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Mouse Tissue mtDNA Copy Number Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

Mouse Tissue mtDNA Copy Number Protocol developed in the Picard lab at Departments of Psychiatry and Neurology, Robert N Butler Columbia Aging Center, Columbia
University Irving Medical Center, New York, NY, USA

Attachments

DOCX

Tissue mtDNA Copy Nu...

150KB

Consumables, with catalog numbers:

1

BD Vacutainer Tubes (BD #363083)
Tris HCl (Sigma #T3253)
Tween 20, 10% (Sigma #P1379)
Nuclease free water (Thermofisher Scientific #AM9939)
Proteinase K (20 mg/mL) (Thermofisher Scientific #AM2548)
Stericup Quick Release-GV Sterile Vacuum Filtration System – 500 mL (Millipore Sigma S2GVU05RE)
BrandTech 96-well semi-skirted plate (BrandTech #781375/VWR #10141-434)
BrandTech 8-strip domed tube caps (BrandTech #781340/VWR #80087-132)
2x TaqMan Universal MasterMix Fast (LifeTech #4444965)
MicroAmp™ Optical 384-Well Reaction Plate with Barcode (ThermoFisher #4309849) MicroAmp™
Optical Adhesive Film (ThermoFisher #4311971) Validated
mtDNA/nDNA Primer & Probe sequences (idtDNA.com)
Additional consumables not listed here: 1.5 mL Eppendorf tubes, pipette tips as needed.

2

Lysis buffer for mtDNA measurement

3 *Keep everything sterile. The below calculations provide sufficient lysis buffer for 1 L of complete lysis buffer, or about 5,000 reactions.*

4 **Tris-HCl (0.38M, pH 8.5)** (Sigma #T3253)

- 18.168 g of Tris HCl (MW = 157.60 g/mol) in 200 mL of nuclease-free dH_2O
- pH to 8.5 with 5M NaOH (about 12.5 mL, but measure pH continuously)
- Note: Accurate pH is critical for proper extraction. Make sure your pH meter has been recently calibrated.
- Make up to 300 mL with nuclease-free dH_2O
- Sterile filter Tris-HCl buffer using a SteriCup *(Make 6 mL or 12 mL aliquots, depending on if you want to run 1 or 2 plates at once)*

5 **Tween 20, 10%** (Sigma #P1379)

- 540 mL of ultrapure dH_2O
- 60mL of Tween 20 (wash tip thoroughly or leave in solution)
- Sterile filter Tween 10% using a SteriCup (Make 12 mL or 24 mL aliquots, depending on if you want to run 1 or 2 plates at once)

NB: Thawing these 10% Tween aliquots can be slow. I recommend using a 37ºC bead bath.

6 **ddH_2O (Sterile, nuclease-free)** (Thermofisher Scientific #AM9939)
(Make 5 mL aliquots or use direct from bottle)

7 **Proteinase K (20 mg/mL)** (Thermofisher Scientific #AM2548)
- To be put in fresh from 20 mg/mL (1:100 dilution, final 200 µg/mL)

Store aliquots at -30°C

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	Recipe for 1ml Lysis Buffer
	300 µL – Tris HCl
	600 µL – Tween 20
	90 µL – nuclease free H2O
	10 µL – Proteinase K

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	Examples of Lysis Buffer Calculations (including a bit of excess)			
	Component	**48 Samples**	**96 Samples (1 plate)**	**2 Plates**
	Tris	3 mL	6 mL	12 mL
	Tween	6 mL	12 mL	24 mL
	Proteinase K	100 µL	200 µL	400 µL

	Nuclease-free water	900 µL	1800 µL	3.6 mL
	Total Volume	10 mL	20 mL	40 mL

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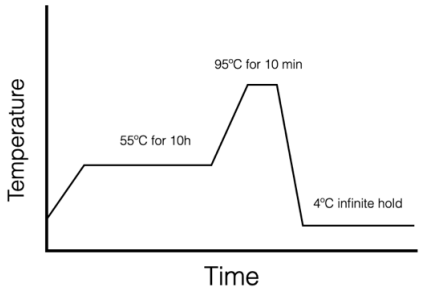
Lysis for Mouse Tissue

- Thaw all reagents and prepare lysis buffer as described above.
- Add 180 µL of lysis buffer in each well of a 96-well semi-skirted plate. We use BrandTech #781375. Supplied in US by VWR #10141-434.
- Add 20 µL of sample into each well.
- We recommend one extra well with 200 µL lysis buffer only to use as a "no template control".
- Seal wells with 8-strip domed caps. We use BrandTech #781340. Supplied in US by VWR #80087-132.
- To ensure proper mixing of the sample and buffer, vortex vigorously.
- To pull the sample/buffer mixture to the bottom of the wells, quickly centrifuge at 1,000g for 10 sec before lysis.
- Proceed to heat-activated lysis of the samples in a Thermocycler for 16 hours at 55^{o}C,
  followed by heat inactivation for 10 minutes at 95^{o}C.

11 o



This digested sample can be used directly as template DNA in qPCR for cf-DNA measurements.

- Before performing qPCR, vortex and centrifuge again the template DNA-containing tube(s)/plate(s) at 1,000g for 10 sec at room temperature. If not using within 24 - 48h, freeze the DNA samples at -80°C or colder.

6. qPCR data analysis and error checking

12 Compute the mean, standard deviation, and coefficient of variation (CV) for the cycle thresholds (Ct) across each triplicate for each sample.

13 If the CV across the 3 triplicates is >2%, check the triplicates to see if any of the wells has a Ct that is >1 unit different from the other two, which could indicate a qPCR failure in that well.

14 In the case where amplification has failed in a well, remove the outlier.

15 The average Ct for each sample should be used compute copies/mL.

Appendix A: qPCR Reagents and MasterMix Recipe

16

	Recipe for each well of qPCR	
	TaqMan Universal MasterMix Fast	10 µL
	ND1 Primers F+R	+ 0.6 µL
	ND1 Probe	+ 0.4 µL
	B2M Primers F+R	+ 0.6 µL
	B2M Probe	+ 0.4 µL
	Total Reagent Volume	**= 12 µL**
	+ Sample Volume	+ 8 µL
	Total Reaction Volume	**= 20 µL**

The reagents used may depend on the qPCR equipment available, the genomic locations of interest, or other experimental parameters.

Below, we provide a summary of possible reagents as well as other validated primers and probes.

17 **2x TaqMan Universal MasterMix Fast** (Life Tech #4444965)
MicroAmp™ Optical 384-Well Reaction Plate with Barcode (ThermoFisher #4309849)
MicroAmp™ Optical Adhesive Film (ThermoFisher #4311971)
Validated mtDNA/nDNA Primer & Probe sequences (idtDNA.com)

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	qPCR Target	Sequences (5′→3′)
	*Mouse mt-*COX1-F	ACCACCATCATTTCTCCTTCTC
	*Mouse mt-*COX1- R	CTCCTGCATGGGCTAGATTT
	*Mouse mt-*COX1-Probe:	HEX/AAGCAGGAG/ZEN/CAGGAACAGGATGAA/3IABkFQ
	Mouse B2M-F	GAGAATGGGAAGCCGAACATA
	Mouse B2M-	CCGTTCTTCAGCATTTGGATTT
	Mouse B2M-Probe	FAM/CGTAACACA/ZEN/GTTCCACCCGCCTC/3IABkFQ

19 - Reconstitute primers in appropriate volume of nuclease free water to achieve 100 µM stock concentration.
- Combine 120 µL each of 100 µM forward and reverse primers with 960 µL nuclease free water to achieve 10 µM working concentration.
- Store primers at -30ºC or -80ºC until use.

- Reconstitute probe in appropriate volume of nuclease free water to achieve 100 µM stock concentration.
- Dilute 100 µM stock probe 20x to achieve 5 µM working concentration.
- Store probes at -30^{o}C or -80^{o}C until use (avoid freeze-thaw) and protect probes from light.