Detection of mitochondrial DNA (mtDNA) deletion by qPCR

Sylwia Kabacik,Ales Tichy

Abstract

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Protocol

Step 1.

Prepare standard curve by serial dilution of control human DNA sample in water, the final DNA concentrations should span at least between 10ng and 100pg. To save time, standard curve can be prepared in advance and stored at 4°C for 24h. If experiments are to be compared they have to be analysed using the same DNA sample for standard preparation, or ideally the same standard.

Step 2.

Reagents required

• PerfeCTa® MultiPlex qPCR SuperMix (Quanta Biosciences, Inc. Gaithersburg, MD, USA)

• Following primers and probes:

Name	Sequence
b2M-F	GCT GGG TAG CTC TAA ACA ATG TAT TCA
b2M-R	CCA TGT ACT AAC AAA TGT CTA AAA TGG
FAM-b2M- BHQ1	FAM-CAG [C]CT [A]TT [C]TG [C]CA GCC T-BHQ1
mtMin-F	CTA AAT AGC CCA CAC GTT CCC
mtMin-R	AGA GCT CCC GTG AGT GGT TA
HEX-mtMin- BHQ1	HEX-CAT [C]AC [G]AT [GG]A [T]CA [C]AG GT-BHQ1
mtMaj-F	CTG TTC CCC AAC CTT TTC C
TmtMaj-R	CCA TGA TTG TGA GGG GTA GG
Texas Red-mtMaj- BHQ2	TexasRed-GAC C[C]C [C]TA A[C]A ACC CCC-BHQ2

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Nucleotides in brackets represent locked nucleic acids (LNA) bases.

Step 3.

Prepare qPCR mastermix reaction according to the following template multiplying the volumes by number of samples required plus some excess.

For 1 sample:

PerfeCTa® MultiPlex qPCR SuperMix 2x (5 µl)

Water (2.2µl)

b2M primers forward and reverse 10µM (0.3 µl)

mtMinArc primers forward and reverse 10µM (0.3 µl)

mtMajArc primers forward and reverse 10µM (0.3 µl)

B2M-FAM probe (0.3 µl)

mtMinArc-HEX probe (0.3 µl)

mtMaj-TexasRed probe (0.3 µl)

Total volume (9 µl)

Step 4.
Add 1ng of sample DNA or 1µl of standard per well (total volume for each reaction should be 10µl), measure each sample in triplicate

Step 5.
Set the cycling parameters as follows: 10 min at 95°C, then 40 cycles of 10 s at 95°C and 60s at 60°C.

Step 6.
Using the standard curve, convert the Ct values to ng. An acceptable results should give the reaction efficiency between 93 and 103% and R2 values higher than 0.998.

Step 7.
To obtain mitochondrial DNA content and mitochondrial deletion level, normalise mtMinArc and mtMajArc DNA values to b2M DNA values respectively for each sample.

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