Mitophagy Analysis (by Relative Quantification of Mitochondrial DNA)

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METHOD

1. Total DNA containing genomic DNA and mitochondrial DNA is purified from mice cells using Gentra Puregene Kit (QIAGEN) according to the recommended protocols.
2. To prepare PCR template, aforementioned total DNA is adjusted to a concentration of 0.1ï 1 ng/ε L in TE buffer.
3. To perform real-time quantitative PCR, prepare a set of primers for a reference gene, GAPDH (5N-AAC GAC CCC TTC ATT GAC -3Nj and 5N-TCC ACG ACA TAC TCA GCA C-3Nj) and for the target gene, mitochondrial 12s ribosomal RNA (5N-AAC TCA AAG GAC TTG GCG GTA CTT TAT ATC-3Nj and 5N-GAT GGC GGT ATA TAG GCT GAA TTA GCA AGA G-3Nj)
4. Real-time quantitative PCR is carried out using QuantiTect SYBR Green PCR kit (QIAGEN) with a set of primers described above, and 0.5 unit of Uracil DNA Glycosylase (Life technologies) to prevent carry-over contamination.
5. Detailed conditions for PCR are as follows; PCR is carried out with a 7900HT Sequence Detection System (Applied Biosystems) with initial activation at 50 °C for 2 min and 95 °C for 15 min, amplification by 40 cycles of 95 °C for 20 s and 60 °C for 60 s. Data acquisition and analysis were carried out on a 7900HT SDS 2.0 (Applied Biosystems). Samples are prepared in triplicate for each condition (reference gene GAPDH and mitochondria gene mt 12s ribosomal RNA), and data are acquired and analyzed on a 7900HT SDS 2.0 (Applied Biosystems).

MATERIALS

REAGENTS

ÅGentra Puregene Kit (QIAGEN, 158667)

ÅQuantiTect SYBR Green PCR kit (QIAGEN, 204145 etc.)

ÅUracil DNA Glycosylase (Life technologies, 18054-015)

EQUIPMENT

Å Realtime PCR machine and analyzing software (e.g. Applied Biosystems 7900HT Sequence Detection System and 7900HT SDS 2.0, etc.)

TROUBLESHOOTING and TIPS

1. To obtain the valid signal:

   To confirm the validity of the experiment, you need to check whether the amplification efficiency of target gene (mtDNA) is approximately equal to that of the reference gene (GAPDH). See user bulletin of realtime PCR machine and analyzing software (e.g., User Bulletin #2 of Applied Biosystems).

2. No PCR signal:

   Make sure that the culture cells are not human- or monkey-derived cells. This experimental protocol is only applicable for mice-derived cells. To set up the experimental protocol for human- or monkey-derived cells, selection of the target sequence (this time specific sequence in mtDNA coding 12s ribosomal RNA) is important and preconditioning is needed.