UvA Universiteit van Amsterdam

Muskusrat detection in eDNA with a qPCR Taqman assay.

v 1.6

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Materials:

Rnase/Dnase free H2O	(10977-035, ThermoFischer)
Muskrat-tail DNA (100 ng/µl)	(UvA)
PrimeTime Gene expression Master Mix	(1055770, IDT)
Internal control (GFP plasmid)	(13031-DNA.cg, Addgene)
LowTE buffer	(12090015, ThermoFischer)

Primers and probes ordered @IDT:

Cytochrome c oxidase I	COX_fw2	5'-AAACCACCAGCCATAACA-3'
	COX_rv2	5'-GTGATTCCTGCGGCTAATA-3'
probe	COX_Pb2	5'-6-FAM-TCACGGCTG-ZEN-TACTACTACTCCTTTCTCT-LABkFQ-3'
Cytochrome b	CYTB_fw2	5'-GAGGCCAACCAGTTGAATATC-3'
	CYTB_rv2	5'-TGATTCCTGCAATGGGTATAAAG-3'
probe	CYTB_Pb2	5'-6-FAM-AGCAGCTTC-ZEN-AATTGCTTACTTTGCCA-LABkFQ-3'

Internal control (GFP)	EGFP-1-F	5 '-GACCACTACCAGCAGAACAC-3'
	EGFP-2-R	5'-GAACTCCAGCAGGACCATG-3'
probe	EGFP-Pb	5'-TAMRA-AGCACCCAGTCCGCCCTGAGCA-BHQ-3'

1. Dilute the internal control to 0.1 pg/µl.
2. Dilute the 100 ng muskrat tail DNA with low TE to 10, 1, 0.1, 0.01, 0.001 and 0.0001 ng/µl in order to create a 6-point calibration line.
3. Pipet 1 µl per calibration point in the qPCR plate and add 10 µl RNase/Dnase free water for a total of 11 µl.
4. Create a negative template control (NTC) by using only 11 µl Rnase/Dnase free water.
5. Pipet duplicates of 10 µl per eDNA sample.
6. Add RNase/DNase free water to a volume of 11 µl
7. Make a master mix with primers, probes, GFP plasmid and PrimeTime Gene expression mix. Single **30 µl** reaction:
   - 15 µl Primetime gene expression master mix
   - 1.5 µl Muskrat primer/probe mix
   - 1.5µl internal control primer/probe mix
   - 1 ul internal control (0.1 pg/reaction)
8. Add 19 µl Mastermix to the 11 µl samples, NTC and calibration points

9. Cover plate with an optical seal and close securely.
10. Short spin
11. Vortex briefly and spin down the samples
12. Run the plate according the following program:

    95 C 3 min

    95 C 15 sec] 45 X

    60 C 1 min]
13. Analyze the samples in the qPCR software depending on which qPCR machine was used

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If the CT of the internal control of a sample is 2 CT or more above the average of the rest of the samples, redo PCR with a diluted sample.

Fw and Rv Primer and probe stocks (original IDT tubes) should be at 100 µM each

Muskrat Primer/probe mix:

15 µM Fw primer Cytb (100 µM/15 µM=6.67x dilution)
15 µM Rv primer Cytb (100 µM/15 µM=6.67x dilution)
15 µM Fw primer Cox (100 µM/15 µM=6.67x dilution)
15 µM Rv primer Cox (100 µM/15 µM=6.67x dilution)
5 µM probe Cytb (100 µM/5 µM= 20x dilution)
5 µM probe Cox (100 µM/5 µM= 20x dilution)

For a 500 µl primer/probe mix add:

500/6.67 = 75 µl (100 µM) Fw Cytb)
500/6.67 = 75 µl (100 µM) Rv Cytb
500/6.67 = 75 µl (100 µM) Fw Cox
500/6.67 = 75 µl (100 µM) Rv Cox
500/20 = 25 µl (100 µM) probe Cytb
500/20= 25 µl (100 µM) probe Cox
----------+
350 µl
Add 150 µl lowTE
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500 µl primer/probe mix(concentration:15µM for each primer and 5 µM for each probe)

Add 1,5 µl primer/probe mix to a 30 µl reaction -> dilution factor is 30/1,5=20
In the PCR reaction the final concentrations are:
Primers:
15µM=15000 nM-> 15000/20=750 nM final concentration
Probe:
5 µM=5000 nM-> 5000/20=250 nM final concentration

Internal control Primer/probe mix:

15 µM Fw primer GFP (100 µM/15 µM=6.67x dilution)
15 µM Rv primer GFP (100 µM/15 µM=6.67x dilution)
5 µM probe GFP (100 µM/5 µM= 20x dilution)

For a 500 µl primer/probe mix add:

500/6.67 = 75 µl (100 µM) Fw GFP
500/6.67 = 75 µl (100 µM) Rv GFP
500/20= 25 µl (100 µM) Probe GFP
----------+
175 µl
Add 325 µl lowTE
----------+
500 µl primer/probe mix(concentration:15µM for each primer and 5 µM for each probe)

Add 1,5 µl primer/probe mix to a 30 µl reaction -> dilution factor is 30/1,5=20
In the PCR reaction the final concentrations are:
Primers:
15µM=15000 nM-> 15000/20=750 nM final concentration
Probe:
5 µM=5000 nM-> 5000/20=250 nM final concentration