

## Coypu eDNA detection with a qPCR Taqman assay.

v 1.6

18-07-2022

### Materials:

Rnase/Dnase free H <sub>2</sub> O	(10977-035, ThermoFischer)
coypu-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	brCOX3_Fw	CCCTATCCGCTCTTCTACTTAC
	brCOX3_Pb	FAM-TGCTTCTACAACCCTCCTAACCCCT-BHQ
	brCOX3_Rv	TGTACCTTCTCGTACTACATCAC
Cytochrome b	brCYTB_Pb	FAM-CCAATTAGCATCTGTTTCCTACTTTTGCAT-BHQ
	brCYTB_Fw	AGGGCAACCAGTAGAACA
	brCYTB_Rv	ATGAGGCTTGTTACAGGCA
Internal control (GFP)	EGFP-1-F	5'-GACCACTACCAGCAGAACAC-3'
	EGFP-2-R	5'-GAACTCCAGCAGGACCATG-3'
	probe	5'-TAMRA-AGCACCCAGTCCGCCCTGAGCA-BHQ-3'

1. Dilute the internal control to 0.1 pg/μl.
2. Dilute the 100 ng coypu 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, internal control and PrimeTime Gene expression mix.

Single **30 μl** reaction:

15 μl Primetime gene expression master mix

1.5 μl coypu primer/probe mix

1.5 μl 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.

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**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

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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

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175 µl

Add 325 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  $\mu$ l primer/probe mix to a 30  $\mu$ l reaction -> dilution factor is  $30/1,5=20$

In the PCR reaction the final concentrations are:

Primers:

15 $\mu$ M=15000 nM->  $15000/20=750$  nM final concentration

Probe:

5  $\mu$ M=5000 nM->  $5000/20=250$  nM final concentration