CAST* gene in principle. With regard to the rapid DNA extraction method on-site, a simplified procedure is needed in the future.

**Key words:** Genotyping, IMP, Meat quality, NT5E, Real-time PCR