Molecular Diagnosis of Mitochondrial Disorders

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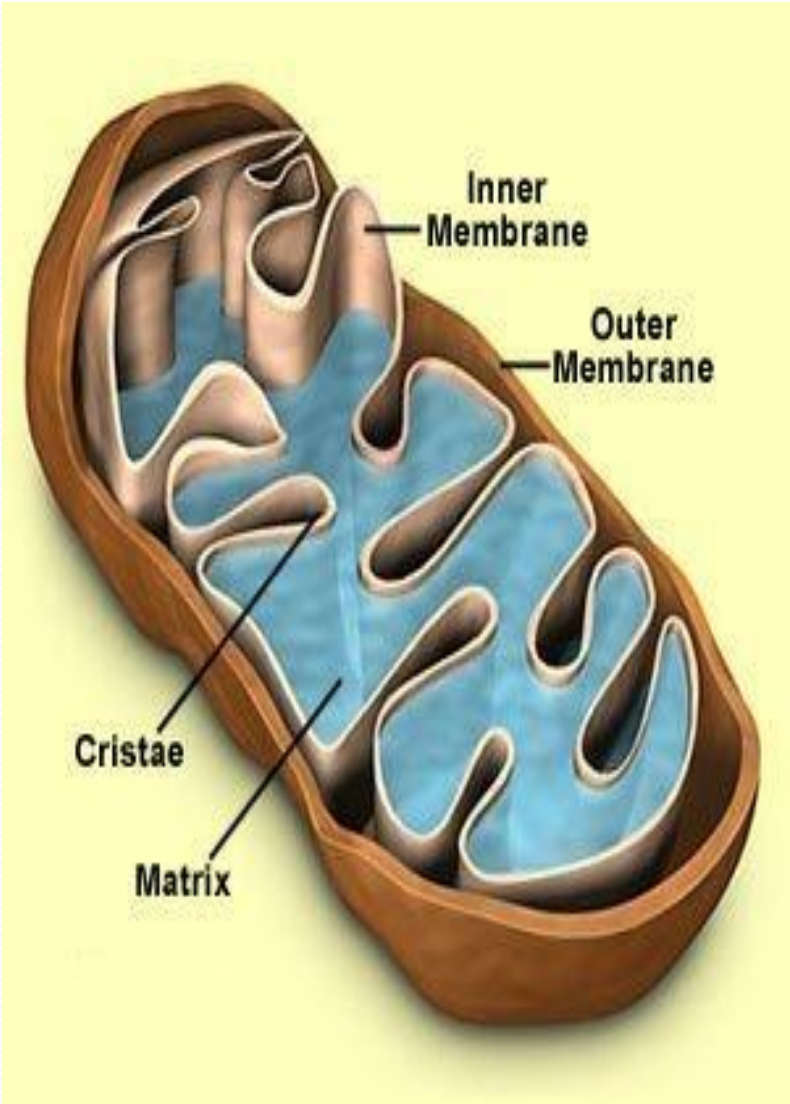
Case

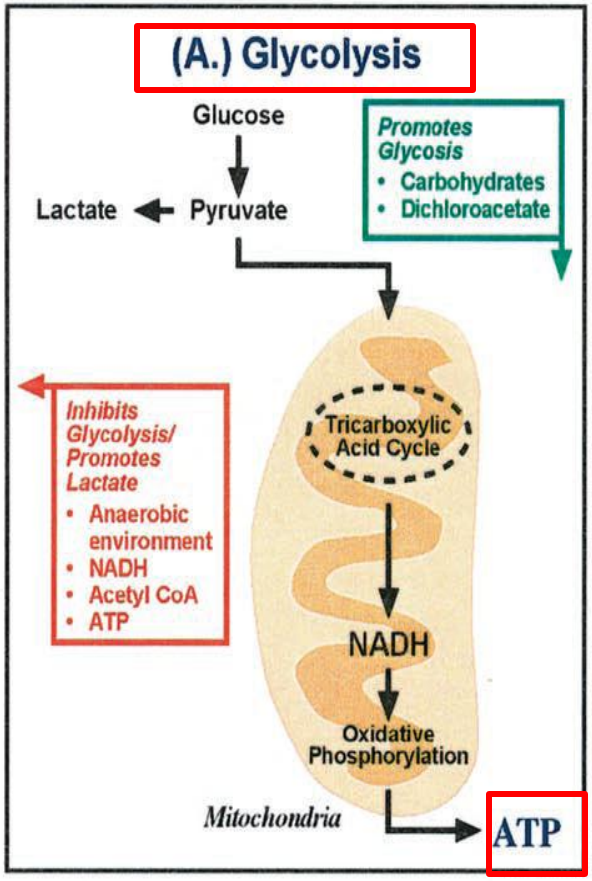
- **Clinical history:** Normal birth to unrelated Hispanic parents. Abnormal phenylalanine on newborn screening. Follow-up plasma amino acids showed elevated tyrosine, but normal phenylalanine while on a regular diet. At 7 wks he was noted to have conjugated hyperbilirubinemia, tyrosine and methionine high. He had significant failure to thrive associated with feeding difficulties and fat malabsorption.
- The progression of his liver disease with hypoglycemia and coagulopathy led to liver transplantation at 7 weeks of age. Blood mtDNA content was 62% of controls. Normal MRI of the brain
- His subsequent clinical course was dominated by hypotonia and psychomotor regression. He died at 23 months from a cardiac arrest.

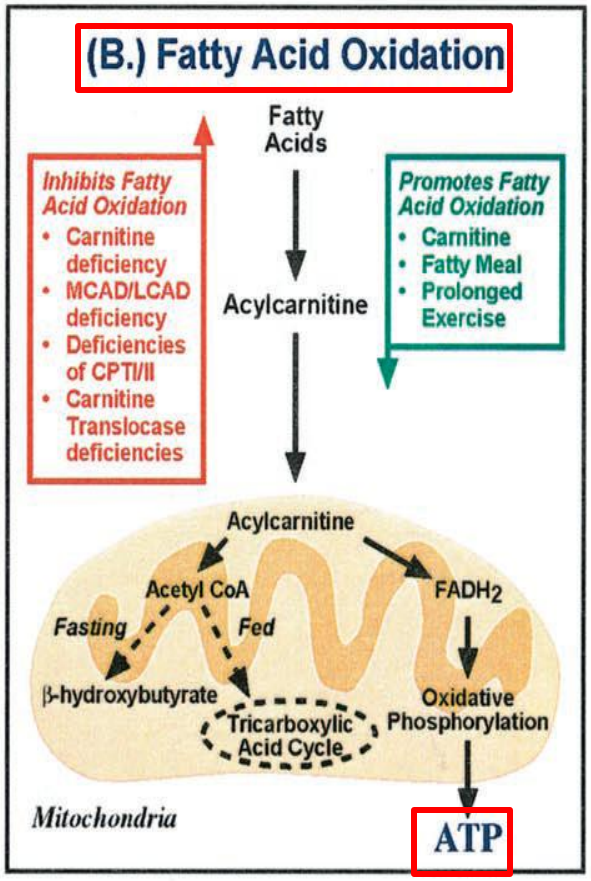
Mitochondria

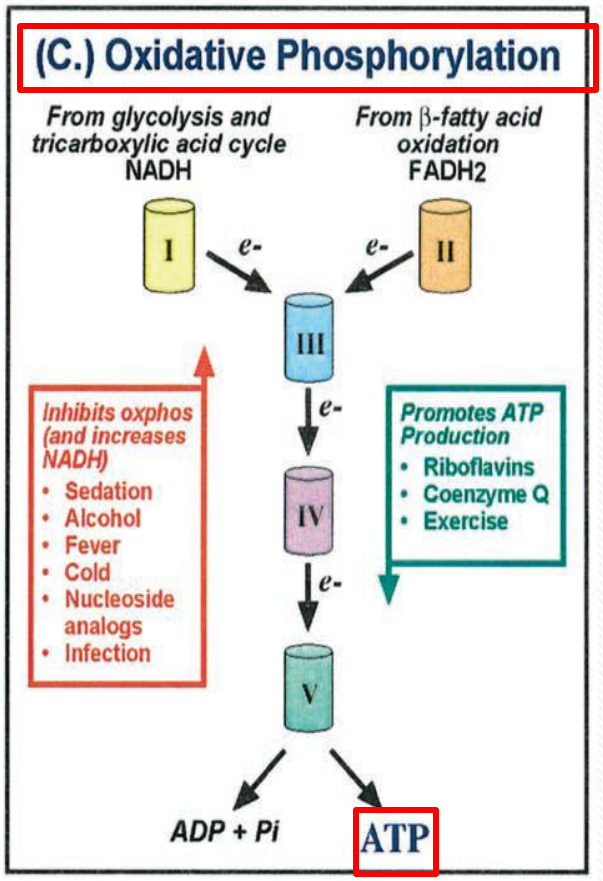
A mitochondrion (singular of mitochondria) is part of every cell in the body that contains genetic material. Mitochondria are responsible for processing oxygen and converting substances from the foods we eat into energy for essential cell functions. Mitochondria produces energy in the form of ATP, which is then transported to the cytoplasm of a cell for use in numerous cell functions.



Mitochondrial Functions

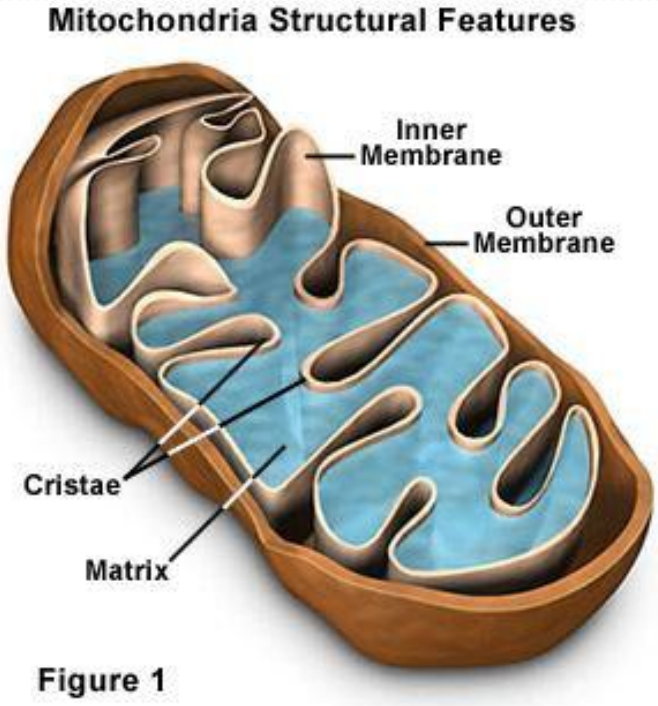






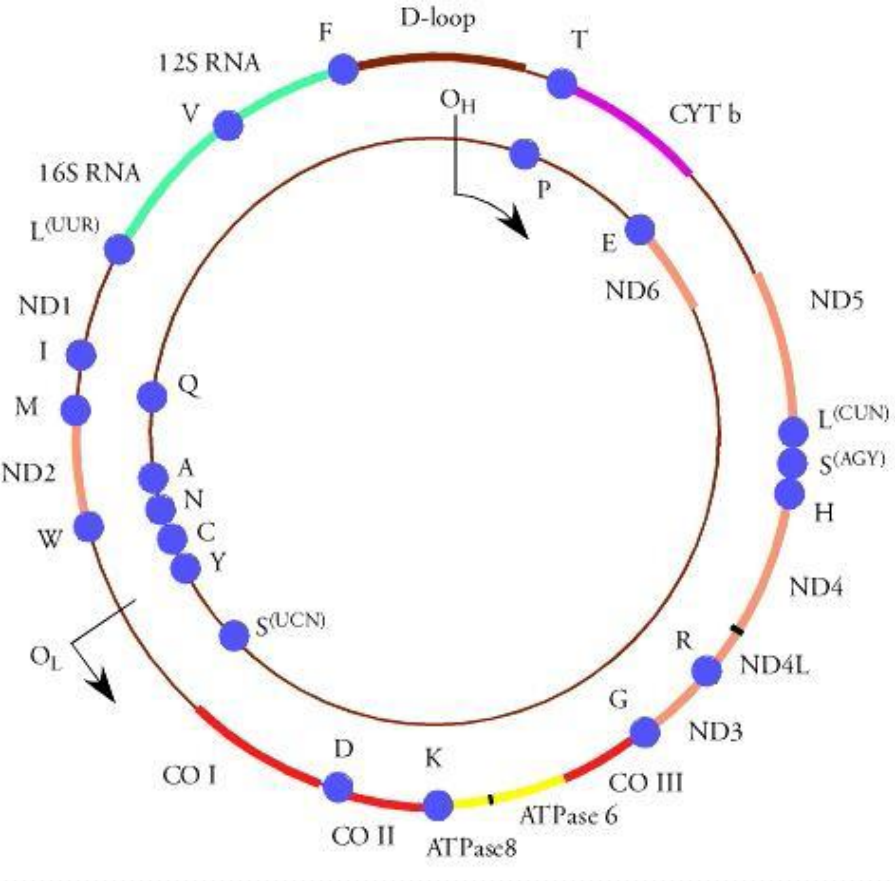
Clay et al. Chest 2001 120:634-648

Mitochondrial Functions



- >1500 genes
  - Nuclear DNA
  - mtDNA
- ATP generation
  - ATP production via oxidative phosphorylation
- Energy resource:
  - – supplies 90% of energy for the body

Mitochondrial Genome



- Double stranded, circular 16.5Kb
- No intron, 80 - 93% coding gene
- No repeat
- Lack histone and DNA repair mechanism damage, mutations (free radicals)
- 37 gene: 22 tRNA, 2 rRNA &13 protein
- Heteroplasmy

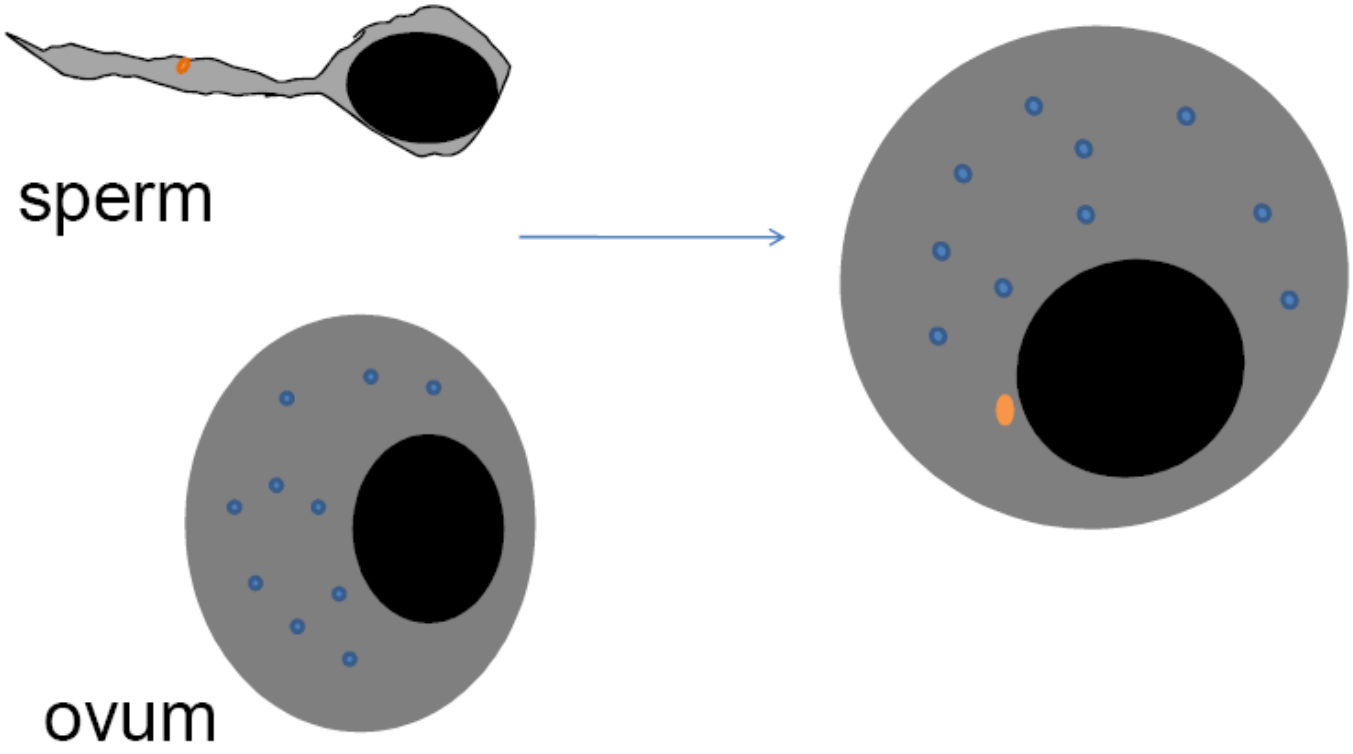
mtDNA Encodes for

- 13 protein subunits of the respiratory chain (of a total of approx. 67)
- 16S and 12S mt rRNAs
- 22 mt tRNAs
- Genetic code differs slightly

	standard	mtDNA
• UGA	stop	Arg
• AGA	Arg	stop
• AGG	Arg	stop
• AUA	Ile	Met

Mitochondrial DNA Inheritance

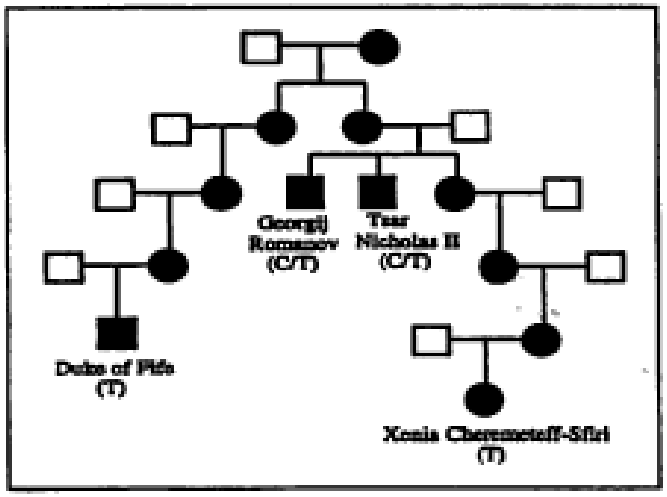
Maternal Inheritance



Mitochondrial Genome

- Highly polymorphic
  - >1000 polymorphisms (http://www.mitomap.org/MITOMAP)
  - 200 mutations

- Genealogy and Ancestry



Romanov Family

Mitochondrial Disease - Clinical Heterogeneous

Definition

Clinically heterogeneous disorders that are due to mitochondrial respiratory chain dysfunction, caused by mutations in the mtDNA OR nDNA that encodes for any of the following:

1. structural protein of the OXPHOS complexes
2. protein required for assembly of OXPHOS complexes
3. proteins involved in mtDNA translation
4. proteins involved in mtDNA maintenance
5. proteins involved in mitochondrial fusion and fission

Wallace, et al. PNAS 96:1817-1819 1999

Mitochondrial Disease Prevalence

- Incidence of 1:5000 live births (Smeitink 2006)
- 20% are due to mtDNA mutations (200 pathogenic mutations), 80% to nuclear DNA mutations

Phenotype Recognition

- Very Difficult Disorders to Diagnose
- Several hundred clinical presentations
- Frequency: as low as 1:8000 (1:3000)

Mitochondrial disorders

- Multisystem or single organ
- Affect organs with high energy usage
  - Brain and neurons, heart, retina, muscle, liver, kidneys, respiratory system, endocrine organs
- Wide scope of presentation > family members; same mutation

Mitochondrial Disease: Clinical Heterogeneous

Organs	Presentations
Nervous system	visual/hearing loss, fit, myoclonus, migraine, stroke, encephalopathy, focal deficit, ataxia, hypo/hypertonia, peripheral neuropathy , antibiotic-induced ototoxicity, cataracts, mental retardation/degeneration
Musculoskeletal	Myopathy, rhabdomyolysis, ptosis, exercise intolerance, ophthalmoplegia, chronic fatigue
Cardiac	Cardiomyopathy, conducting defect
Endocrine	Endocrine diabetes, pancreatic insufficiency, hyperthyroidism, systemic lipomatosis
Blood and bone marrow	Sideroblastic anemia, pancytopenia, petechia, acrocyanosis
Liver	Hepatitis, cirrhosis
GIT	diarrhea, dysmotility, intestinal obstruction, FTT, vomiting

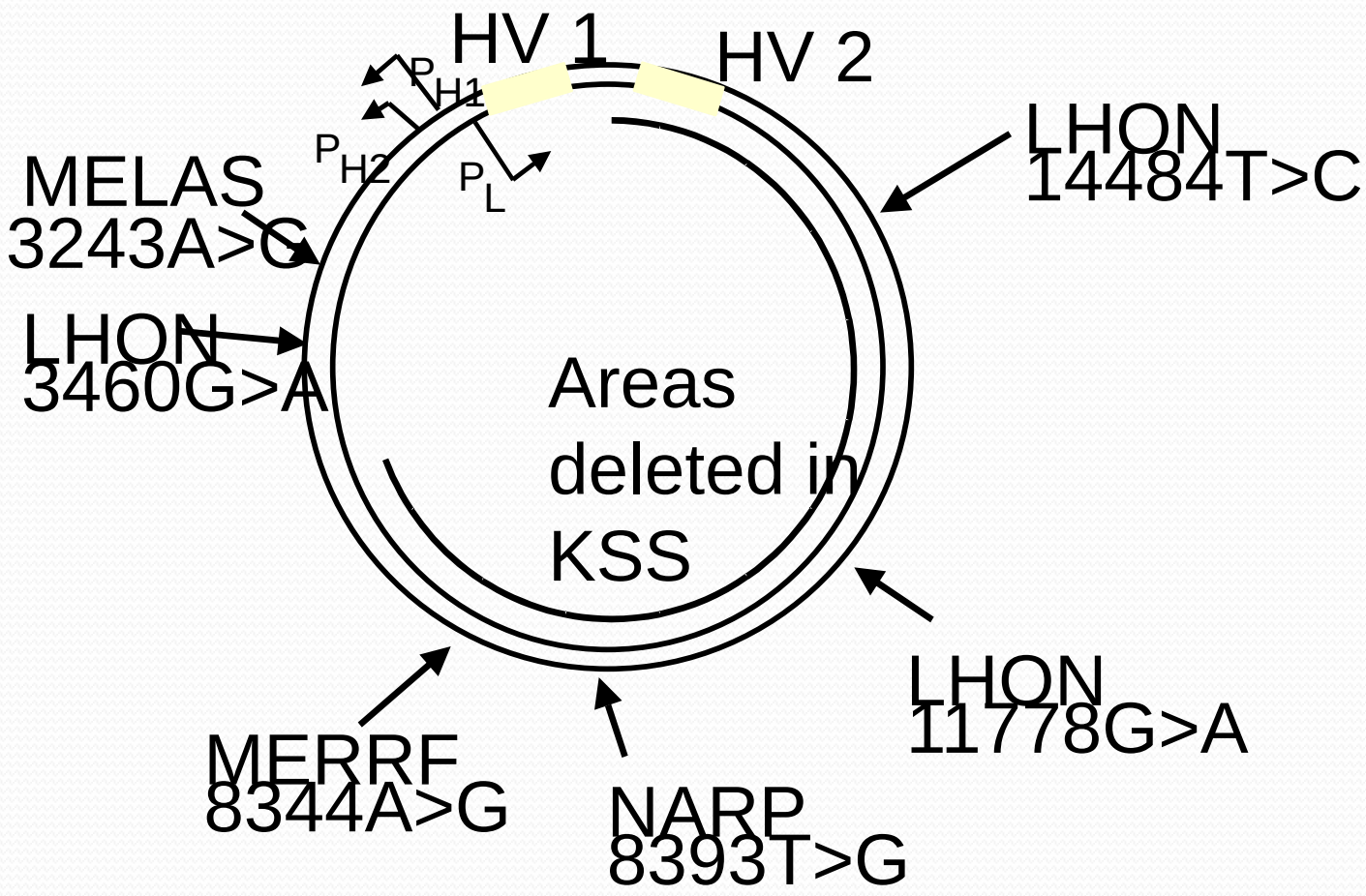
Mitochondrial Diseases Prevalence

- Minimum prevalence of pathogenic mtDNA mutations: 1:8000
- Maternal inheritance

- Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (**MELAS**)
- Myoclonic epilepsy with ragged red fibers (**MERRF**)
- Neuropathy, ataxia, retinitis pigmentosa (**NARP**)
- Deafness
- Leber hereditary optic neuropathy (**LHON**)
- Kearnes Sayre syndrome (**KSS**)
- Pigmentary retinopathy, chronic progressive external ophthalmoplegia (**CPEO**)

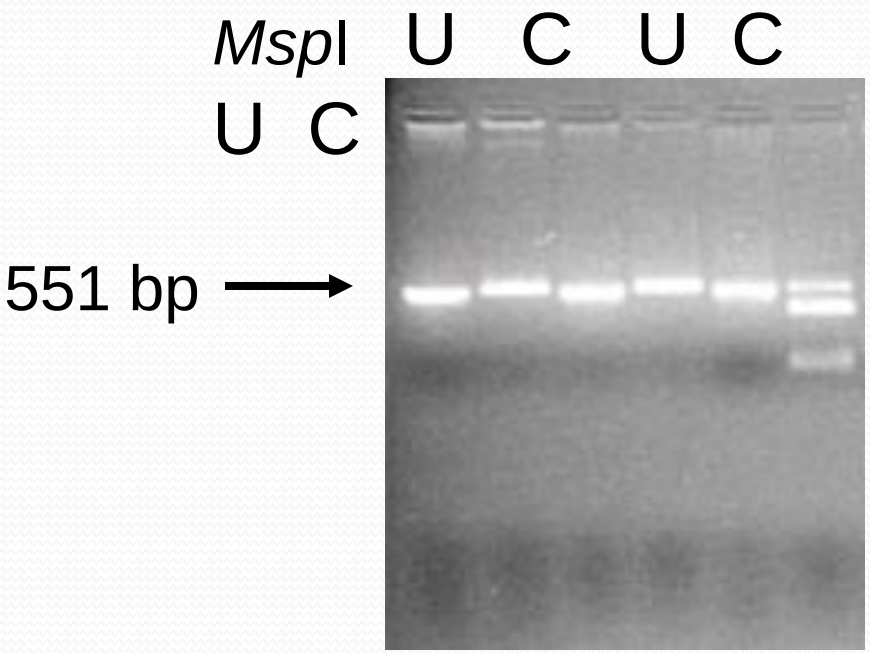
Chinnery et al. Lancet 2000

Mitochondrial Mutations Associated with Disease



Detection of NARP Mitochondrial Point Mutation (ATPase VI 8993 T→C or G) by PCR-RFLP

U = Uuncut, no *Msp*I
C = Cut, with *Msp*I



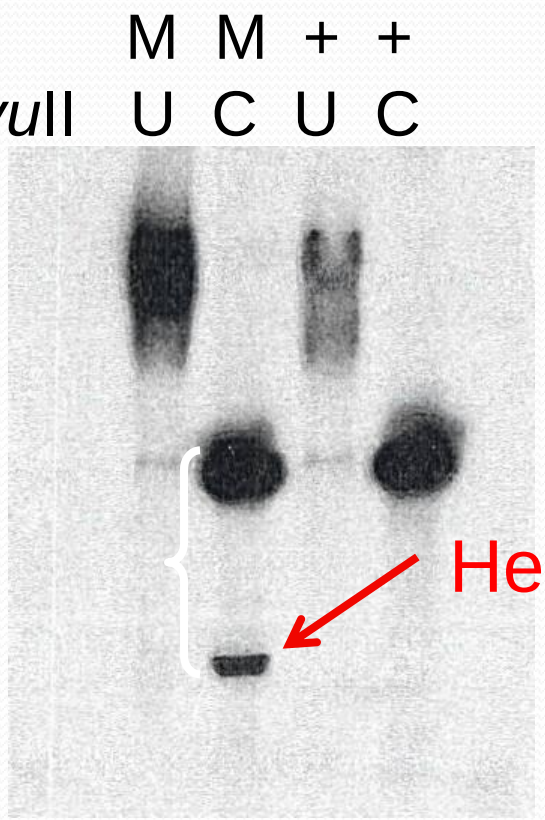
Agarose gel

The presence of the mutation creates an *Msp*I restriction enzyme site in the amplicon.

Detection of KSS Mitochondrial Deletion Mutation by Southern Blot

The restriction enzyme, *Pvu*II cuts once in the circular mitochondrial DNA.

M = Mutant
+ = Normal
U = Uncut, No *Pvu*II
C = Cut with *Pvu*II



Autoradiogram

New Class of Mitochondrial Disease

- Nuclear genes
  - Nuclear genes which affect mtDNA levels: POLG; MPV17, EFG1
  - Nuclear gene which affects mito protein assembly: SURF1
- Inheritance:
  - Autosomal Recessive
  - Autosomal Dominant
  - X-linked

Diagnostic Criteria in Adults and Children

Major Criteria

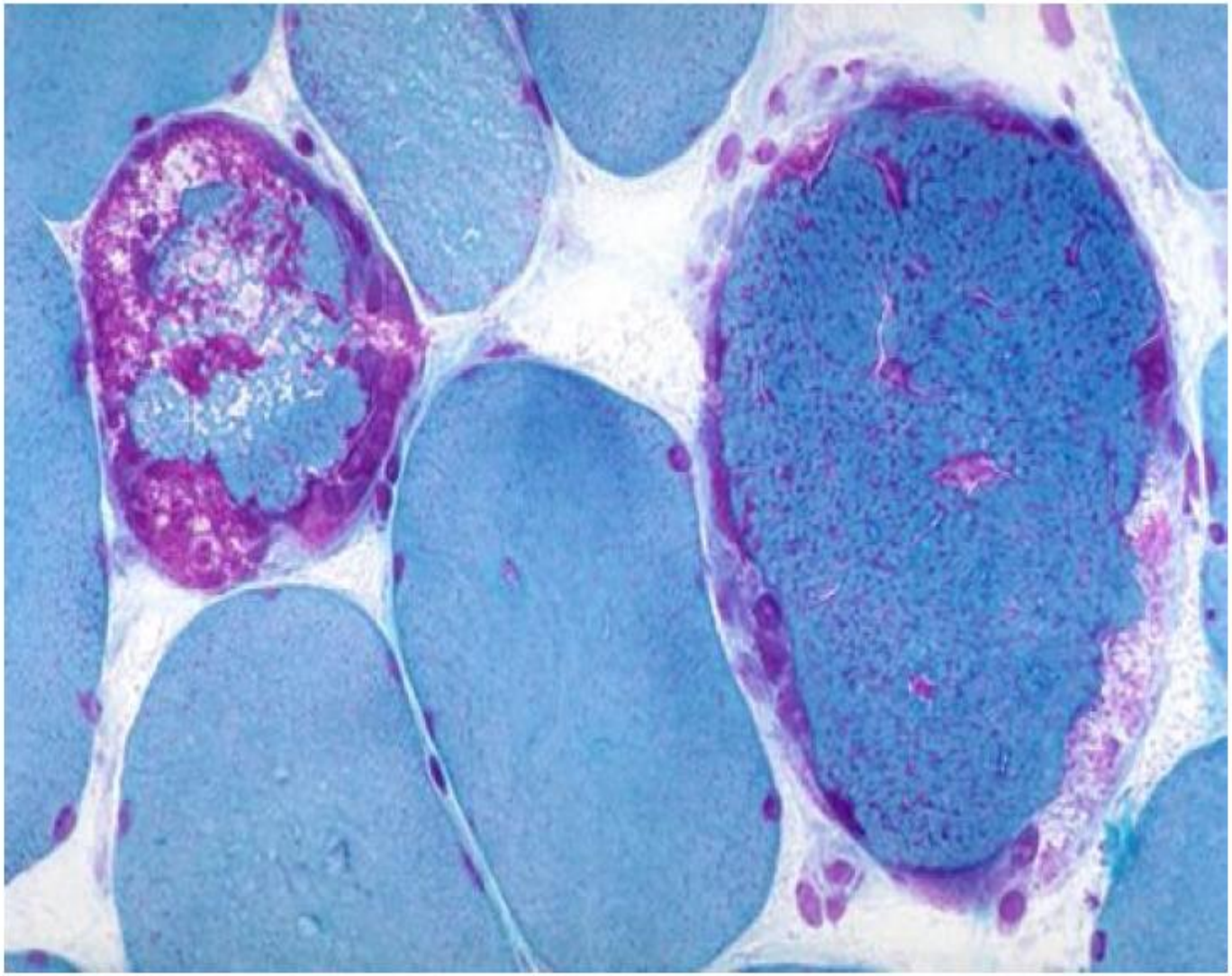
- Clinical presentation, ↑lactate
- Histology
  - >2% RRF
  - 2-5% COX-negative fibers
- Enzymology
  - <20% RC in a tissue or <30%RC>=2 tissues
  - <30# RC in a cell line
- Functional
  - Fibroblast ATP synthesis rates >3 SD below normal
- Molecular
  - Nuclear or mtDNA mutation of undisputed pathogenicity

Minor criteria

- Clinical presentation, +/-
- Histology
  - >2% RRF age 30-50y
  - >2%SSMA (<16y)
  - Abnormal mitochondrial (EM)
- Enzymology
  - 20-30% RC in a tissue or 30-40% RC>=2 tissues
  - 30-40% RC in a cell line
- Functional
  - Fibroblast ATP synthesis rates 2-3 SD below normal
- Molecular
  - Nuclear or mtDNA mutation of undisputed pathogenicity

Bernier et al. Neurol 2002 59:1406-11

Red Ragged Fibers



ARUP Approach

- Next Generation Sequencing (NGS) (Shale Dame and Bob Chou)
  - Mitochondrial genome sequencing
  - 128 Mito Nuclear genes sequencing

  Point mutations and small ins/del Low heteroplasmy

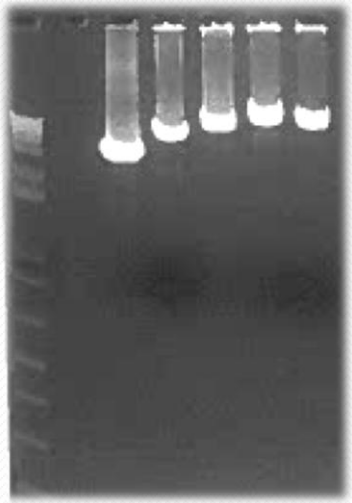
- Large deletions and duplications in mitochondrial genome and >100 nuclear gens by high density exonic CGH Microarray (Tracey Lewis)

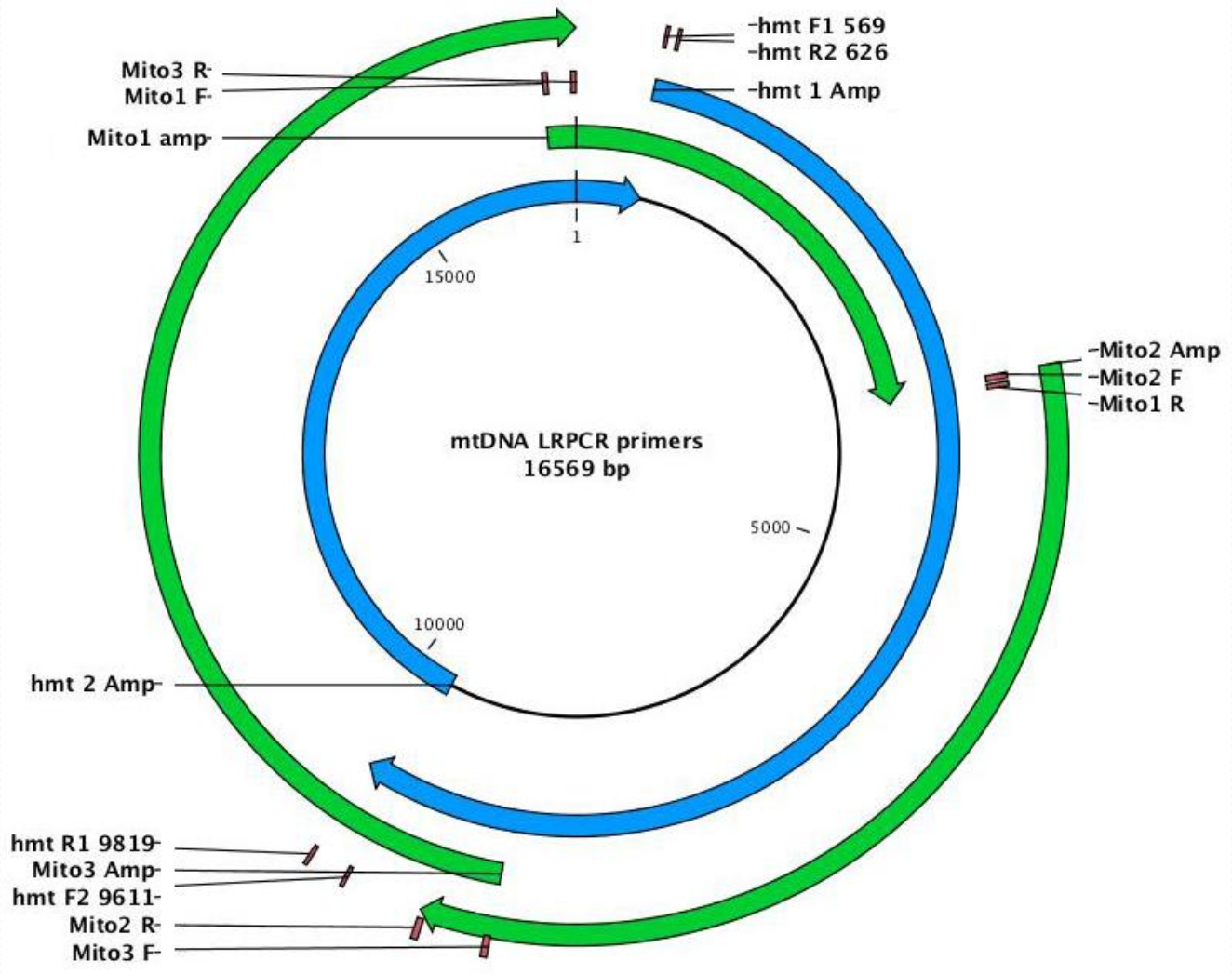
  20% of del in mito DNA and 5-10% large del/dup in nuclear genes

Mitochondrial Genome NGS Assay

- Long range PCR (LRPCR) enrichment
- Library prep, barcode/pooling
- Single end HiSeq reads (100bp)
- CLCBio data analysis

Mito Genome NGS: Long Range PCR Enrichment



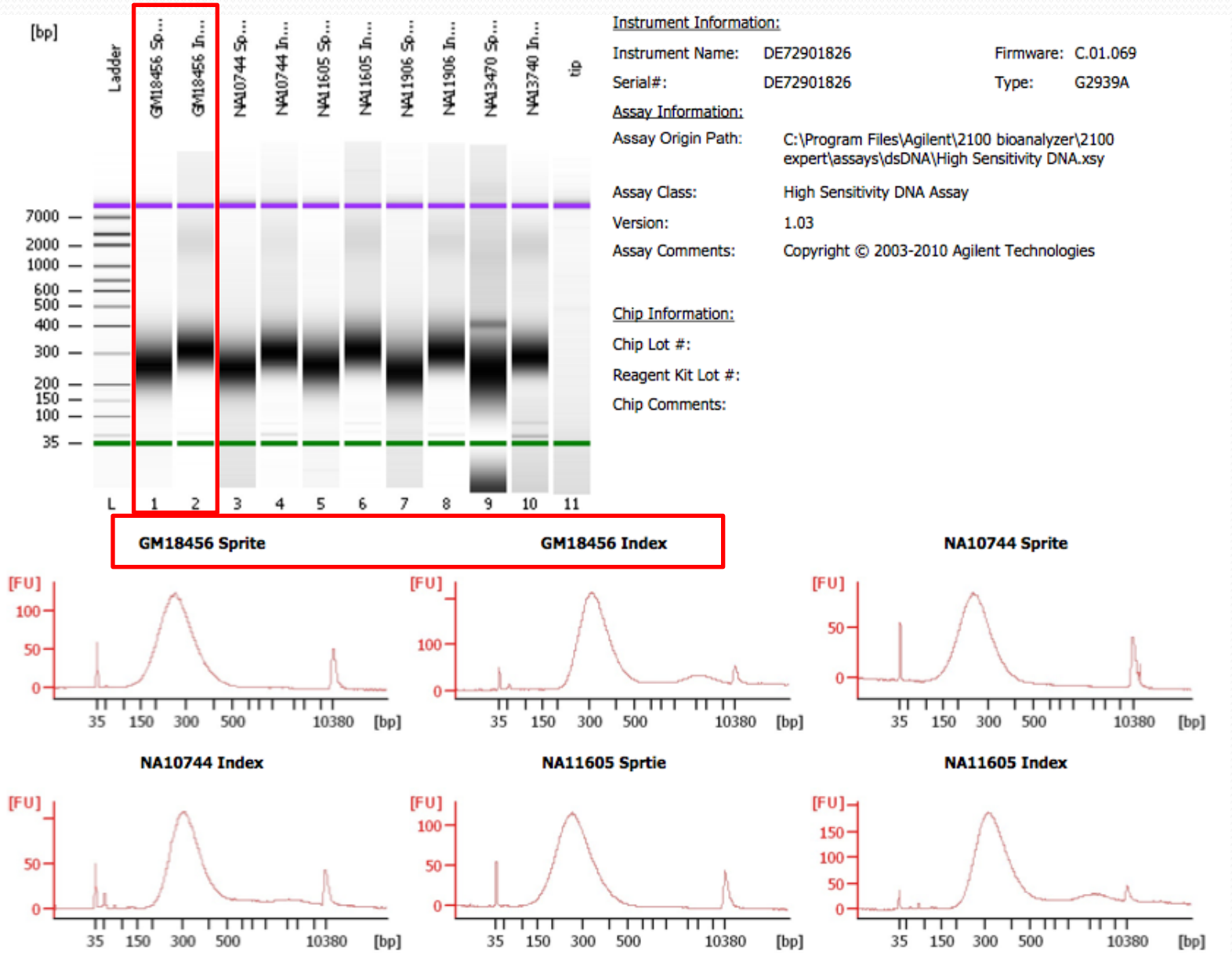


Illumina Library Prep

- Sonicated samples are placed in the SPRI-TE for library prep
  - Blunted
  - Adenylated
  - Ligation of adapters
- Post SPRI-TE, samples are PCR amplified with multiplex PE primers and one of 12 index primers (4, 6 and 8 samples pooling)

SPRI-TE

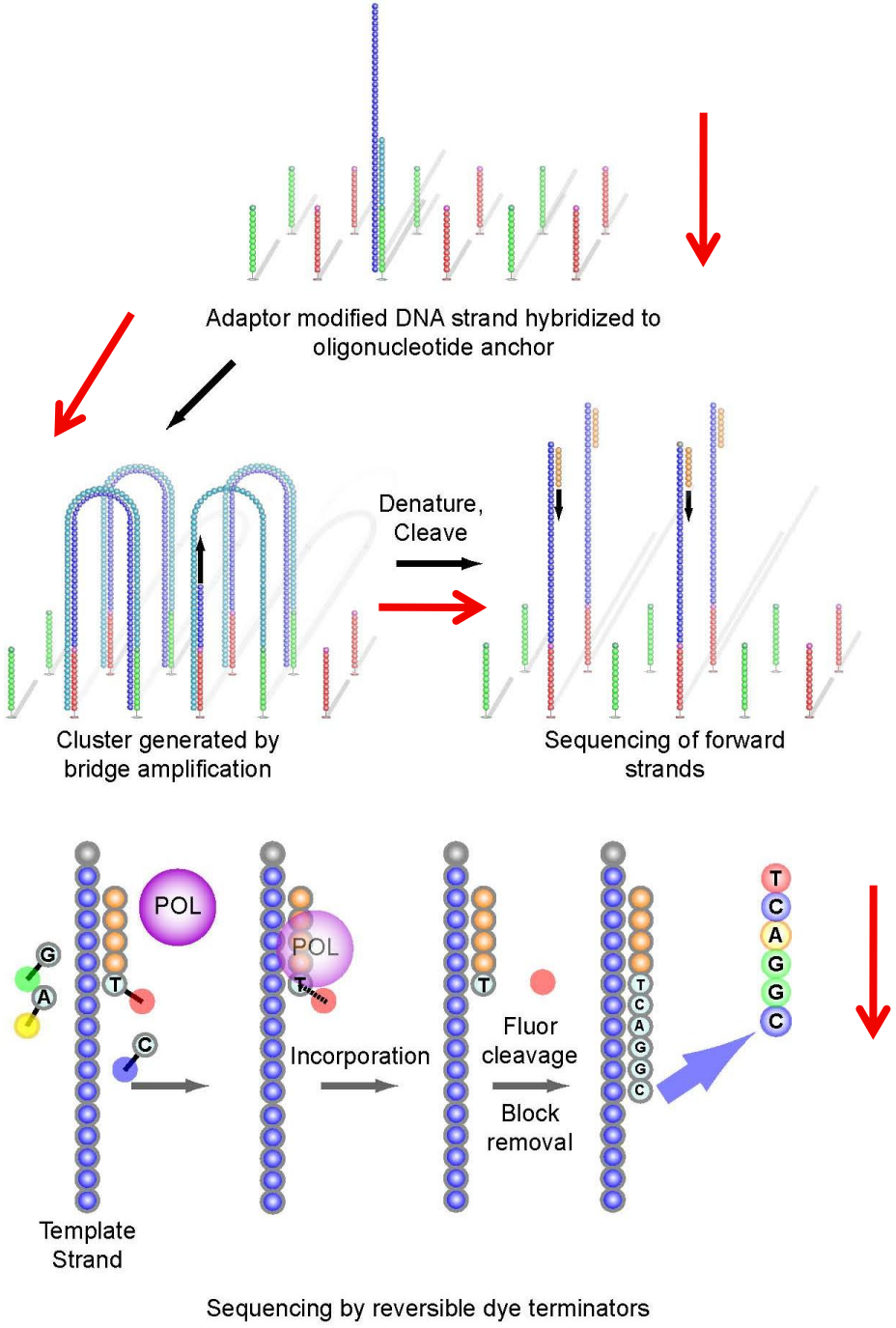
Library Prep



Illumina

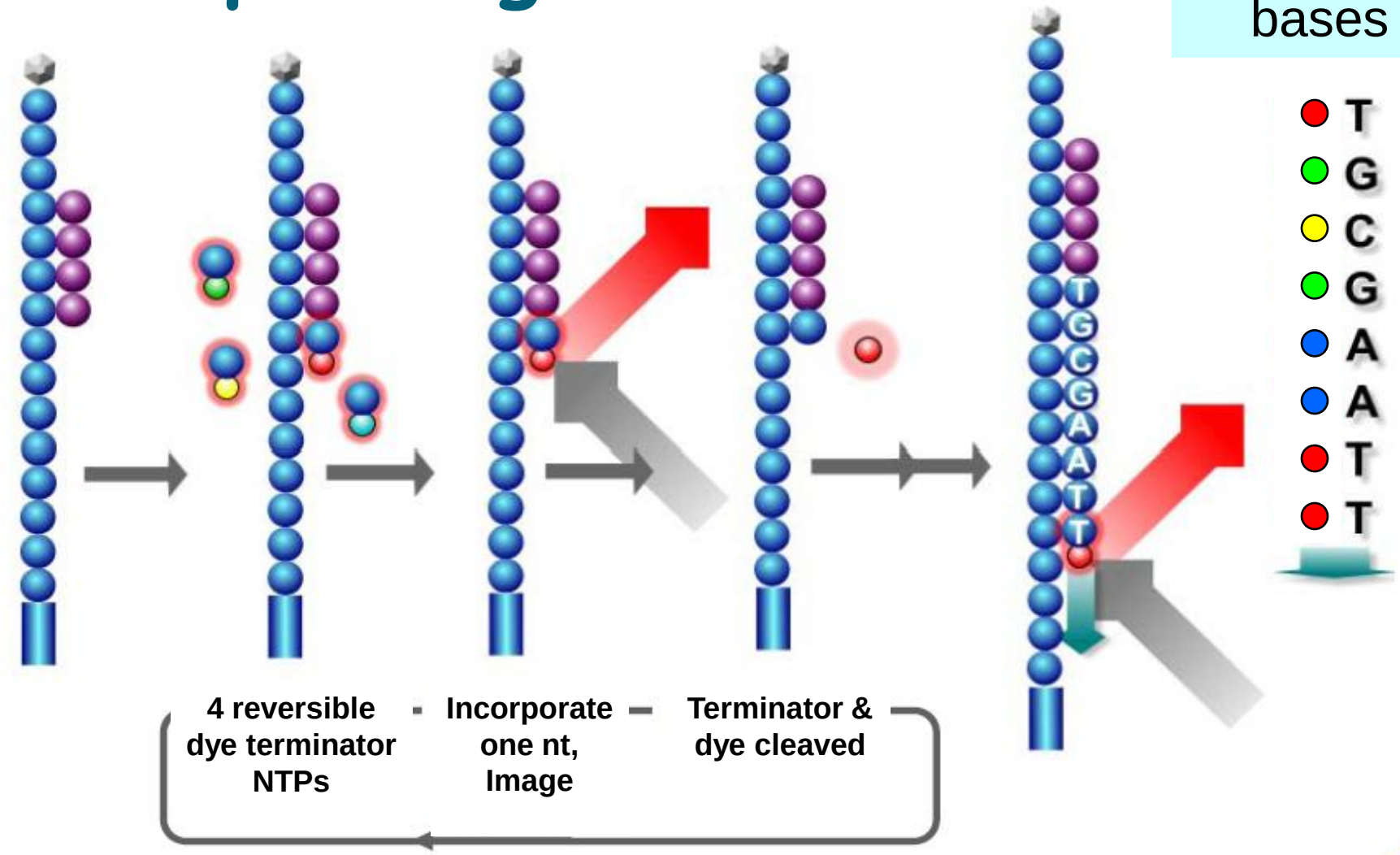
Sequencing by reversible dye terminators

Illumina HiSeq 2000



Sequencing

Read 100 bases



Three step cycle

illumina

Mitochondrial Genome NGS

SPRI-TE

Illumina HiSeq 2000

Long Range PCR

Amplicons equimolar pooled

SPRI-TE and index

Illumina HiSeq

Sequence Alignment

Variant calls

Day 1

Days 2-3

Days 4-9

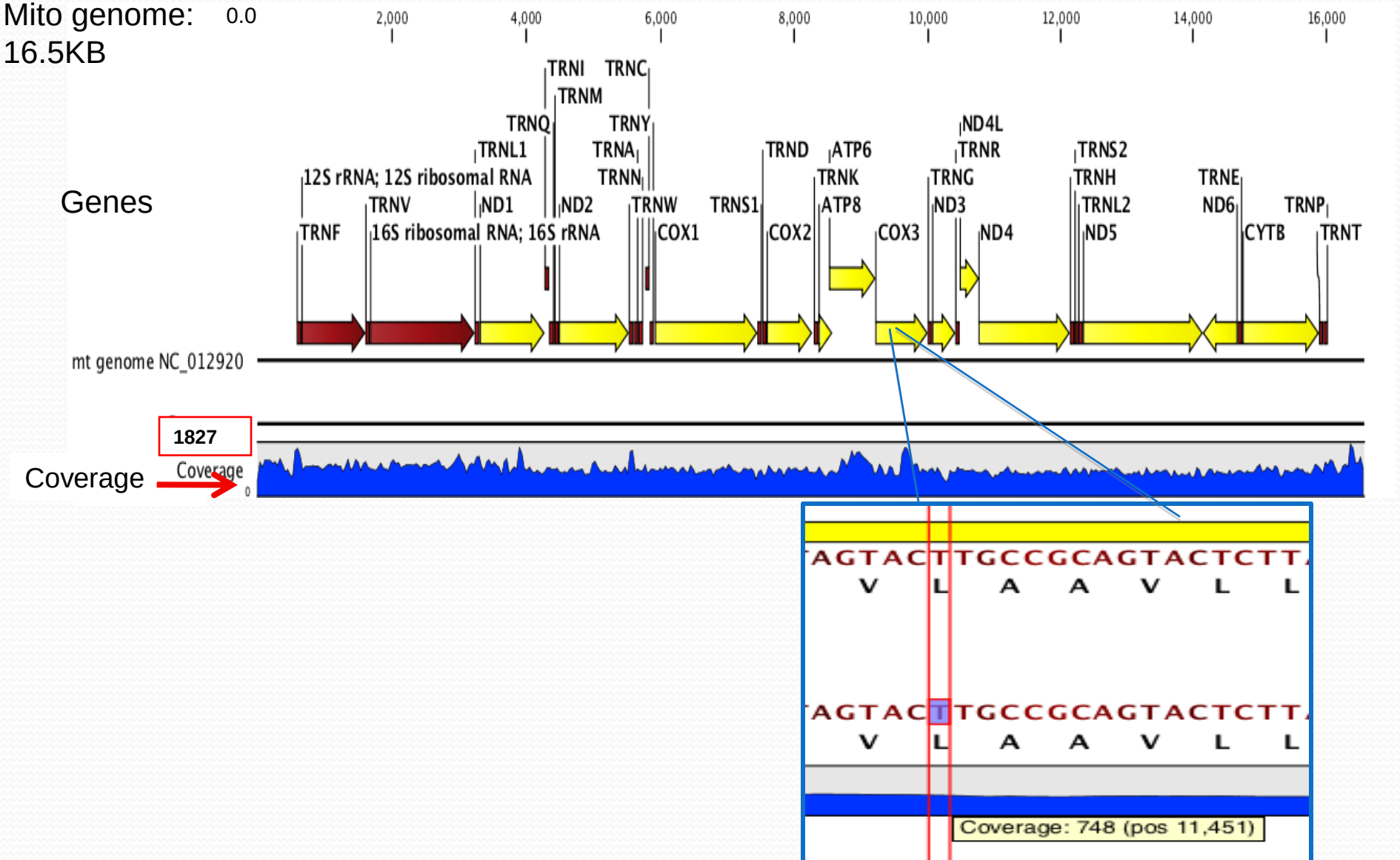
Days 10+

Data Analysis

- Raw HiSeq files converted to FastQ
- CLCbio
  - Alignments
  - SNP/DIP calls
  - Sequence annotation
    - Reference sequence dependant
    - Manual
- Data report
  - Excel spreadsheet and .html

Mitochondrial Genome NGS

- CLCbio Genomics Workbench



Mito Genome NGS Data Analysis

- Alignment/variant call parameters
  - Aligned to fully annotated reference sequence
  - Minimum coverage: 200-fold
  - Minimum minor allele frequency: 3%
  - Report nonsynonymous single nucleotide polymorphisms (SNP) and deletion/insertion polymorphisms (DIP) variants
  - Filter out common polymorphisms

CLCBio Output

m.3460G>A

Sample ID: NA11605		Flowcell ID: 81C03ABXX	
Fastq file: NA11605_7_1		Cluster kit ID: 0745788 L/N 5836181	
Start date of run: 022111		Index sample, Single read	
Date of analysis: 03082011		Technician: S. Dames	

Reference Position	Amino Acid Change	Frequencies	Coverage	Clinical Significance
3460	Ala52Thr	99.7	6517	Significant: Peripapillary microangiopathy; Gene ND1

Results

Ref Position	Reference	Allele Variations	Frequencies	Coverage	Overlapping Annotations	aa change
152	T	C	99.8	11834	D-Loop T-del, T-c	
263	A	G	99.9	8657	D-Loop A-a, A-g	
310	T	T/C	86.1/13.8	4679	D-Loop T-del T-c T-tc T-ttc	
311	-	C/-	75.6/24.2	7194	D-Loop, C-del, C-ccins(n), C-t	
750	A	G	99.9	22202	a-g (consensus)	
1438	A	G	99.9	13670	a-g (consensus)	
4769	A	G	99.9	11075	a-g syn (consensus), Gene: ND2	
8592	G	A	99.9	11723	g-a syn, Gene: ATP6	
8860						Thr112Ala
8979						
8993						**Leu156Arg**
10394	C	T	99.7	10477	c-t syn, Gene: ND3	
12358	A	G	99.8	10602	a-g t-a, Gene: ND5	Thr8Ala
15326	A	G	99.9	12516	a-g t-a (consensus), Gene: CYTB	Thr194Ala
15340	A	G	99.9	12876	a-g syn, Gene: CYTB	
16519	T	C	99.9	27287	D-Loop T-c	

m. 8993 T>G, in ATP6 gene Leu156Arg
Neuropathy, ataxia, retinitis pigmentosa (NARP)

All SNPs/DIPs filtered >200-fold coverage and
> 10% heteroplasmy, 16 variants calls

NA11605 Concordance

NA11605		Huntsman		ARUP		Sanger Verified	Clinical Significance
Reference Position	Amino Acid Change	Frequencies	Coverage	Frequencies	Coverage		
750	synonymous	99.9	12114	99.9	16470	Yes	common
1438	synonymous	99.9	11689	99.9	8629	Yes	common
1935	synonymous	96.7/3.3	11742	Not detected		<20%	unknown
2803	synonymous	92.8/7.2	11917	Not detected		<20%	Not reported
3460	**Ala52Thr**	**99.7**	**11055**	**99.7**	**6517**	**Yes**	**Significant: Peripapillary microangiopathy**
3549	synonymous	99.7	10059	99.8	18930	Yes	common
4330	frame shift	93.9/6.1	10492	Not detected		<20%	Not reported
4506	frame shift	96.3/3.7	9410	Not detected		<20%	Not reported
4580	synonymous	99.9	7748	99.8	6969	Yes	common
4769	synonymous	99.8	7777	99.9	6621	Yes	common
5204	frame shift	96.2/3.8	9362	Not detected		<20%	Not reported
6419	Lys172Asn	Not detected		91.4/8.4	4707	Not sequenced	Not reported
7028	synonymous	99.8	9195	99.6	7834	Yes	common
7444	**Thr112Ala**	**99.7**	**5884**	**99.8**	**6134**	**Yes**	**Possible: LHON associated**
8860	Thr112Ala	100	15219	99.9	23371	Yes	not significant
9053	Ser176Asn	86.7/13.3	1108	87.4/12.6	23249	<20%	not significant
11899	synonymous	99.9	8934	99.8	7395	Yes	common
15326	Thr194Ala	99.9	8647	99.8	7305	Yes	Possible significance
15904	noncoding	99.8	10380	99.9	6626	Yes	common

Mitochondrial Genome NGS Validation

Results:

- Reproducibly detected all reported SNP/DIP variants in Coriell samples (8/8)
- Currently sequencing 18 additional samples
- Can detect low levels of heteroplasmy (<10%)
  - All selected variants Sanger verified with >30% heteroplasmy
  - Low level heteroplasmy has been verified by “variant-specific” PCR

Mitochondrial 128 Gene Nuclear Panel

- RainDance enrichment
- Library prep
- Single end HiSeq reads
- CLCBio data analysis

mt 128 Gene Nuclear Panel

Mito Nuclear Genes	Number of Genes
Mitochondria DNA integrity	12
Complex assembly	22
Fatty acid metabolism	14
Coenzyme Q10	5
Respiratory chain disorders	16
OXPHOS subunits	8
OXPHOS assembly:4	4
Enzymes	25
Transcription	5
Carriers	5
Mitochondria maintenance	12

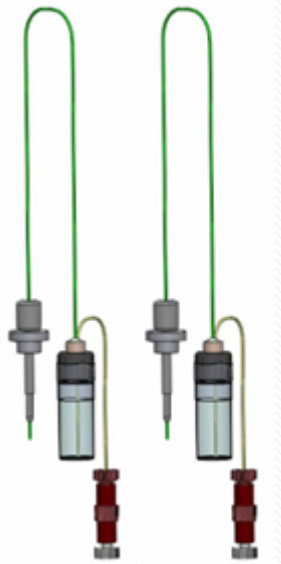
RainDance Library Prep

RDT Reagent Inputs

RDT 1000 Consumables

Primer Libraries

Genomic DNA Template

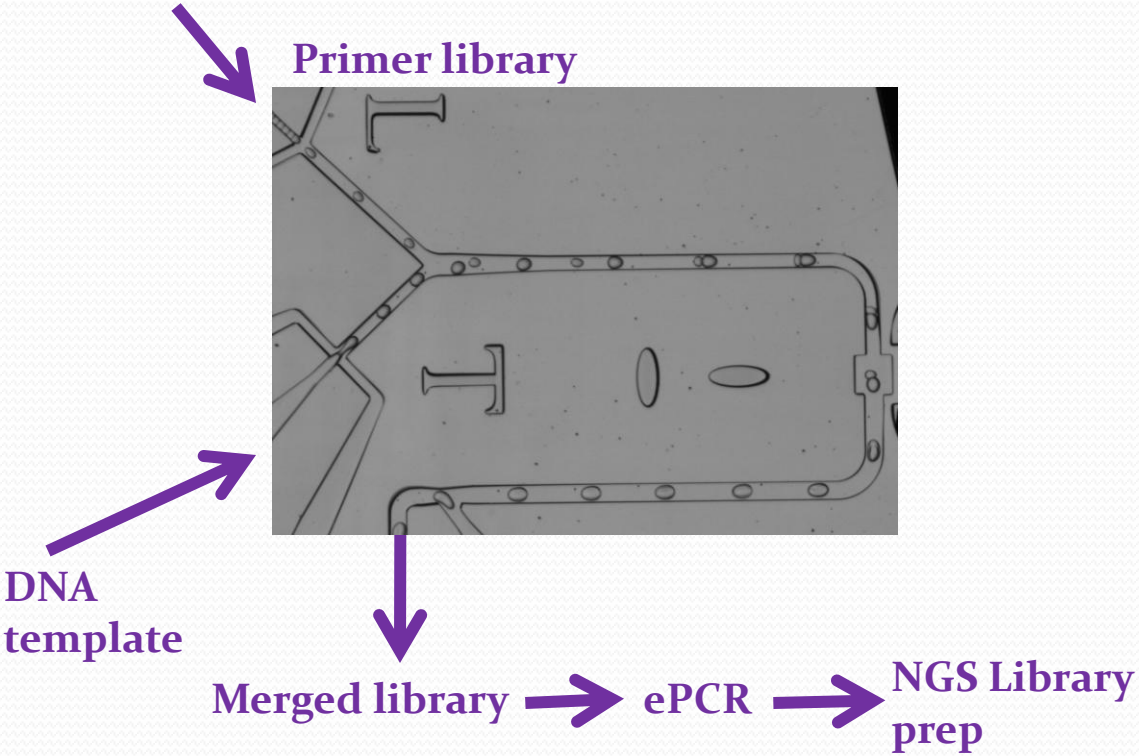


RDT 1000 Input/Output Vials

RDT 1000 Chip

Courtesy of Take Ogawa, RainDance

RainDance RDT 1000-Emulsion PCR



Advantages

Evenness of PCR

Specific primer, No pseudogene amp

Limits

Primer design

Chip expensive

Example: 128 nuclear genes for mito disorder in 1304 amplicons

Courtesy of Take Ogawa, RainDance

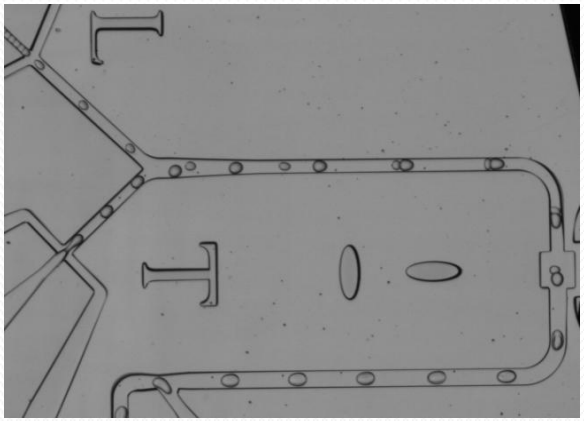
mt 128 Nuclear Gene Panel

RainDance Enrichment

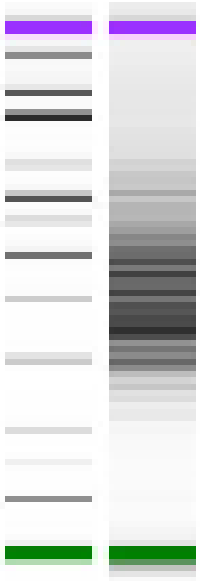
- Automated
- Emulsion PCR based
- 1,304 Amplicons
- All exons and splices site junctions
- Single tube amplification

Primer library

DNA template



Merged Library



Sonicate + RainDance

PCR amplify → ligation

Days 1-3

Sonicate and SPRI-TE

Day 4

Illumina HiSeq

Sequence Alignment

Days 5-10

Variant calls

Days 11+

Data Analysis

- Whole genome, NG, artificial chromosome, or masked genome?
- Quality metrics
  - Minimum coverage
  - Q scores
  - Heterozygous frequencies
  - Seed/window length
  - Cost to open gaps

Mitochondrial Nuclear NGS

Alignment Method	SNP/DIP
Whole genome	37,045
exon filtered	388
CDS filtered	91
nonsynonymous filtered	36
Masked Genome	1,651
CDS filtered	118
nonsynonymous filtered	55

- Masked alignments are useful
- Polymorphism database

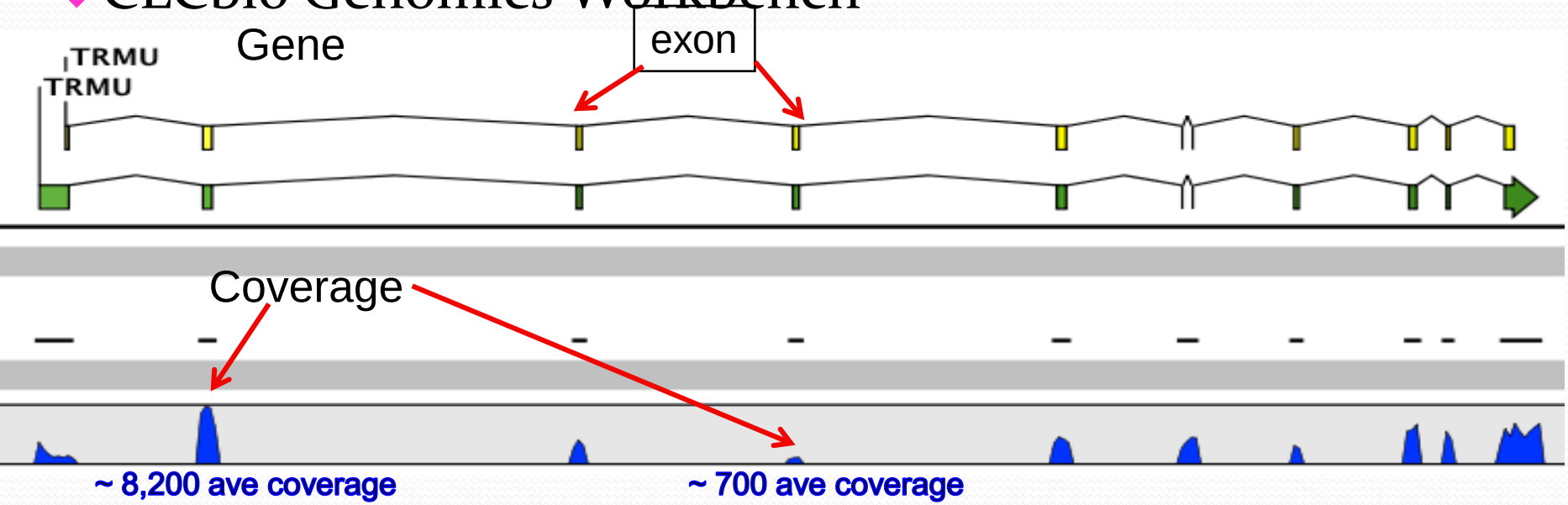
Data Analysis

- Minimum coverage: 50-fold
- Q score: 30
- Heterozygous allele frequencies: 30-70%
- Seed/window length: +/- 11

Are these quality metrics reasonable?

mt 128 Nuclear Gene Panel

- CLCbio Genomics Workbench



- Alignment/variant call parameters:
  - Aligned to dbSNP132 annotated and masked reference sequence
  - Minimum coverage: 50-fold
  - Heterozygous allele frequency range: 30-70%
  - Report all CDS SNP/DIP variants
  - Filter out common polymorphisms

Mito Nuclear Gene Panel-Results

Mapping	Reference	Variants	Allele	Frequencies	rs	Amino Acid Change	URL	Clinical Information
ACADL	211060050	T/G	60.7/39.2	1598/1033	rs2286963	Lys333Gln	rs2286963	Clinical source, LCAD DEFICIENCY
DBT	100672060	C	100	2377	rs12021720	Ser384Gly	rs12021720	Clinical source, MAPLE SYRUP URINE DISEASE, INTERMEDIATE, TYPE II
NDUFV2	9117867	T/C	50.6/49.3	2767/2696	rs906807	Val29Ala	rs906807	Clinical source, VARIANT OF UNKNOWN SIGNIFICANCE (PD)
TRMU	46731689	G/T	53.3/46.7	340/298	rs11090865	Ala10Ser	rs11090865	Clinical source, DEAFNESS, MITOCHONDRIAL, MODIFIER OF [TRMU, 28G-T, ALA10SER]
ETFDH	159603550	C/T	52.9/47.1	880/782	not reported	Leu127Phe	CM093456	Leu127Arg and Leu127His are disease causing
ETFDH	159605751	T/G	59.3/40.6	797/546	not reported	Leu138Arg	CM024518	disease causing mutation
HADHB	26477126	ACT/---	63.6/36.3	1586/904	not reported	Met1_Thr2insThr	not reported	unknown
PDSS1	26991092	T/C	64.5/34.2	2038/1080	not reported	Val44Ala	not reported	unknown
PDSS1	26991113	A/C	59.1/38.9	2191/1443	not reported	Asp51Ala	not reported	unknown
SDHA	225535	G/T	53.4/46.5	1427/1241	not reported	Gly105Val	not reported	unknown
SDHA	225536	A/T	53.2/46.7	1431/1256	not reported	Gly105Gly	not reported	unknown
SDHA	225593	C/T	51.0/49.0	1506/1446	not reported	Tyy124Tyr	not reported	unknown
SDHA	225645	G/A	55.3/44.7	1127/912	not reported	Met142Val	not reported	unknown
SDHA								
TSFM								
TSFM								
ACAD9								
ACAD9								
ACAT1								
COQ2								
COQ2								
COQ2	84205872	A/C	50.4/49.6	504/496	rs6818847	Val66Leu	rs6818847	polymorphism
COX10	14095309	G	76.8	3886	rs2230354	Pro233Pro	rs2230354	polymorphism
COX10	14095348	G/A	52.1/47.9	2152/1979	rs34362247	Pro246Pro	rs34362247	allele frequenct NA
COX15	101473218	G	100	421	rs2231687	Phe374Leu	rs2231687	polymorphism
COX6B1	36142187	C/T	54.8/45.1	2715/2235	rs7991	Thr14Thr	rs7991	polymorphism
CPT1A	68549340	G	99.9	3920	rs2228502	Phe417Phe	rs2228502	Minor allele frequency <1%

Two mutations found in ETFDH gene: Leu127Phe and Leu138Arg disease causing Multiple Acyl-CoA Dehydrogenase Deficiency, MADD

After filtered synonymous, known SNPs/DIPs and intronic sequence; 27 variant calls left

Copy Number Aberration in Mitochondrial Diseases

- Majority of the mutations: point mutations

- **However, Deletions/ Duplications are:**
  - **20% of mtDNA mutations**
  - **5-10% of nuclear genes**

Mito aCGH array design

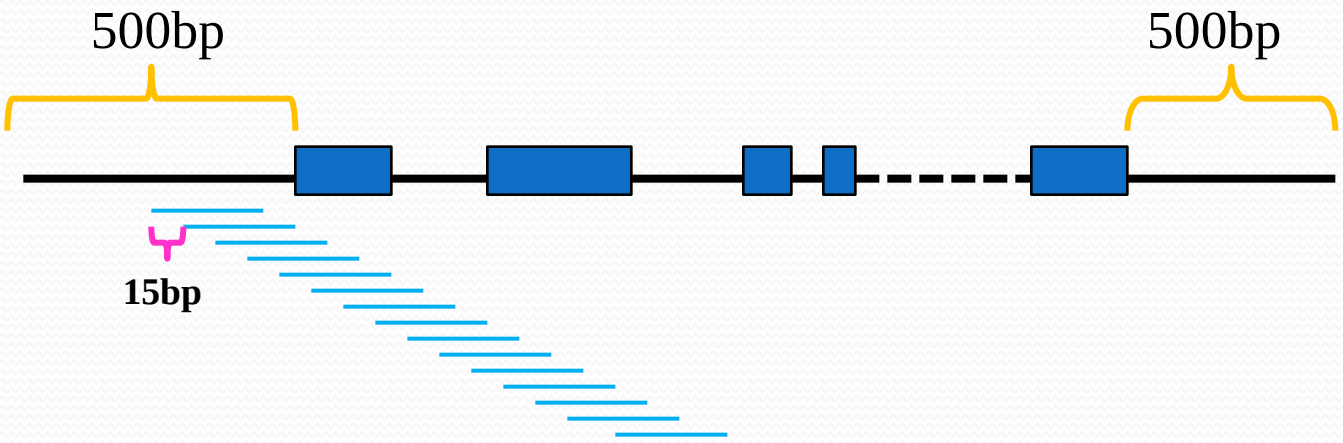
- Gene content:
  - Mitochondrial DNA
  - 101 nuclear genes:
    - 22 for OxPhos subunits
    - 11 genes for OxPhos assembly factors
    - 29 enzymes
    - 9 transcription/translocation
    - 11 carriers
    - 19 for mtDNA maintenance/mitochondria biogenesis

Mito aCGH design

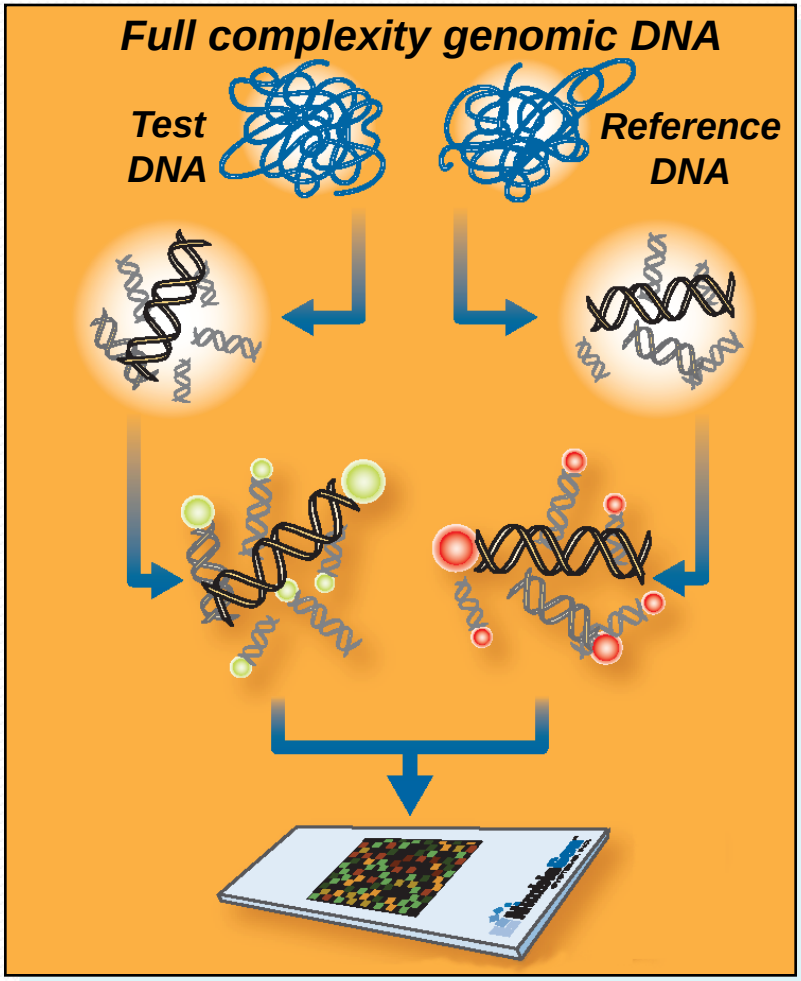
- Mito genome:16.5KB

Roche/NimbleGen
3X720K

- 101 Nuclear Genes:

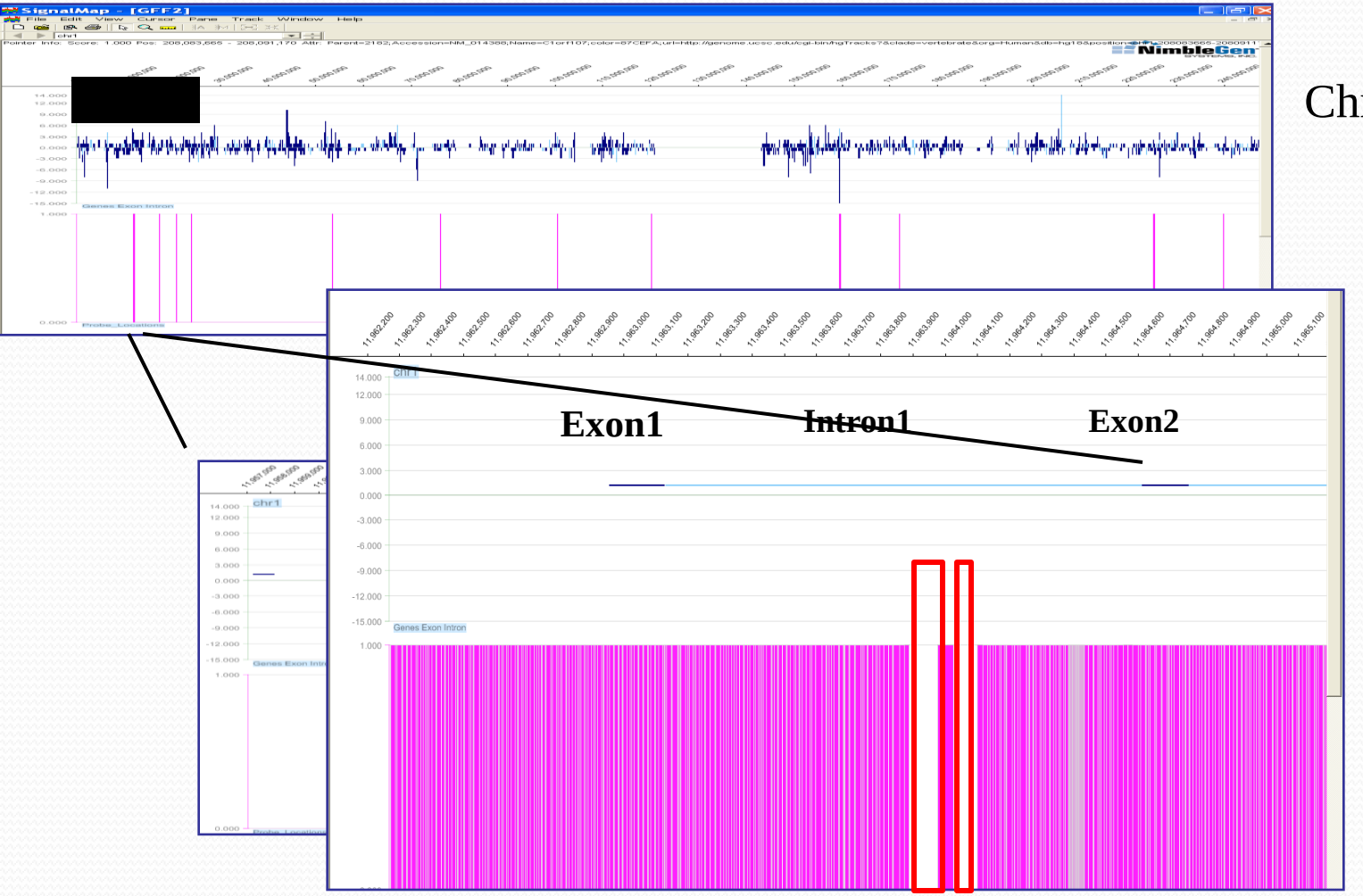


Array CGH Protocol



1. Random fragmentation of DNA
2. Cy3 & Cy5 random prime label
3. Combine labeled test and reference DNA and hybridize
4. Scan array, Cy3 and Cy5 channels
5. Extract images and normalize Cy dye intensities
6. Calculate Log_2 Ratio and perform segmentation analysis

View the Probe Designs in SigMap



Chr 1 12 genes

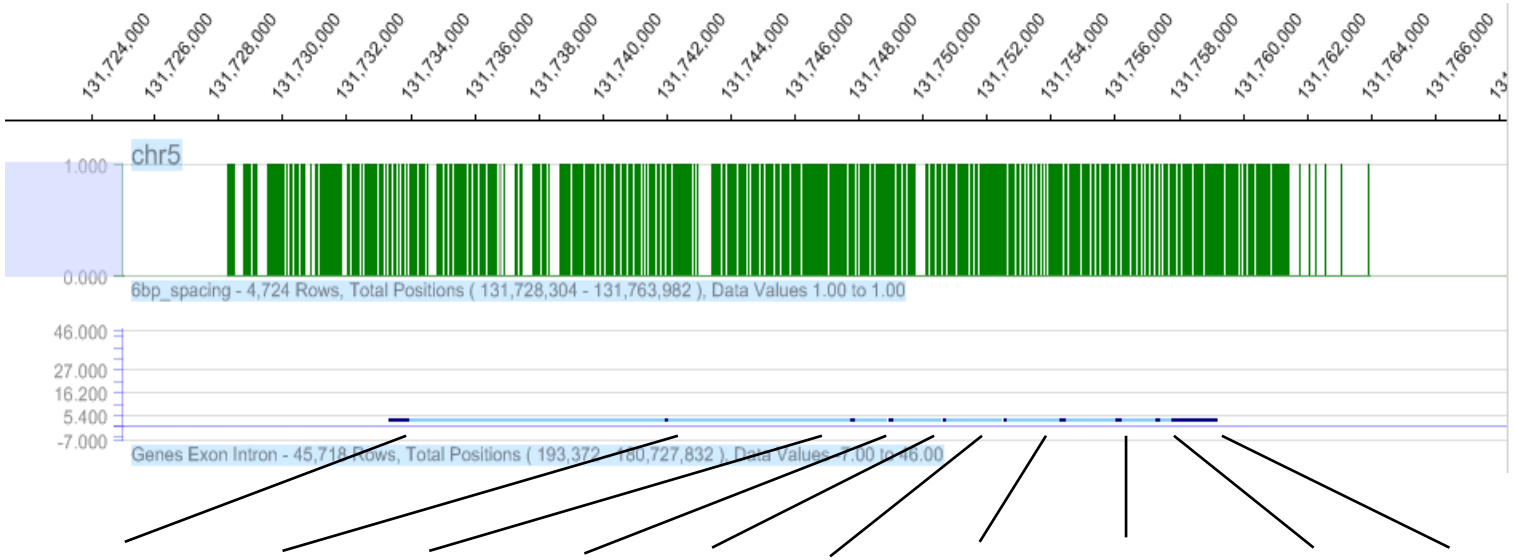
SDHB gene

Exonic level of SDHB gene

Gene Examples, SLC22A5

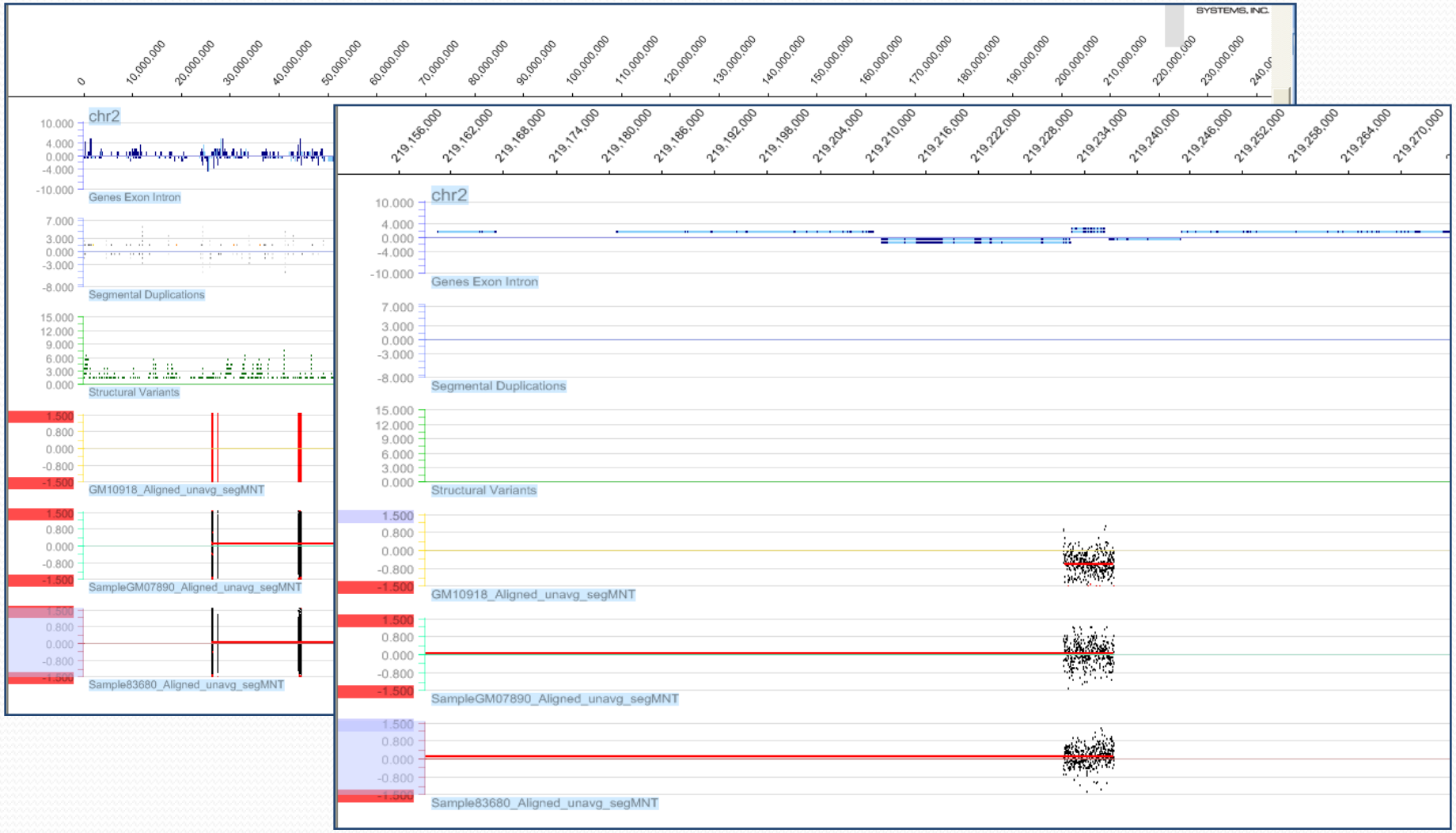
Gene = 25.9 Kb
Region = 35.9 Kb

Total Probes = 4724
Average Probe Spacing = 7.5 bp



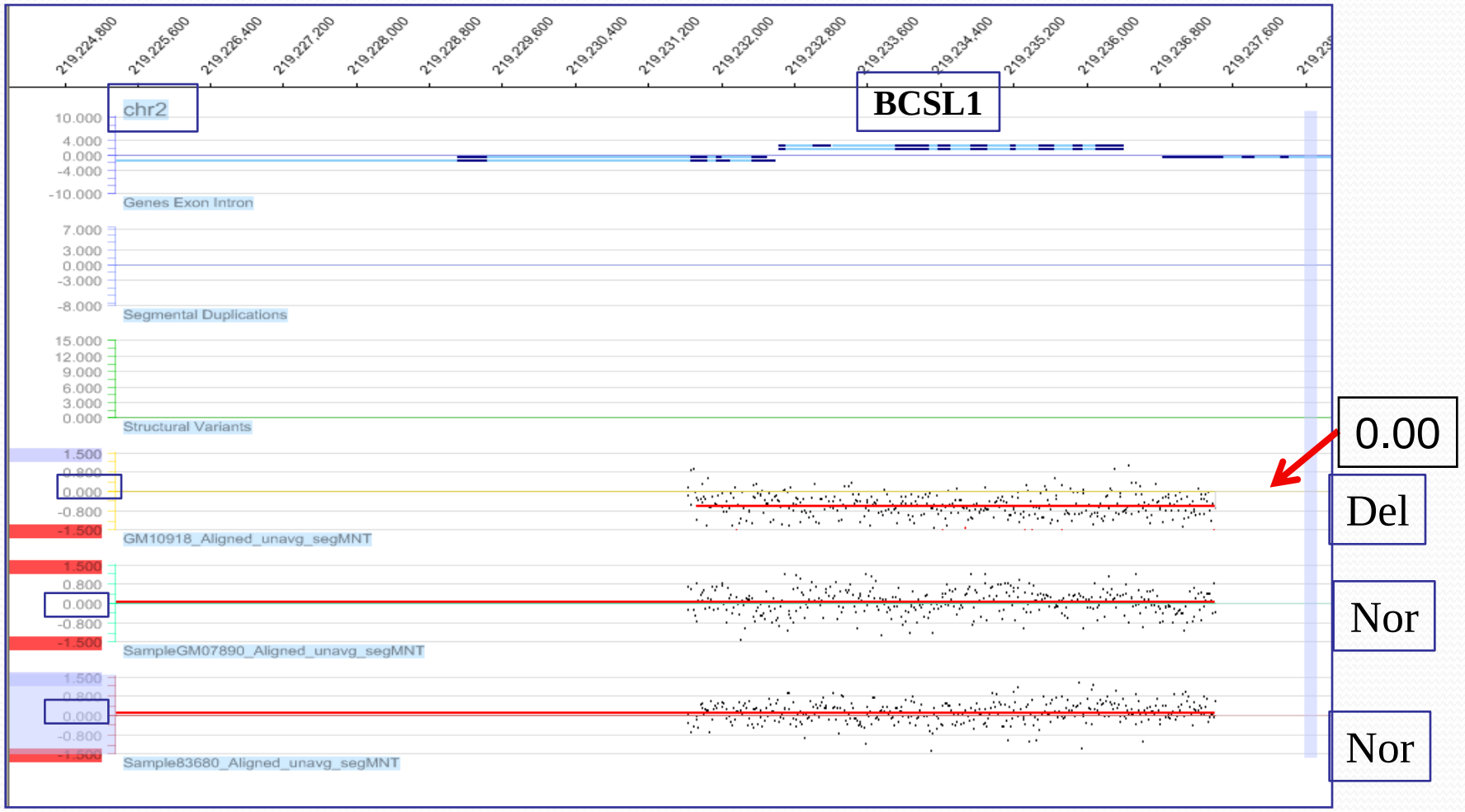
Size, bp	**656**	**103**	**154**	**171**	**126**	**100**	**214**	**182**	**135**	**1429**
Exonic Probes	**69**	**17**	**26**	**26**	**22**	**17**	**35**	**31**	**22**	**221**
Size	**5kb**	**8011**	**5664**	**1025**	**1524**	**1768**	**1667**	**1527**	**1059**	**372**	**5kb**
Intron Probes	**535**	**1096**	**836**	**157**	**193**	**284**	**278**	**249**	**177**	**62**	**372**

Mito aCGH results- Chr2 Nine Genes



Deletion of BCSL1 gene

CHR	START	STOP	SIZE	PROBES	LOCATION	LOG2_RATIO
Chr2	219231599	219237104	5505	385	BCSL1 entire	-0.63



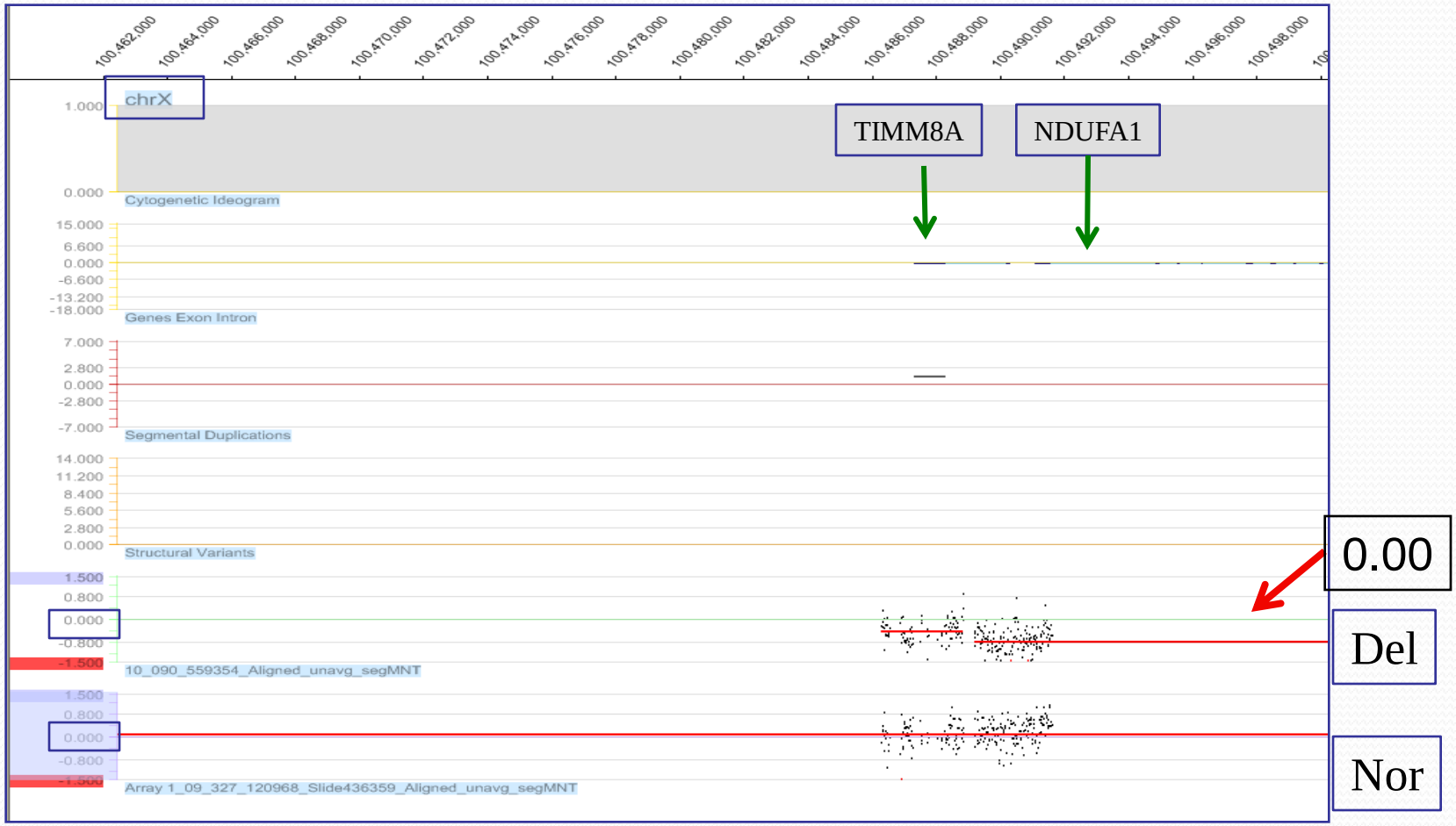
BCSL1 Mutations Causing Mitochondrial Disease

- *BCSL1* gene encoding proteins necessary for assembly of Complex III in OXPhos

- Patients with *BCSL1* mutations:
  - Mitochondrial encephalomyopathies
  - GRACILE syndrome = growth retardation, aminoaciduria cholestasis, iron overload, lactic acidosis, early death

Deletion of the TIMM8A and NDUFA1 Gene

CHR	START	STOP	SIZE	PROBES	LOCATION	LOG2_RATIO
ChrX	100485999	**118889999**	344	344	TOMM8A and NDUFA1	-0.74



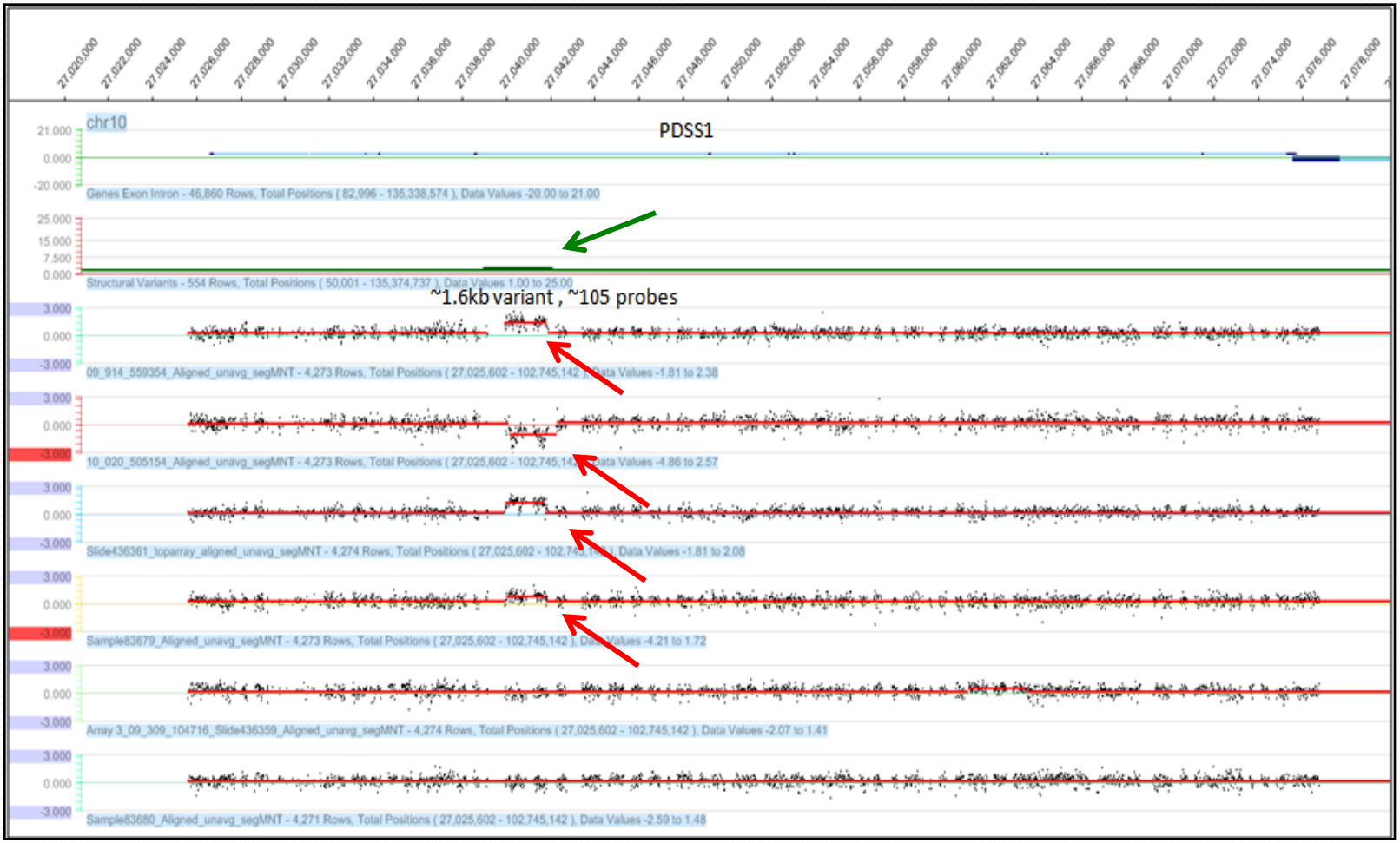
Confirmed by 44K Constitutional CGH array

TIMM8A in Mitochondrial Disease

- TIMM8A protein mediate the import and insertion of hydrophobic membrane protein into the mitochondrial inner membrane

- TIMM8A mutation: a progressive neurodegenerative disorder (Mohr-Tranebjaeg syndrome)

Common CNS on Chr10

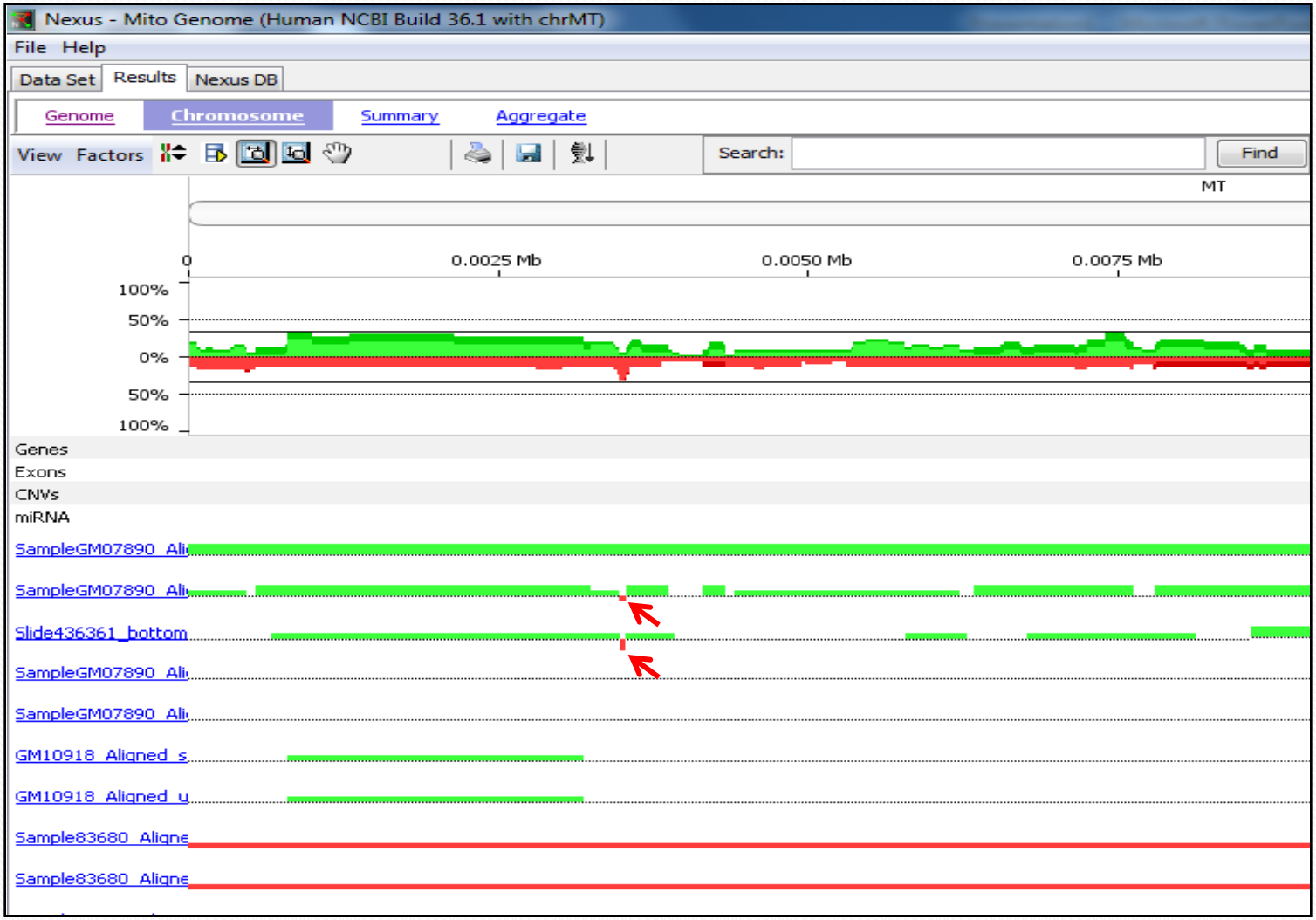


Common CNS , 1.6Kb in 1.5 probes , in PDSS1 gene

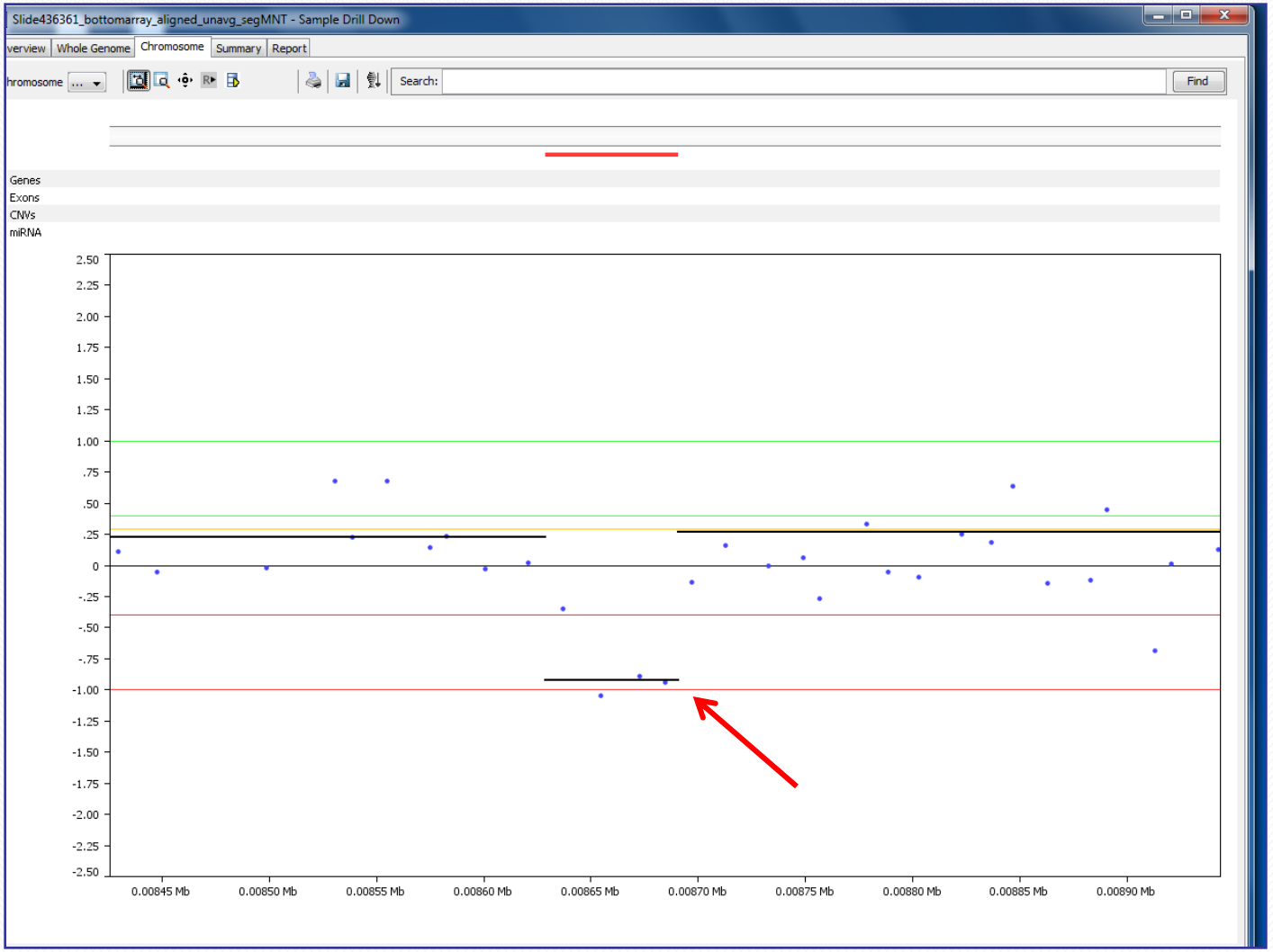
15 positive samples tested with 100% concordance

Sample	Chr	Gene(s)	Probes	Del/Dup	Size, kb
559354	chr1	SDHB, PINK	2517	0.59	3634
GM10918	chr2	BCS1L	385	-0.63	6
130421	chr4	WFS1	2139	-0.71	34
108377	chr6	BCKDHB	12280	-0.50	242
300327	chr9	APTX	1449	0.48	30
120968	chr9	SURF1	417	0.40	2846
GM07890	chr13	SLC25A15,	3665	-0.45	7214
106561	chr17	ATPAF2	976	-0.46	21
118417	chr18	NDUFV2	1765	-0.61	33
556003	chrX	PDHA1, ABC	5537	0.45	55013
556003	chrX	TIMM8A	344	-0.73	18404
505154	chrX	PDHA1	2181	0.59	54937
505154	chrX	ABCB7	3132	0.43	71
505154	chrX	TIMM8A, ND	628	0.72	44601
505154	chrX	TAZ	700	0.42	34411

aCGH for Mitochondrial DNA (Nexus)



SNPs Causing Probe Drop-off



aCGH for Mitochondrial DNA:D-Loop

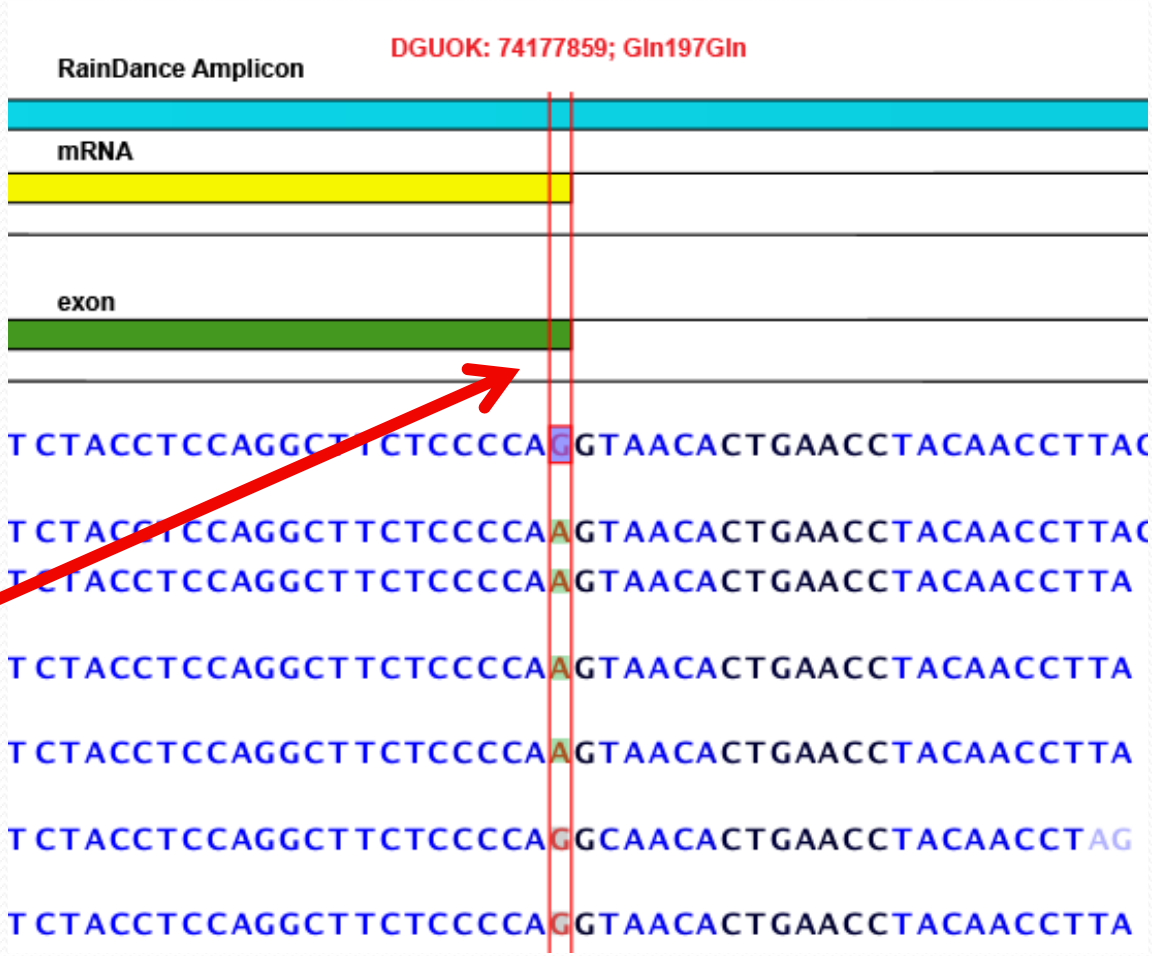
Case cont:

- The patient's sample has been tested for next generation sequencing for mitochondrial genome and 128 nuclear gene mutations.
- Two mutations have been detected in *DUGOK* genes.

DGUOK Mut1:

Reference Seq

c.591G>A
p.Gln197Gln
Splice site



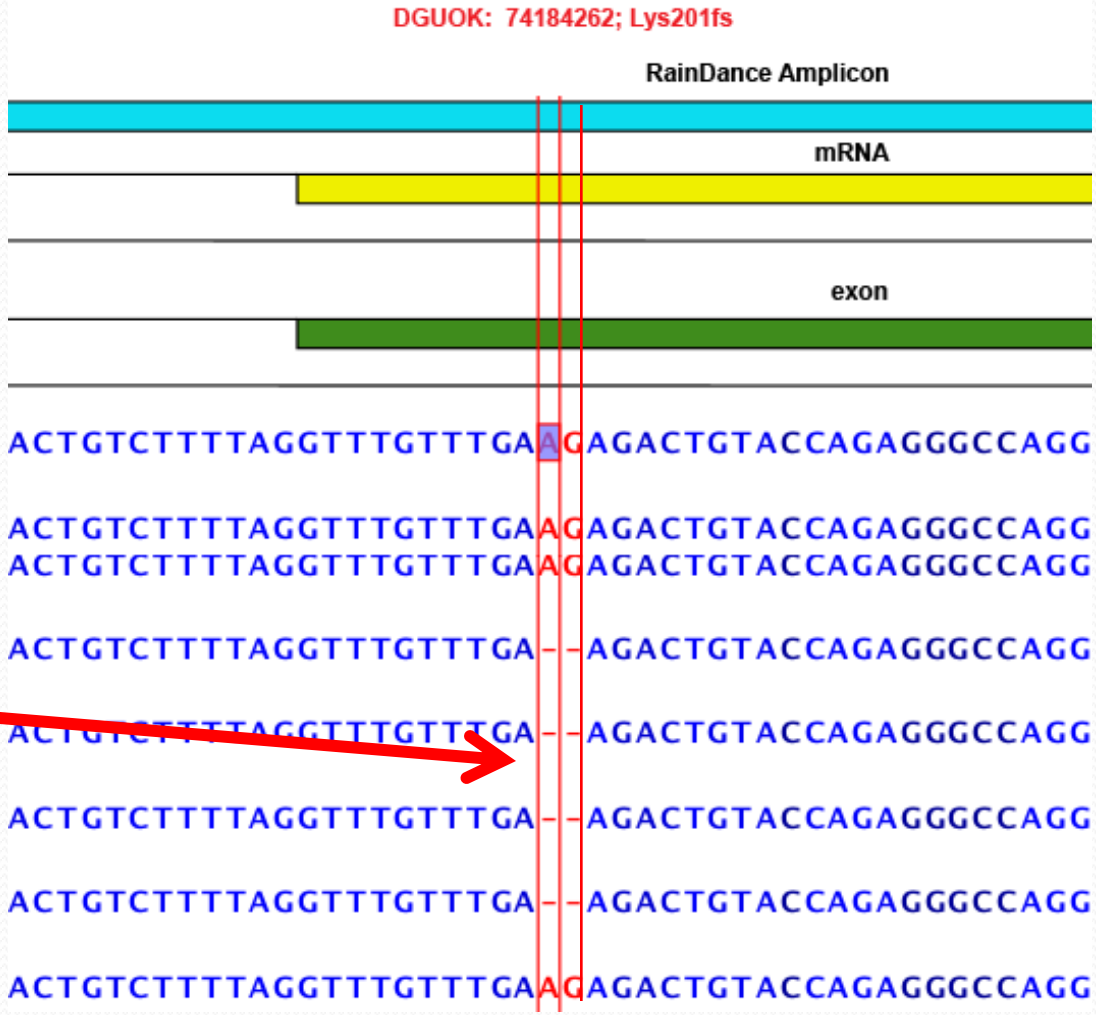
Mapping	Reference	Variation	Reference	Allele	Frequencies	Counts	Coverage	Amino	rs	Mutation
DGUOK	74177859	SNP	G	A/G	53.7/46.3	2695/2327	5023	Gln197Gln	not reported	**MDS compound het with AKA R202TfsX13.**

Courtesy of Shale Dames

DGUOK Mut 2

Reference Seq

c.605-606delAG
p.Lys201fs



Mapping	Reference	Variation	Reference	Allele	Frequencies	Counts	Coverage	Amino	rs	Clinical
DGUOK	74184262	DIP	AG	AG/--	59.9/39.9	2977/1983	4967	Lys201fs	not reported	MDS. AKA R202TfsX13. Introduces stop codon at aa position Glu214Ter (alt trans VCLKTVPEGQGGGERN*)

Courtesy of Shale Dames

Conclusions

- Next generation sequencing technology provides opportunities for mutation detections in large gen panel
- The mitochondrial genome and 128 nuclear panel has been developed and will offer as the first clinical NGS assays in ARUP
- The NGS assay in accompany with aCGH for deletions and duplication will improve the sensitivity of the test
- Variants detected need confirmation and causality needs evidence
- Clinical and family information is critical in assessing significance

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