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C.elegans mtDNA measurement by qPCR

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Preparation of DNA template

1. 30 early L4 worms were collected to a PCR tube containing 50 µl WLB.
2. Incubate 100 min at 65ºC.
3. Heat inactivate PK at 95ºC for 15min.
4. Cool on ice.

Setup of qPCR reaction

5. Setup reaction for each column:
   primer mix, 4 µl
   SYBR Green Real-time PCR Master Mix (TOYOBO QPK-201), 10 µl
   worm lysate, 3-4µl
   Add ddH_2O to final volume of 20µl
6. Both mtDNA and nDNA needed to be measured in each lysate for 3-4 technical repeats.
7. Primers:

Primers:	mtDNA-F	GCTTTTTCTTTATATGTTTTGTG
	mtDNA-R	TCACCTTCAGAAAAATCAAATGG
	nDNA-F	AGGCTAAGCCGGGGTAAGTT
	nDNA-R	GCCAAAAGCTTAAACTGCGG

8. The mtDNA/nDNA ratio was then calculated using the comparative ΔΔCt method.

Worm Lysis Buffer (WLB):

50 mM KCl,
10 mM Tris-HCl (pH 8.3),
2.5 mM $MgCl_2$,
0.45% NP-40,
0.45% Tween 20,
0.01% gelatin,
fresh proteinase K (200 µg/ml)