**HRM (High-resolution melting) analysis**

Preparation of primers and templates

Primers allow the amplification of 100bp fragments, which have an RNA editing site at the middle of each fragment.

cDNA is prepared by use of PrimeScript RT reagent Kit (TaKaRa BIO INC.) by following the manufacture's protocol. The cDNA was diluted to 1/100. WT genomic DNA is used as a control of unedited sequence.

HRM analysis

HRM reaction of the following mixture containing SYBR *Premix Ex Taq* Ⅱ (TaKaRa BIO INC.) is performed with LightCycler (Roche Applied Science).

| | |
|---|---|
| SYBR *Premix Ex Taq* Ⅱ (2×conc.) | 5μl |
| F primer (5μM) | 0.4μl |
| R primer (5μM) | 0.4μl |
| template | (*) |
| total | up to 10μl |

(*)

| | |
|---|---|
| WT genomic DNA | 1ng |
| plasmid | 25pg |
| 1/100 dilution of cDNA | 3μl |

HRM analysis reaction condition

| | | | |
|---|---|---|---|
| denature | 95℃ | 1min | |
| PCR | 95℃ | 20sec | 40cycles |
| | 60℃ | 20sec | |
| | 72℃ | 20sec | |
| denature | 95℃ | 30sec | |
| renature | 70℃ | 30sec | |
| melting | 70→90℃ | | |

Data analysis

The amplicons are analyzed by melting: The change in fluorescence caused by the release of the intercalating dye from a DNA duplex as it is denatured by increasing temperature is precisely monitored. The presence of heteroduplexes that melt at lower

temperature alters the shape of the melting curve.

An absolute quantification analysis is performed to check the amplification curve and the Crossing Point (CP) value. Then, a *T*m calling analysis is done to generate melting curves representing the fluorescence signal (at 450-500nm) with increasing temperature, melting peaks corresponding to the negative derivative (-d/d*T*) of the fluorescence signal, and to calculate *T*m values.

The editing efficiency of a particular site is determined by comparing the shape of melting peaks and *T*m values of RT-PCR products to known dilution mixes of edited and unedited plasmids containing the same RNA editing site.