**Supplementary File 3 | Oligonucleotide sequences**

**Sequences of DNA oligonucleotides for use in radioactive MPAT assay**

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| **Oligonucleotide name** | **Oligonucleotide sequence** |
| LIGN | [Phos]-ATG TGA GAT CAT GCA CAG TCA TA-[SpcC3] |
| ANTI-LIGN | GAC TGT GCA TGA TCT CAC AT |
| MPAT\_12S\_Fw1 | ACC TGG CGG TGC TTC ATA TC |
| MPAT\_12S\_Fw2 | AAG TGT ACT GGA AAG TGC AC |
| MPAT\_16S\_Fw1 | ACC CAA CCT CCG AGC AGT AC |
| MPAT\_16S\_Fw2 | TAT ACC CAC ACC CAC CCA AG |
| MPAT\_ND1\_Fw1 | CCG AAC TAG TCT CAG GCT TC |
| MPAT\_ND1\_Fw2 | TAC AAT CTC CAG CAT TCC CC |
| MPAT\_ND2\_Fw1 | CAC CCT TAA TTC CAT CCA CC |
| MPAT\_ND2\_Fw2 | CTA TCT CCC CTT TTA TAC |
| MPAT\_ND3\_Fw1 | TGC GGC TTC GAC CCT ATA TC |
| MPAT\_ND3\_Fw2 | GAC TAC AAA AAG GAT TAG AC |
| MPAT\_ND4/4L\_Fw1 | TG ACT TCT AGC AAG CCT CGC |
| MPAT\_ND4/4L\_Fw2 | ACC CCG ACA TCA TTA CCG G |
| MPAT\_ND5\_Fw1 | CAT GCC TCA GGA TAC TCC TC |
| MPAT\_ND5\_Fw2 | CAT CGT TGT ATT TCA ACT AC |
| MPAT\_CYTB\_Fw1 | TTC TCC GAT CCG TCC CTA AC |
| MPAT\_CYTB\_Fw2 | TAA TTG AAA ACA AAA TAC TC |
| MPAT\_CO1\_Fw1 | GAC CAA ACC TAC GCC AAA ATC |
| MPAT\_CO1\_Fw2 | CCA ACC CCA TGG CCT CCA TG |
| MPAT\_CO2\_Fw1 | ATT CCT AGA ACC AGG CGA CC |
| MPAT\_CO2\_Fw2 | TAT AGC ACC CCC TCT ACC CC |
| MPAT\_CO3\_Fw1 | GTC TCT ATT TTA CCC TCC TAC |
| MPAT\_CO3\_Fw2 | TGT ATG TCT CCA TCT ATT GAT G |
| MPAT\_ATP86\_Fw2 | CCC ACT TCT TAC CAC AAG GC |
| MPAT\_ATP86\_Fw2 | AGT AAG CCT CTA CCT GCA CG |
| MPAT\_Lys\_Fw1 | ACT GTA AAG CTA ACT TAG CAT TAA CC |
| MPAT\_Lys\_Fw2 | AAG AGA ACC AAC ACC TCT TTA C |
| MPAT\_Glu\_Fw1 | GTT CTT GTA GTT GAA ATA CAA CGA TG |
| MPAT\_Glu\_Fw2 | GAT GGT TTT TCA TAT CAT TGG TC |
| MPAT\_Met\_Fw1 | GTA AGG TCA GCT AAA TAA GCT ATC GG |
| MPAT\_Met\_Fw2 | TAT CGG GCC CAT ACC CCG AAA ATG |
| MPAT\_SerAGY\_Fw1 | GAG AAA GCT CAC AAG AAC TGC TAA C |
| MPAT\_SerAGY\_Fw2 | AAG AAC TGC TAA CTC ATG CC |
| MPAT\_Val\_Fw1 | CAG AGT GTA GCT TAA CAC AAA GCA CC |
| MPAT\_Val\_Fw2 | CAC CCA ACT TAC ACT TAG GAG |
| MPAT\_His\_Fw1 | TAA ATA TAG TTT AAC CAA AAC ATC AG |
| MPAT\_His\_Fw2 | ATC AGA TTG TGA ATC TGA CAA CAG |

**Sequences of DNA oligonucleotides for use in Next-generation sequencing MPAT assay for mitochondrial tRNAs**

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| **Oligonucleotide name** | **Oligonucleotide sequence** |
| MPAT\_LeuUUR\_Fw | GTT AAG ATG GCA GAG CCC GGT AAT C |
| MPAT\_Asn\_Fw | TAG ATT GAA GCC AGT TGA TTA GGG TG |
| MPAT\_Gln\_Fw | TAG GAT GGG GTG TGA TAG GTG GCA C |
| MPAT\_Phe\_Fw | GTT TAT GTA GCT TAC CTC CTC AAA GC |
| MPAT\_LeuCUN\_Fw | AAG GAT AAC AGC TAT CCA TTG GTC |
| MPAT\_Lys\_Fw | ACT GTA AAG CTA ACT TAG CAT TAA CC |
| MPAT\_His\_Fw | TAA ATA TAG TTT AAC CAA AAC ATC AG |
| MPAT\_Val\_Fw | CAG AGT GTA GCT TAA CAC AAA GCA CC |
| MPAT\_Ile\_Fw | GAA ATA TGT CTG ATA AAA GAG TTA C |
| MPAT\_Ala\_Fw | AAG GGC TTA GCT TAA TTA AAG TGG C |
| MPAT\_Glu\_Fw | GTT CTT GTA GTT GAA ATA CAA CGA TG |
| MPAT\_SerUCN\_Fw | GAA AAA GTC ATG GAG GCC ATG GGG |
| MPAT\_Pro\_Fw | GAG AAT AGT TTA AAT TAG AAT CTT AG |
| MPAT\_Met\_Fw | GTA AGG TCA GCT AAA TAA GCT ATC GG |
| MPAT\_Trp\_Fw | GAA ATT TAG GTT AAA TAC AGA CCA AG |
| MPAT\_Asp\_Fw | GTA TTA GAA AAA CCA TTT CAT AAC TTT G |
| MPAT\_Gly\_Fw | CTT TTA GTA TAA ATA GTA CCG TTA AC |
| MPAT\_Thr\_Fw | GTC CTT GTA GTA TAA ACT AAT ACA CC |
| MPAT\_Tyr\_Fw | GGT AAA ATG GCT GAG TGA AGC ATT GG |
| MPAT\_Cys\_Fw | AGC TCC GAG GTG ATT TTC ATA TTG |
| MPAT\_Arg\_Fw | GGT ATA TAG TTT AAA CAA AAC GAA TG |
| MPAT\_SerAGY\_Fw | GAG AAA GCT CAC AAG AAC TGC TAA C |

**Primers and probe sequences for Q-PCR**

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| --- | --- |
| **Oligonucleotide name** | **Oligonucleotide sequence** |
| B2M F | TGC TGT CTC CAT GTT TGA TGT ATC T |
| B2M R | TCT CTG CTC CCC ACC TCT AAG T |
| B2M probe | 6-Fam-TTG CTC CAC AGG TAG CTC TAG GAG G-Tamra |
| mt3211 F | CAC CCA AGA ACA GGG TTT GT |
| mt3298 R | TGG CCA TGG GTA TGT TGT TAA |
| mt3242 Probe | 6-Fam-TTA CCG GGC TCT GCC ATC T-Tamra |
| mt9827 F | CGT CAT TAT TGG CTC AAC |
| mt9974 R | GAT GGA GAC ATA CAG AAA TAG |
| mt9852 Probe | 6-Fam-ACT ATC TGC TTC ATC CGC CAC TAA-Tamra |

**DNA oligonucleotides used for construction of radiolabelled dsDNA probes**

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| --- | --- | --- |
| **Transcript** |  | **Oligonucleotide sequence** |
| 12S | F | CAC TGA AAA TGT TTA GAC GGG |
| R | GGC TCC TCT AGA GGG ATA TG |
| 16S | F | TAG ATA TAG TAC CGC AAG GG |
| R | GAC TTG TTG GTT GAT TGT AG |
| ND1 | F | AAC CTC AAC CTA GGC CTC C |
| R | AAT GCT AGG GTG AGT GGT AGG |
| ND2 | F | TCC CAG AGG TTA CCC AAG |
| R | GAG TAG TGT GAT TGA GGT GGA G |
| ND4L/ND4 | F | ACT ACC ACT GAC ATG ACT TTC C |
| R | GGA GTC ATA AGT GGA GTC CG |
| ND5 | F | GTA GCA TTG TTC GTT ACA TGG |
| R | ACT GCT GCG AAC AGA GTG |
| CO1 | F | CTT ATT CGA GCC GAG CTG |
| R | GGT ATA GAA TGG GGT CTC CTC |
| CO2 | F | GCG CAA GTA GGT CTA CAA GAC GC |
| R | GCA TGA AAC TGT GGT TTG CTC C |
| ATP8/6 | F | CCC ATA CTC CTT ACA CTA TTC C |
| R | GTT AGC GGT TAG GCG TAC |
| CYTB | F | CTA CCT TCA CGC CAA TGG |
| R | TTT GTT AGG GAC GGA TCG |

**DNA oligonucleotides used for construction of template to produce radiolabelled ssRNA probes**

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| --- | --- | --- |
| **Transcript** |  | **Oligonucleotide sequence** |
| ND3 | F | ATA AAC TTC GCC TTA ATT TTA ATA ATC |
| R | TAA TAC GAC TCA CTA TAG GGA TTC GGT TCA GTC TAA TCC TTT TTG TAG |
| ND6 | F | GGG GTT TTC TTC TAA GCC TTC |
| R | TAA TAC GAC TCA CTA TAG GGC CCC CGA GCA ATC TCA ATT AC |
| COX3 | F | CCT GAG AAC CAA AAT GAA CG |
| R | TAA TAC GAC TCA CTA TAG GGG CCA GGG CTA TTG GTT GAA TG |

**Oligonucleotides for production of MitoRibo-Seq libraries**

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| --- | --- |
| **Oligonucleotide name** | **Oligonucleotide sequence** |
| Preadenylated 3’-adaptor | rATGGAATTCTCGGGTGCCAAGG |
| 5’ adaptor | GUUCAGAGUUCUACAGUCCGACGAUC |
| RT\_Primer | GCCTTGGCACCCGAGAATTCCA |
| RP1 | AATGATACGGCGACCACCGAGATCTACA CGTTCAGAGTTCTACAGTCCGA |
| RPIX (Where N is primer number) | CAAGCAGAAGAC GGCATACGAGAT N6GTGACT GGAGTTCCTTGGCACCCGAGAATTCCA |