

Test Description

The MolQ Germline Cancer Predisposition Panel include genes associated with both common and rare hereditary cancers.

Patient Demographic

Name: Ms. Nidhi Gulati
Sex: Female
Date of Birth/Age: 53 years
Disease: Endometrioid carcinoma

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Therapy and Cancer Research (IPTCR)
Pathologist: Not Provided

Specimen

Booking ID: 012406080159
Site: NA
Sample Type: Blood
Date of Collection: 08-06-2024
Date of Booking: 08-06-2024

CLINICAL SYNOPSIS

Nidhi Gulati, is a known case of endometrioid carcinoma. She has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

Variants of Uncertain Significance related to the clinical phenotype were detected.

Gene (Transcript)#	Location	Variant	Zygosity	Disease (OMIM/Ref)	Inheritance	Classification [§]
<i>PMS2</i> (-) (NM_000535.7)	Intron 6	c.705+3A>G (Splice site variant)	Heterozygous	Lynch syndrome type 4	Autosomal Dominant	Uncertain significance (PP3)
<i>POLE</i> (-) (NM_006231.4)	Exon 43	c.5988C>G (p.Phe1996Leu)	Heterozygous	Colorectal cancer susceptibility/ Endometrial cancer susceptibility	Autosomal Dominant	Uncertain significance (PP2, PP3)

[§]Genetic test results are reported based on the recommendations of American College of Medical Genetics.

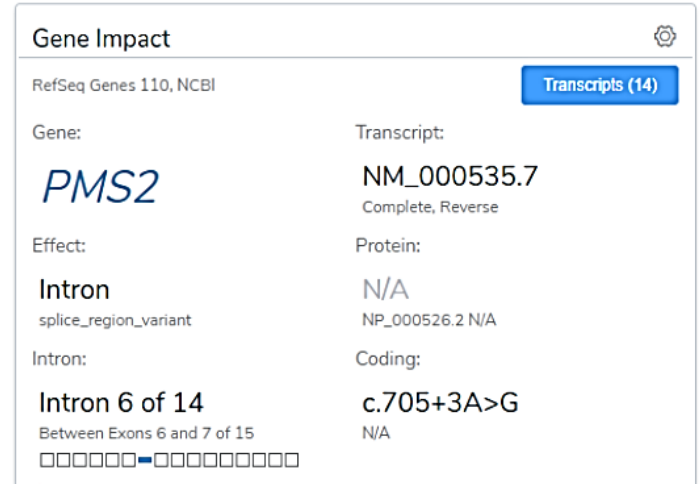
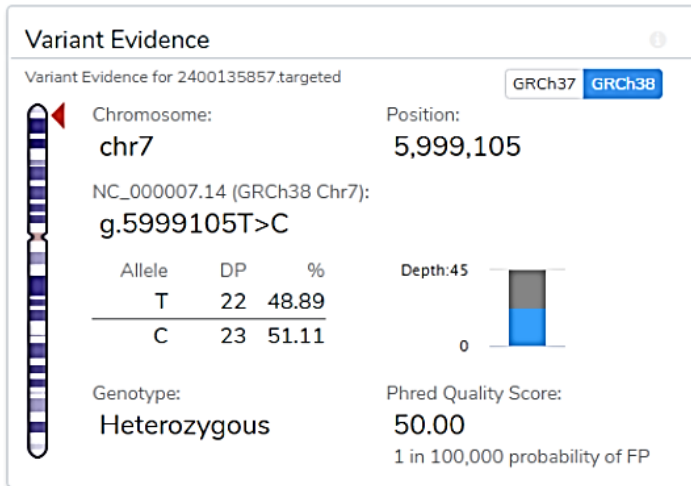
[#]Due to lack of clinical evidence for this variant, it is classified as variant of uncertain significance.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PMS2 c.705+3A>G

Variant description: The submitted sample shows heterozygous variant in intron 6 of gene *PMS2* (chr7: g.5999105T>C). A splice region variant "A" to "G" detected at nucleotide position 705+3. *In silico predictions:* The nucleotide c.705+3A>G in *PMS2* is predicted conserved by GERP++ and PhyloP across 100 vertebrates. *Population frequency and Internal database:* The c.705+3A>G variant is observed in 5/30,782 (0.0162%) alleles from individuals of gnomAD South Asian background in gnomAD All. The c.705+3A>G variant is novel (not in any individuals) in 1kG All. This variant is absent in reference laboratory's internal database.

OMIM Phenotype: Lynch syndrome-4 (LYNCH4), also known as hereditary nonpolyposis colorectal cancer type 4 (HNPCC4), is caused by heterozygous mutation in the *PMS2* gene (600259) on chromosome 7p22. Lynch syndrome-4 (LYNCH4), or hereditary nonpolyposis colorectal cancer type 4 (HNPCC4), is an autosomal dominant disorder characterized primarily by the development of early-onset colorectal cancer. It is associated with the development of a variety of epithelial tumors that include endometrial cancer, stomach cancer, and ovarian cancer (summary by Thompson et al., 2004).



Based on the above evidence\$, **this variant (PMS2: c.705+3A>G) is classified as Uncertain significance variant.**

POLE p.Phe1996Leu

Variant description: The submitted sample shows heterozygous variant in Exon 43 of gene *POLE* (chr12: g.132634202G>C). A missense variant “C” to “G” detected at nucleotide position 5988 leading to change in amino acid sequences from Phenylalanine to Leucine at codon 1996. *In silico predictions:* The p.Phe1996Leu missense variant is predicted to be damaging by both SIFT and PolyPhen2. The phenylalanine residue at codon 1996 of *POLE* is conserved in all mammalian species. The nucleotide c.5988 in *POLE* is predicted conserved by GERP++ and PhyloP across 100 vertebrates. *Population frequency and Internal database:* The p.Phe1996Leu variant is observed in 8/30,590 (0.0262%) alleles from individuals of gnomAD South Asian background in gnomAD All. The p.Phe1996Leu variant is novel (not in any individuals) in 1kG All. This variant is absent in reference laboratory’s internal database.

OMIM Phenotype: Colorectal cancer-12 (CRCS12) is an autosomal dominant disorder characterized by a high-penetrance predisposition to the development of colorectal adenomas and carcinomas, with a variable tendency to develop multiple and large tumors. Onset usually occurs before age 40 years. The histologic features of the tumors may be unremarkable (Palles et al., 2013) or show microsatellite instability (MSI). Elsayed et al. (2015) reported 2 Dutch families and an unrelated Dutch patient with CRCS12 confirmed by genetic analysis. In the first family, a mother was diagnosed with an MSI colorectal tumor and 2 adenomas at age 40 years. She later developed multiple colorectal polyps as well as microsatellite-stable endometrial cancer at age 50. Her son was diagnosed with MSI adenocarcinoma at age 30. Tumor tissue from these patients showed loss of MSH2 (609309) and/or MSH6 (600678) protein expression, suggestive of Lynch syndrome (see, e.g., 120435), although there were no germline mutations in these genes. The mother's tumor showed a hypermutator phenotype, with multiple somatic mutations in several genes. In the second family, the proband was diagnosed with multiple polyps at age 34. An adenoma with cancer showed MSI associated with immunohistochemical loss of MSH2 and MSH6 protein staining, also in the absence of germline variants in these genes. The patient had a family history of colorectal and other forms of cancer, but DNA and tumor tissue samples from her relatives were not available. The unrelated Dutch patient was diagnosed with a microsatellite-stable colon cancer and polyposis at age 33. No tumor tissue was available for analysis. The MSI observed in some of these patients was in contrast to the microsatellite stability observed in the tumors from the family reported by Palles et al. (2013). *POLE* exonuclease domain mutations (EDMs) have been reported in 7-12% endometrial cancers (ECs) and defined a subgroup of endometrial cancers with ultrahigh somatic mutation frequencies, high tumor infiltrated lymphocytes and favorable outcomes.

Variant Evidence

Variant Evidence for 2400135857.targeted

GRCh37 GRCh38

Chromosome: **chr12** Position: **132,634,202**

NC_000012.12 (GRCh38 Chr12): **g.132634202G>C**

Allele	DP	%
G	26	47.27
C	29	52.73

Depth: 55

Genotype: **Heterozygous**

Phred Quality Score: **48.00**
1 in 10,000 probability of FP

Gene Impact

RefSeq Genes 110, NCBI

Transcripts (1)

Gene: **POLE** Transcript: **NM_006231.4**
Complete, Reverse

Effect: **Missense** Protein: **p.Phe1996Leu**
missense_variant NP_006222.2 (AA 1,996 of 2,287)

Exon: **Exon 43 of 49** Coding: **c.5988C>G**
Coding 5987 of 6861 (87%)

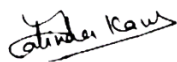
Based on the above evidence\$, ***this variant (POLE: c.5988C>G) is classified as Uncertain significance variant.***

RECOMMENDATIONS

- Segregation analysis of the variant by Sanger sequencing is recommended in affected and unaffected members of the family.
- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- Genetic counselling is advised for interpretation on the consequences of the variant(s).
- The significance/classification of the variants might change based on parental and family members genetic testing.
- For questions about this report, or for assistance in locating nearby genetic counseling services, please contact the laboratory: contact@molq.in.
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.

REFERENCES

1. Cui J, Chen X, Zhai Q, Chen N, Li X, Zhang Y, Wang H, Bian X, Gao N, Chen D, Chen Z, Zhang S, Chen Y. A novel somatic mutation in POLE exonuclease domain associated with ultra-mutational signature and MMR deficiency in endometrial cancer: a case report. Diagn Pathol. 2023 Feb 10;18(1):19. doi: 10.1186/s13000-023-01287-y. PMID: 36765365; PMCID: PMC9912575.



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APPENDIX 1: TEST METHODOLOGY

Method

Next Generation Sequencing:¹⁻⁵ DNA extracted from blood, saliva, amniotic fluid, CVS or any other standard source is used for targeted capture-based Library preparation. Targeted capture provides an efficient and sensitive means for sequencing specific genomic regions in a high-throughput manner. The libraries were sequenced to mean >85-100x coverage on Illumina Novaseq 6000 sequencing platform with Paired End 2x150 chemistry.

Illumina DRAGEN Bio-IT Platform was followed for identification of variants in the sample. The sequences obtained are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build (GRCh38). Haplotype caller has been used to identify variants which are relevant to the clinical indication.

In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the commercially available algorithm. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset. Clinically relevant mutations were annotated using published variants in literature, Commercial datasets and a set of diseases databases.

Common variants are filtered based on allele frequency in 1000Genome, ExAC, gnomAD, dbSNP and reference laboratory's internal database.

Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2. Based on annotations data, ACMG rules-based classification performed for classification of variants identified through next generation sequencing study.

Average Sequencing Depth (x)	Percentage Target Base Pairs Covered		
	1x	≥5x	≥20x
83.59	99.02	98.83	98.47

Title	Data
Total data generated (Gb)	9.04
Total reads aligned (%)	100.00
Reads that passed alignment (%)	99.68
Data ≥ Q30 (%)	92.12

***The classification of the variations is done based on American College of Medical Genetics as described below⁶**

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Benign	A variant which is known not to be responsible for disease has been detected. Generally, no further action is warranted on such variants when detected.
Likely Benign	A variant which is very unlikely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of Pathogenicity.
Pathogenic	A disease-causing variation in a gene which can explain the patient's symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

DISCLAIMER

- Interpretation of variants in this report is performed to the best knowledge of the laboratory based on the information available at the time of reporting. The classification of variants can change over time and MolQ cannot be held responsible

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for this. Please feel free to contact MolQ Laboratory (contact@molq.in) in the future to determine if there have been any changes in the classification of any variations. Re-analysis of variants in previously issued reports in light of new evidence is not routinely performed, but may be considered upon request.

- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is 70-75%. The CNVs detected with this assay have to be confirmed by alternate method such as MLPA & Microarray.
- Due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants present in areas of insufficient coverage as well as those variants which currently do not correlate with the provided phenotype may not be analyzed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- The mutations have not been validated/confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines² can be given upon request.
- Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS).
- The transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by reference laboratory.

LIMITATIONS

- Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Accurate interpretation of test results may require knowing the true biological relationships in a family. Failing to accurately state the biological relationships in {my/my child's} family may result in incorrect interpretation of results, incorrect diagnoses, and/or inconclusive test results.
- Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- Triplet repeat expansions, translocations, large deletion or duplications and chromosomal rearrangements events are currently not reliably detected by next generation sequencing.
- This assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, other gene rearrangements like inversion or translocation and does not detect single or multiexon deletions or duplications.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to: mislabelled samples, inaccurate reporting of clinical/medical information, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).
- It should be noted that this test does not sequence all bases in a human genome, not all variants have been identified or interpreted, and this report is limited only to variants with evidence for causing or contributing to disease/clinical details provided to MolQ Laboratory.
- Testing has been performed assuming that the sample received belongs to the above named individual and any stated relationships between individuals are accepted as true.
- The results should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic

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background. Please note that variant classification and/or interpretation may change over time if more information available. Reinterpretation of multi gene next generation sequencing data is recommended on an annual basis and may be requested by a medical provider.

REFERENCES

2. Meyer L.R. et al., The UCSC Genome Browser database: extensions and updates 2013. *Nucleic Acids Res.*, 41(D1):D64-9, 2013.
3. McKenna, A., et al., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.*, 20(9): 1297-303, 2010.
4. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. *Nature*, 526(7571): 68-74, 2015.
5. Lek M. et al., Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, 536(7616):285-91, 2016.
6. McLaren W. et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics.*, 26(16):2069-70, 2010.
7. Richards S. *et al.*, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, *Genet Med.*, 17(5):405-24, 2015.
8. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.*, 19(2):249-255, 2017.

APPENDIX 2: GENE LIST WITH COVERAGE

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ABRAXAS1	100.00	FANCG	100.00	PTCH1	100.00
ACVRL1	100.00	FANCI	100.00	PTCH2	100.00
AIP	100.00	FANCL	100.00	PTEN	100.00
AKT1	100.00	FANCM	100.00	PTPN11	100.00
ALK	100.00	FGFR2	100.00	RAD50	100.00
ANKRD26	100.00	FH	100.00	RAD51	100.00
APC	100.00	FLCN	100.00	RAD51C	100.00
AR	100.00	GALNT12	100.00	RAD51D	100.00
ATM	100.00	GATA2	100.00	RAF1	100.00
ATR	100.00	GPC3	100.00	RASA2	100.00
AXIN1	100.00	GPR101	100.00	RASAL1	100.00
AXIN2	100.00	GREM1	100.00	RB1	100.00
BAP1	100.00	HAVCR2	100.00	RECQL	100.00
BARD1	100.00	HNF1A	100.00	RECQL4	100.00
BLM	100.00	HNF1B	100.00	REST	100.00
BMPR1A	100.00	HOXB13	100.00	RET	100.00
BRAF	100.00	HRAS	100.00	RFWD3	100.00
BRCA1	100.00	IKZF1	100.00	RHBDF2	100.00
BRCA2	100.00	KIF1B	100.00	RINT1	100.00
BRIP1	100.00	KIT	100.00	RIT1	100.00
BTNL2	100.00	KITLG	100.00	RNASEL	100.00
BUB1B	100.00	KRAS	100.00	RNF43	100.00
CASR	100.00	LIG4	100.00	RPS20	100.00
CBL	100.00	LSP1	79.12	RRAS	100.00
CD70	100.00	LZTR1	100.00	RUNX1	100.00
CD82	100.00	MAD2L2	100.00	SAMD9	100.00
CDC73	100.00	MAP2K1	100.00	SAMD9L	100.00
CDH1	100.00	MAP2K2	100.00	SBDS	100.00
CDK4	100.00	MAP3K1	100.00	SDHA	100.00
CDKN1B	100.00	MAX	100.00	SDHAF2	100.00
CDKN1C	100.00	MC1R	100.00	SDHB	100.00
CDKN2A	100.00	MEN1	100.00	SDHC	100.00
CDKN2B	100.00	MET	100.00	SDHD	100.00
CEBPA	100.00	MITF	100.00	SHOC2	100.00
CEP57	100.00	MLH1	100.00	SLX4	100.00
CFTR	100.00	MLH3	100.00	SMAD4	100.00
CHEK2	100.00	MRE11	100.00	SMARCA2	100.00
CPA1	100.00	MSH2	100.00	SMARCA4	100.00
CTNNA1	100.00	MSH3	100.00	SMARCB1	100.00
CYLD	100.00	MSH6	100.00	SMARCE1	100.00
DDB2	100.00	MSR1	100.00	SOS1	100.00
DDX41	100.00	MUTYH	100.00	SOS2	100.00
DICER1	100.00	MXI1	100.00	SPINK1	100.00
DIS3L2	100.00	MYSM1	100.00	SPRED1	100.00
DKC1	100.00	NBN	100.00	SRP72	96.83

<i>EFL1</i>	100.00	<i>NF1</i>	100.00	<i>STK11</i>	100.00
<i>EGFR</i>	100.00	<i>NF2</i>	100.00	<i>SUFU</i>	100.00
<i>ELAC2</i>	100.00	<i>NHEJ1</i>	100.00	<i>TERC</i>	0.00
<i>ELANE</i>	100.00	<i>NRAS</i>	100.00	<i>TERT</i>	100.00
<i>ENG</i>	100.00	<i>NSD1</i>	100.00	<i>TGFB1</i>	100.00
<i>EPCAM</i>	100.00	<i>NSUN2</i>	100.00	<i>TGFBR2</i>	100.00
<i>ERCC1</i>	100.00	<i>NTHL1</i>	100.00	<i>TINF2</i>	100.00
<i>ERCC2</i>	100.00	<i>PALB2</i>	100.00	<i>TMEM127</i>	100.00
<i>ERCC3</i>	100.00	<i>PALLD</i>	100.00	<i>TOX3</i>	96.65
<i>ERCC4</i>	100.00	<i>PAX5</i>	100.00	<i>TP53</i>	100.00
<i>ERCC5</i>	100.00	<i>PDGFRA</i>	100.00	<i>TRIP13</i>	100.00
<i>ETV6</i>	100.00	<i>PHOX2B</i>	100.00	<i>TSC1</i>	100.00
<i>EXO1</i>	100.00	<i>PIK3CA</i>	100.00	<i>TSC2</i>	100.00
<i>EXT1</i>	100.00	<i>PMS1</i>	100.00	<i>UBE2T</i>	100.00
<i>EXT2</i>	100.00	<i>PMS2</i>	100.00	<i>VHL</i>	100.00
<i>EZH2</i>	100.00	<i>POLD1</i>	100.00	<i>VPS13B</i>	100.00
<i>FAM111B</i>	100.00	<i>POLE</i>	100.00	<i>WRN</i>	100.00
<i>FANCA</i>	100.00	<i>POLH</i>	100.00	<i>WT1</i>	100.00
<i>FANCB</i>	100.00	<i>POT1</i>	100.00	<i>XPA</i>	100.00
<i>FANCC</i>	100.00	<i>PPM1D</i>	100.00	<i>XPC</i>	100.00
<i>FANCD2</i>	100.00	<i>PRF1</i>	100.00	<i>XRCC2</i>	100.00
<i>FANCE</i>	100.00	<i>PRKARIA</i>	100.00	<i>XRCC3</i>	100.00
<i>FANCF</i>	100.00	<i>PRSS1</i>	100.00	<i>ZFHX3</i>	100.00

MTDNA GENES LIST WITH COVERAGE

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
<i>MT-ATP6</i>	100.00	<i>MT-ND6</i>	100.00	<i>MT-TL1</i>	100.00
<i>MT-ATP8</i>	100.00	<i>MT-RNR1</i>	100.00	<i>MT-TL2</i>	100.00
<i>MT-CO1</i>	100.00	<i>MT-RNR2</i>	100.00	<i>MT-TM</i>	100.00
<i>MT-CO2</i>	100.00	<i>MT-TA</i>	100.00	<i>MT-TN</i>	100.00
<i>MT-CO3</i>	100.00	<i>MT-TC</i>	100.00	<i>MT-TP</i>	100.00
<i>MT-CYB</i>	100.00	<i>MT-TD</i>	100.00	<i>MT-TQ</i>	100.00
<i>MT-ND1</i>	100.00	<i>MT-TE</i>	100.00	<i>MT-TR</i>	100.00
<i>MT-ND2</i>	100.00	<i>MT-TF</i>	100.00	<i>MT-TS1</i>	100.00
<i>MT-ND3</i>	100.00	<i>MT-TG</i>	100.00	<i>MT-TS2</i>	100.00
<i>MT-ND4</i>	100.00	<i>MT-TH</i>	100.00	<i>MT-TT</i>	100.00
<i>MT-ND4L</i>	100.00	<i>MT-TI</i>	100.00	<i>MT-TV</i>	100.00
<i>MT-ND5</i>	100.00	<i>MT-TK</i>	100.00	<i>MT-TW</i>	100.00
<i>MT-TY</i>	100.00				