

The Meaning of Your Mutations¹

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August 10, 2007

This report on coding region mutations was prepared expressly for *Ernest Scott*.

Mitochondrial DNA (mtDNA) is of special interest because of its inheritance pattern – it is inherited through the egg, so your mtDNA came from your mother, who got it from her mother, who got it from her mother...clear back to the common matrilineal ancestor of all humanity, sometimes nicknamed mitochondrial Eve. “Eve” was not the first woman, nor was she the only woman alive at the time. Her contemporaries could still have descendants, whose line zig-zagged back and forth between males and females, but Eve was the only one who had an unbroken line of daughters, through thousands of generations, clear down to the present time.

The Cambridge Reference Sequence

Your mitochondrial DNA was completely sequenced and compared to the Cambridge Reference Sequence (CRS). There is nothing special about the CRS – it was simply the first one to be sequenced in 1981, using a placenta obtained from a maternity hospital located conveniently close to Cambridge University in England (Anderson 1981). Today, mtDNA can be sequenced using a much smaller sample of cells, easily gathered from the inside of your cheeks.

As more samples from around the world were examined, it became clear that all human beings shared 99% or more of their mtDNA sequence. Yet the remaining portion, a mere handful of differences, was enough to sort people into clusters, based on similar patterns in their mtDNA.

These broad clusters are known as haplogroups, and the divisions between haplogroups occurred tens of thousands of years ago. Some haplogroups are common in Africa, others hail from Europe, and still others forge a bond between Asia and the Americas.

Additional mutations have accumulated since the founding mothers of each haplogroup lived, forming subhaplogroups (also called subclades). Even more distinctive *haplotypes* (the complete set of differences from the CRS) have not been classified into subclades, and they may point to an ancestor who lived more recently. Your mtDNA reflects many layers of human history.

¹ Disclaimer: This report is not intended to provide medical advice. If you have any concerns, please consult with your personal physician.

The CRS sample was resequenced a few years ago, using more modern techniques (Andrews 1999). A very few errors were detected, and Family Tree DNA (FTDNA) compares your results to the revised edition, sometimes called the rCRS. However, the acronym CRS will be used throughout the remainder of this report.

A brief biology lesson

A little background will help you understand the significance of your own mutations. However, you may skip ahead to *Your differences* if you're eager to see them.

Mitochondria are essential cell structures, responsible for converting food into energy. Each cell has hundreds of mitochondria, and each mitochondrion has several copies of the mtDNA molecule.

The mtDNA molecule is circular, with 16,569 bases. The numbering begins at an arbitrary point in the middle of the D-loop (displacement loop, a section which spreads apart when the mtDNA molecule begins to replicate). This region is sometimes called the control region (due to its role in promoting replication), in distinction from the coding region.

The control region includes base positions 16024 through 16569 and continues around the circle to include bases 1 through 576. Because the control region is not responsible for producing proteins, mutations can accumulate without obvious adverse effects. Indeed, this stretch is also called the hypervariable region, because it provides the best hunting ground for finding differences among people. Until very recently, mtDNA testing for the consumer market was limited to the control region.

The coding region covers the remaining bases, in positions 577 to 16023. A complete list of the various functional areas is shown in Appendix 1. This region is densely packed, including genes for thirteen different proteins involved in breaking down the big molecules found in food. Each protein is composed of amino acids, which are arranged in a particular order, specified by the genetic blueprint. These particular proteins are enzymes, which facilitate chemical reactions in very small and delicate steps, so that the cell does not burst into flames as it burns food for energy.

In addition to the genes, there are two ribosomal RNAs (rRNA) and twenty-two transfer RNAs (tRNA). Ribosomes are like miniature factories, with two floors and an assembly line for constructing proteins. The two rRNAs are called 12S RNA and 16S RNA, for the size of the factory floors. The tRNAs each embrace a specific amino acid and ferry it to the factory floor, ready for the assembly workers who are following the specifications in the genetic blueprint. The genetic code uses three bases (a codon) to specify one of the twenty-some amino acids. Appendix 2 shows the codons for each amino acid.

The effective mutation rate for the coding region is lower than for the hypervariable region. Many changes would be so harmful that a woman might not even know that she had conceived. This mutation would disappear without a trace. Yet other changes seem to

be relatively benign in their effect, as explained in more detail below. These polymorphisms (poly = many, morph = form), being relatively stable compared to the hypervariable regions, are useful for defining haplogroups. However, parallel and back mutations can occur – the same mutation can occur in different branches of the family tree, or one branch may revert to the ancestral value. Thus it is important to look at the whole picture, not just one location.

Your differences

Your differences from the CRS are shown in Table 1, as presented on your FTDNA personal results page, but color-coded to show the significance of various changes.

The gray boxes show the locations where you have a difference because you are *not* in haplogroup H2 (where the CRS is located). As shown in Figure 1, the CRS is just a small twig on one of the major branches of humanity, and most people (even most people in haplogroup H) will have those five polymorphisms at 750G, 1438G, 4769G, 8860G, and 15326G. You also differ from the CRS at locations leading to haplogroup H (2706G and 7028T) and HV (11719A and 14766T). In other words, you have the ancestral values here, and the actual mutations occurred en route to haplogroup H.

You have the mutations that define superhaplogroup **UK** (11467G, 12308G, and 12372A). Mutations defining deeper levels of subhaplogroups are shown in different colors: 1811G is common to U2, U3, U4, U7 and K (clusters that were recognized by their HVR motifs before their position on the tree was appreciated). U3 has its own mutations at 14139G and 15454C, and a collection of mutations define U3a: 3010A (also found in a number of other haplogroups), 4703C, 6519T, 9266A, 10506G, and 13934T.

The color yellow is reserved for the most recent mutations, the ones that have not been observed often enough to be formally recognized as a subhaplogroup. Population geneticists sometimes call these “private” mutations, using the word in the sense of “*confined to particular persons or groups.*” Private mutations may in fact be very recent or quite old, but regardless of their absolute age, they are the ones that narrow down the pool of matrilineal relatives to your closest cousins. Not everyone will even have a private mutation. Your private mutation is at 2294G.

750G	1438G	1811G	2294G	2706G	"private"
3010A	4703C	4769G	6518T	7028T	U3a
8860G	9266A	10506G	11467G	11719A	U3
12308G	12372A	13934T	14139G	14766T	U2,U3,U4,U7 & K
15326G	15454C				UK
					not HV
					not CRS

Table 1

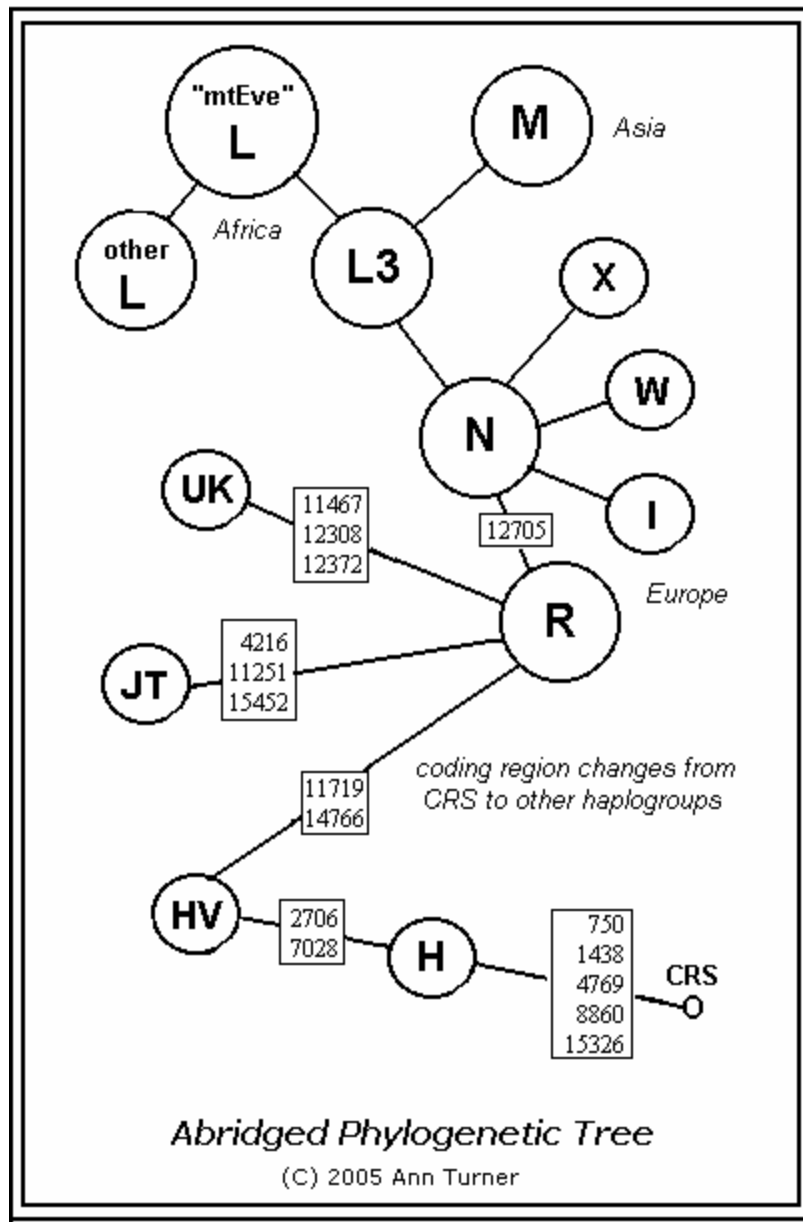


Figure 1

The CRS is a subhaplogroup in H, with a number of mutations that occurred after the clan mother for haplogroup H lived. Anyone who is not in the same subhaplogroup as the CRS will show the five differences listed between H and the CRS. Similarly, anyone who is not in H will show differences at 2706 and 7028, and anyone who is not in H or V will show differences at 11719 and 14766. Haplogroups J and T are closely related, as shown by the mutations they share; likewise U and K spring from a common origin. People in haplogroups X, W and I will all note a difference at 12705. Mutations for subhaplogroups, and mutations from N back to mtEve, are not shown, nor are the numerous branches springing from M and the L subhaplogroups that remained in Africa.

Ranking your mutations

Table 2 shows your polymorphisms arranged in a different order, roughly ranked from low to high according to the degree of interest they hold for you personally. At the lowest level, millions if not billions of people will show the same “mutations.” Polymorphisms at a higher level merit your special attention. Some people will only see mutations at the first two levels.

The table is full of technical details about each of your mutations. If you’re not especially interested in “looking under the hood,” just check column one for the highest code and skip ahead to the next section, *Information about your most distinctive mutations*.

The mutations are coded as follows:

- 1) Polymorphisms where the CRS has the rare value. Most people in the world, even most people within haplogroup H, will show these differences. Your differences may be called mutations, but the mutations actually occurred in the CRS – you have the ancestral versions.
- 2) Polymorphisms defining haplogroups and subhaplogroups, as shown in Table 1. Since these mutations have persisted in many people for thousands of years, there is little reason to suspect they have any great medical significance.
- 3) Polymorphisms in non-coding positions, just a few spots located here and there between the functional areas.
- 4) Polymorphisms in ribosomal RNA, with no known adverse effects. The factory floor has some minor remodeling that does not affect the assembly line.
- 5) Polymorphisms in transfer RNA, with no known adverse effects. The tRNA can still grab the right amino acid and deliver it to the factory.
- 6) Polymorphisms pertaining to amino acids
 - a) Synonymous – Several different three-base codons may be used for the same amino acid. It is like substituting the word “nice” for “pleasant” when describing today’s weather. The words convey the same basic meaning. See Appendix 2.
 - b) Conservative – The amino acid is different, but it has similar properties, such as size or electric charge. It is like saying “The weather will be warm today.” Most people would agree that warm weather is nice and pleasant, but the meaning is not precisely identical.
 - c) Non-conservative – The amino acid is different, with different properties. It is like saying “The weather will be a little breezy today.” The protein function may be affected in subtle ways; however, no disease has been associated with the polymorphism. To carry the analogy further, the weather is still suitable for a picnic, but you might need to arrange the paper napkins so they won’t blow away.
- 7) Polymorphisms linked to a disease. Discretion should be used when sharing these mutations publicly.

These categories are overlapping and not mutually exclusive. For example, the mutation at 14766, defining superhaplogroup HV, is a non-conservative mutation, yet large numbers of people with and without the mutation survive and thrive.

Code	Position	You#	CRS	Function	Amino Acid
1	750	G	A	12S_rRNA	
1	1438	G	A	12S_rRNA	
1	4769	G	A	ND2	synonymous
1	8860	G	A	ATPase6	Thr->Ala
1	15326	G	A	Cytb	Thr->Ala
2	1811	G	A	16S_rRNA	
2	2706	G	A	16S_rRNA	
2	3010	A	G	16S_rRNA	
2	4703	C	T	ND2	synonymous
2	6518	T	C	COI	synonymous
2	7028	T	C	COI	synonymous
2	9266	A	G	COIII	synonymous
2	10506	G	A	ND4L	Thr->Ala
2	11467	G	A	ND4	synonymous
2	11719	A	G	ND4	synonymous
2	12308	G	A	tRNA_Leu	
2	12372	A	G	ND5	synonymous
2	13934	T	C	ND5	Thr->Met
2	14139	G	A	ND5	synonymous
2	14766	T	C	Cytb	Ile->Thr
2	15454	C	T	Cytb	synonymous
4*	2294	G	A	16S_rRNA	

Table 2

The bases C and T are rather similar to each other in chemical structure, and likewise for the bases G and A. Thus most substitutions are C <-> T and G <-> A (called “transitions”). Other combinations (called “transversions”) occur more rarely, and they are less subject to parallel and back mutations. Your mtDNA shows no transversions.

* Ruiz-Pesini has diagrammed the secondary structure of 16s_RNA (the way that the linear chain of bases bends and folds into “loops” and “stems” as it floats in the cell). The “stems” are regions where bases are attracted to each other, and the “loops” are regions where the strands float more loosely in the cell. He noted that most mutations occur on the “loop” sections. The implication is that these regions do not need to be highly conserved, and mutations can accumulate more freely there without affecting the function. Figure 2 zooms in on Figure 4 of his paper (attached to your report). Your mutation at 2294G is located on a “loop” section of the structure. Therefore it’s less likely that any future developments will implicate your mutation.

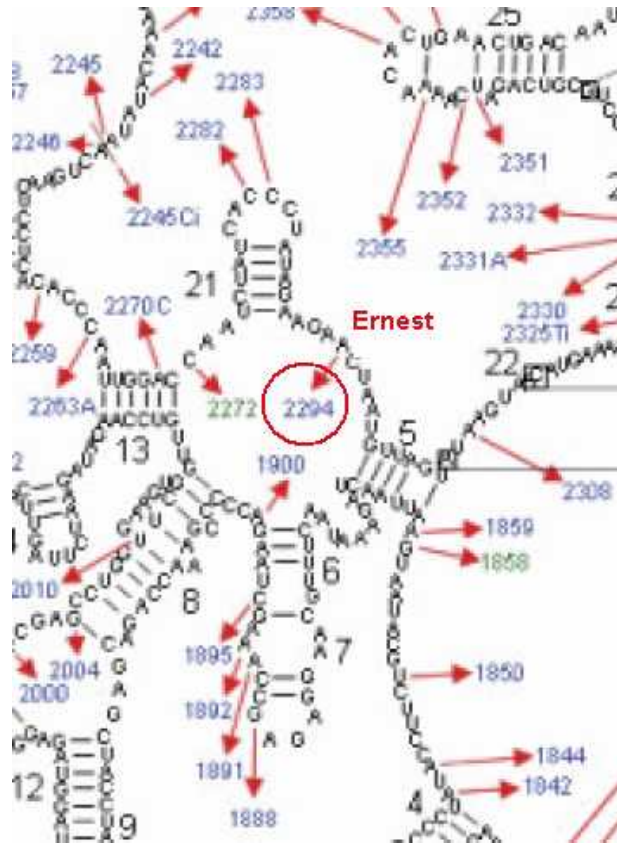


Figure 2

You may also occasionally encounter news items about some condition being associated with a certain haplogroup. These are often preliminary reports, which do not hold up when the study is repeated in a different population. For instance, one study set in northern Italy found that haplogroup J was associated with longevity. Yet a later study in southern Italy did not replicate the finding. Many medical researchers do not take haplogroup structure into account, and they may report a “mutation” that is actually common throughout the world. As Herrnstadt wrote in her article, *An evolutionary perspective on pathogenic mtDNA mutations: haplogroup associations of clinical disorders*,

“As we note here, however, such associations have usually been observed only in single studies and it is difficult to draw broad conclusions on the basis of the available evidence. At a minimum, we suggest that, a haplogroup-group association must be detected in multiple subpopulations or in a large, carefully controlled population survey.”

Information about your most distinctive mutation

Your mutation at 2294G is the most specific one. So far, it appears exclusively in a half-dozen or so examples of haplogroup U3a, most of which are diagrammed in the phylogenetic tree at MitoMap. Figure 3 zooms in on the section showing part of the U3a tree (with the U3a label supplied by me). It shows that your sample in the database retains the ancestral haplotype of U3a-2294G with no additional mutations. Yet there are

a number of branches off 2294G with one or several more mutations, indicating that the founder probably lived many hundreds or even thousands of years ago. In time this cluster may be dignified with a subclade label of its own, perhaps U3a1.

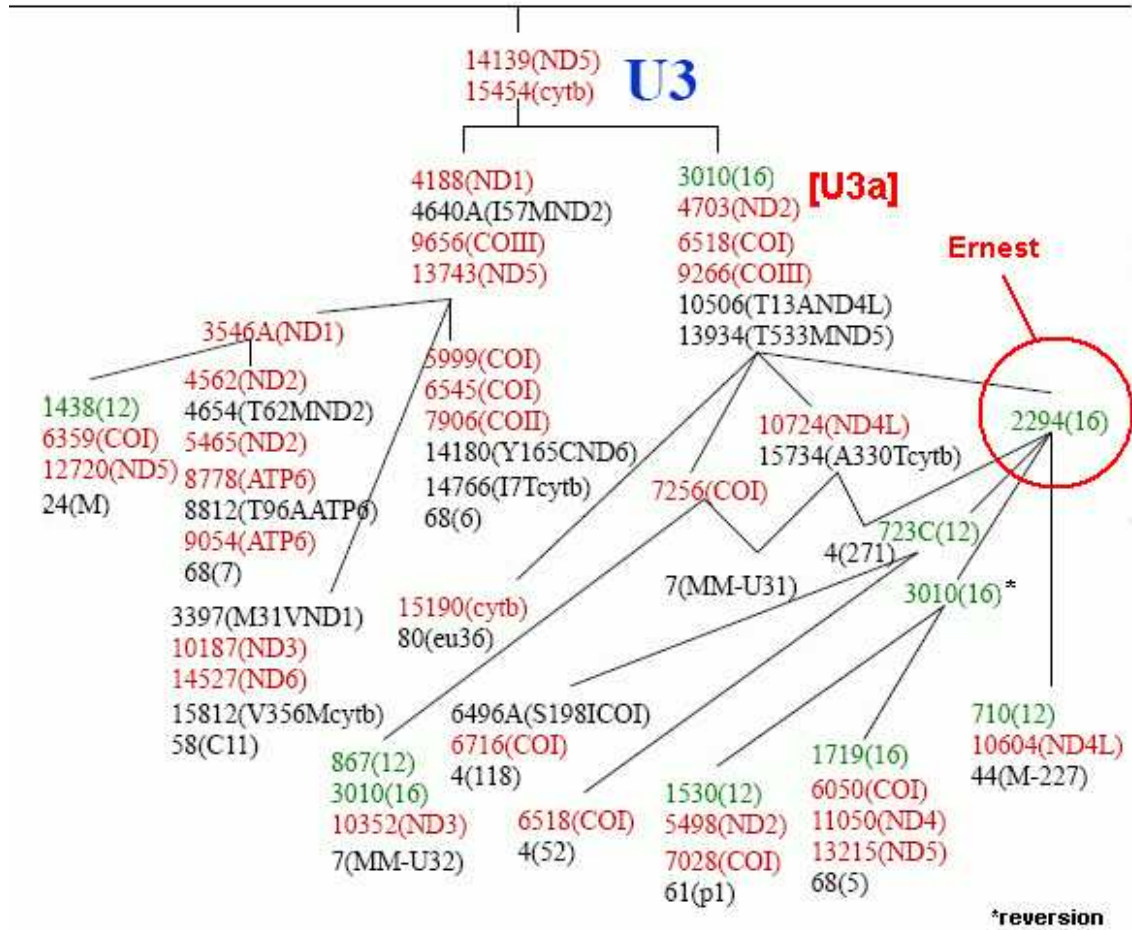


Figure 3
 Enlarged and annotated from the diagram at
<http://www.mitomap.org/mitomap-phylogeny.pdf>
 Samples are coded with the citation number in the bibliography,
 with the sample ID from the original paper in parentheses.
<http://www.mitomap.org/treebib.html>

A good way of keeping track of future developments is to periodically use Google Scholar, <http://scholar.google.com>, with its full-text index and links to sources. The Advanced Search options allow you to limit hits to certain dates. Possible search strategies would be

haplogroup mtDNA U3a
mtDNA 2294G
mtDNA A2294G
mtDNA 2294
etc.

Publications Consulted

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An evolutionary perspective on pathogenic mtDNA mutations: haplogroup associations of clinical disorders
Mitochondrion. 2004 Sep;4(5-6):791-8

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The role of selection in the evolution of human mitochondrial genomes.
Genetics. 2006 Jan;172(1):373-87

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The Medical Implications of Complete Mitochondrial DNA Sequencing.

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Phylogeny of mitochondrial DNA macrohaplogroup N in India, based on complete sequencing: implications for the peopling of South Asia.

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Amino acid properties and consequences of substitutions

<http://www.russell.embl.de/aas/>

Argus Biosciences – tool for extracting polymorphisms from complete sequence data

<http://www.argusbio.com/sooryakiran/gensnip/gensnip.php>

Haplogroup motifs – HVR1 mutations that are commonly found in various haplogroups

<http://www.stats.gla.ac.uk/~vincent/founder2000/motif.html>

Human Mitochondrial Database

<http://www.genpat.uu.se/mtDB/mtDB>

Mitoanalyzer (note: this uses the original CRS, not the rCRS)

<http://www.cstl.nist.gov/biotech/strbase/mitoanalyzer.html>

Mitomap, especially these five pages:

Sequence

<http://www.mitomap.org/mitoseq.html>

rRNA/tRNA point mutations

<http://www.mitomap.org/cgi-bin/tbl9gen.pl>

coding region point mutations

<http://www.mitomap.org/cgi-bin/tbl8gen.pl>

phylogenetic tree

<http://www.mitomap.org/mitomap-phylogeny.pdf>

bibliography for phylogenetic tree

<http://www.mitomap.org/treebib.html>

National Center for Biotechnology Information (NCBI) BLAST

<http://www.ncbi.nlm.nih.gov/blast/>

Neuromuscular Disorders -- Washington University, St. Louis, MO

<http://www.neuro.wustl.edu/neuromuscular/mitosyn.html>

OMIM – Online Mendelian Inheritance in Man

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=omim>

United Mitochondrial Disease Foundation

<http://www.umdj.org/>

Appendix 1

Location of the functional parts in the coding region

Bases	Function	Abbrev
577-647	tRNA phenylalanine	tPhe
648-1601	12S ribosomal RNA	12S
1602-1670	tRNA valine	tVal
1671-3228	16S ribosomal RNA	16S
3230-3304	tRNA leucine 1	tLeu
3305-3306	non-coding nucleotides	
3307-4262	NADH Dehydrogenase subunit 1	ND1
4263-4331	tRNA isoleucine	tIle
4329-4400	tRNA glutamine	tGln
4401-4401	non-coding nucleotide	
4402-4469	tRNA methionine	tMet
4470-5511	NADH dehydrogenase subunit 2	ND2
5512-5579	tRNA tryptophan	tTrp
5580-5586	non-coding nucleotides	
5587-5655	tRNA alanine	tAla
5656-5656	non-coding nucleotide	NC4
5657-5729	tRNA asparagine	tAsn
5761-5826	tRNA cysteine	tCys
5826-5891	tRNA tyrosine	tTyr
5892-5903	non-coding nucleotides	
5904-7445	Cytochrome c oxidase subunit I	CO I
7446-7516	tRNA serine 1	tSer
7517-7517	non-coding nucleotide	
7518-7585	tRNA aspartic acid	tAsx
7586-8269	Cytochrome c oxidase subunit II	CO II
8270-8294	non-coding nucleotides	
8295-8364	tRNA lysine	tLys
8365-8365	non-coding nucleotide	
8366-8572	ATP synthase F0 subunit 8	ATPase8
8527-9207	ATP synthase F0 subunit 6	ATPase6
9207-9990	Cytochrome c oxidase subunit III	CO III
9991-10058	tRNA glycine	tGly
10059-10404	NADH dehydrogenase subunit 3	ND3
10405-10469	tRNA arginine	tArg
10470-10766	NADH dehydrogenase subunit 4L	ND4L
10760-12137	NADH dehydrogenase subunit 4	ND4
12138-12206	tRNA histidine	tHis
12207-12265	tRNA serine2	tSer
12266-12336	tRNA leucine2	tLeu
12337-14148	NADH dehydrogenase subunit 5	ND5

14149-14673	NADH dehydrogenase subunit 6	ND6
14674-14742	tRNA glutamic acid	tGlu
14743-14746	non-coding nucleotides	
14747-15887	Cytochrome b	Cytb
15888-15953	tRNA threonine	tThr
15954-15954	non-coding nucleotides	
15955-16023	tRNA proline	tPro

Appendix 2

Names of the amino acids, with their three-letter abbreviations, one-letter symbols, and codons

Alanine	Ala	A	GCT, GCC, GCA, GCG
Arginine	Arg	R	CGT, CGC, CGA, CGG; AGA, AGG
Asparagine	Asn	N	AAT, AAC
Aspartic acid	Asx	D	GAT, GAC
Cysteine	Cys	C	TGT, TGC
Glutamine	Gln	Q	CAA, CAG
Glutamic acid	Glu	E	GAA, GAG
Glycine	Gly	G	GGT, GGC, GGA, GGG
Histidine	His	H	CAT, CAC
Isoleucine	Ile	I	ATT, ATC, ATA
Leucine	Leu	L	TTA, TTG; CTT, CTC, CTA, CTG
Lysine	Lys	K	AAA, AAG
Methionine	Met	M	ATG
Phenylalanine	Phe	F	TTT, TTC
Proline	Pro	P	CCT, CCC, CCA, CCG
Serine	Ser	S	TCT, TCC, TCA, TCG; AGT, AGC
Threonine	Thr	T	ACT, ACC, ACA, ACG
Tryptophan	Trp	W	TGG
Tyrosine	Tyr	Y	TAT, TAC
Valine	Val	V	GTT, GTC, GTA, GTG

Note that many of the synonymous codons differ in the third base.