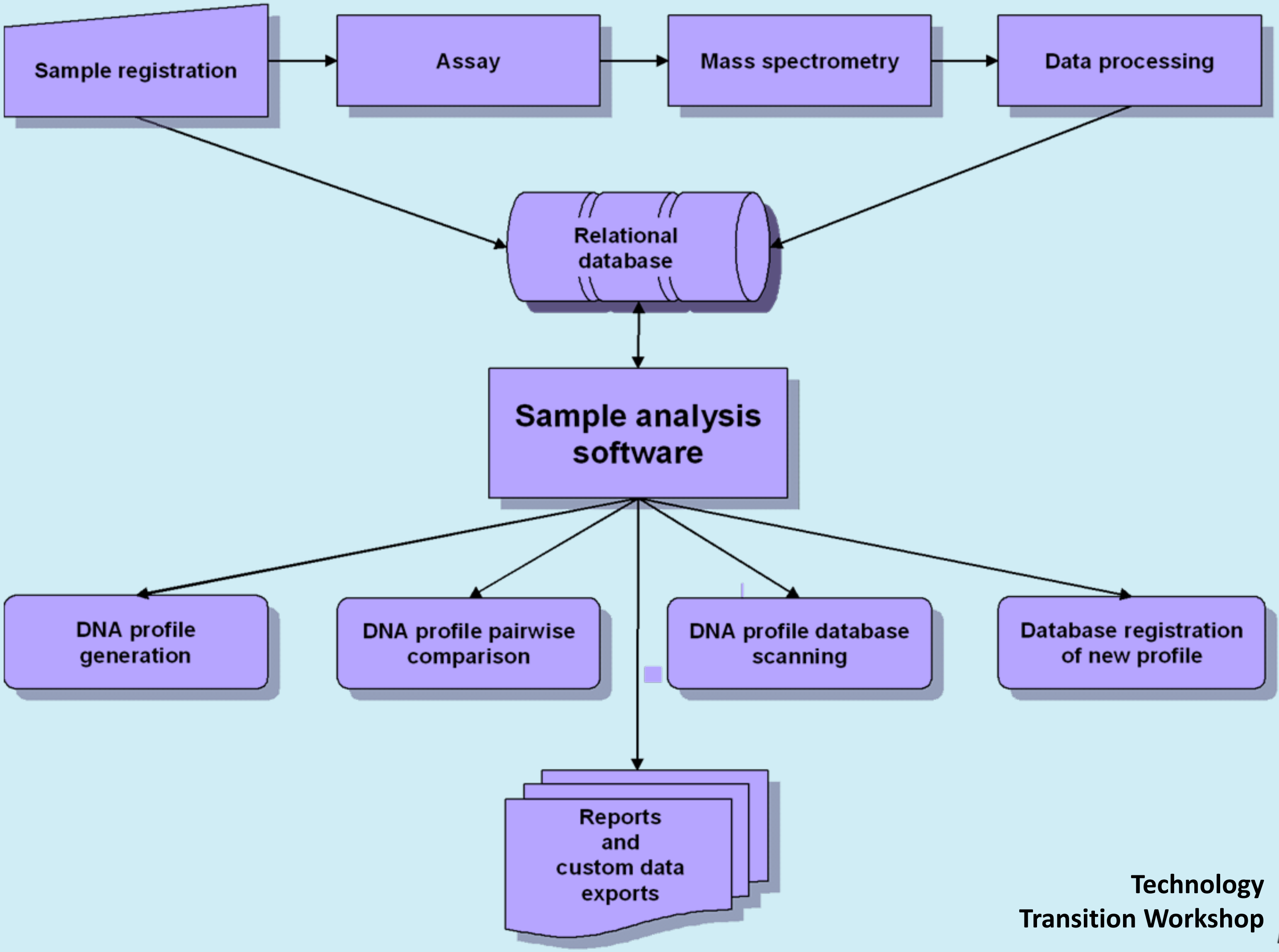
Technology Transition Workshop| *Thomas Hall, Ph.D.*

Overview of Ibis™ Forensic Analysis Software

Outline

- General software interface
- Features
- Data processing
- Data analysis
  - mtDNA
  - SNPs

Forensic Analysis Software


Sample registration

Assay

Mass spectrometry

Data processing

Relational database

Sample analysis software

DNA profile generation

DNA profile pairwise comparison

DNA profile database scanning

Database registration of new profile

Reports and custom data exports

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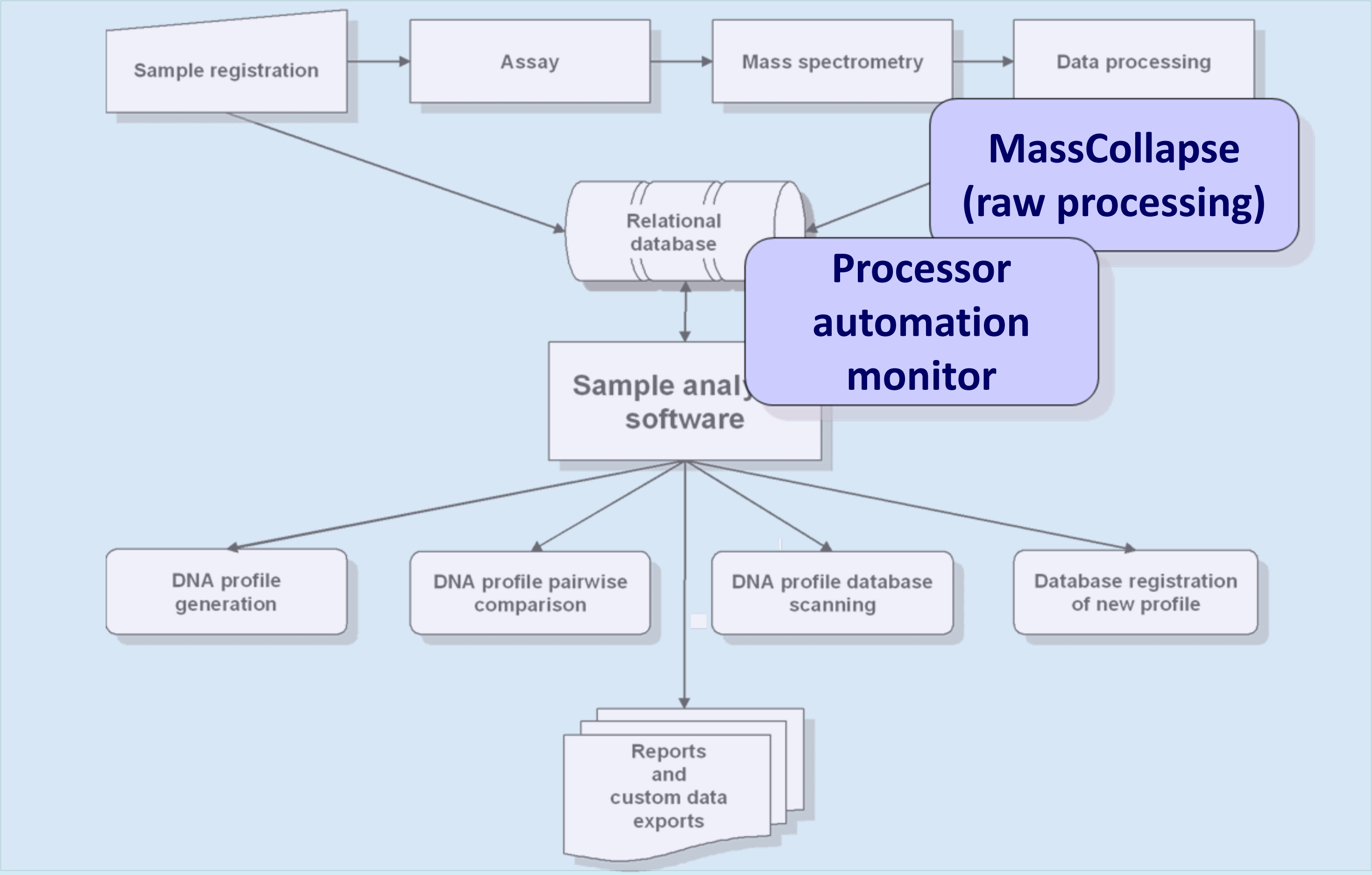
Software Features

- **Utilizes IbisTrack database software for sample tracking**
  - **Integrated system**
  - **MitoDNA analysis module is an independent module**
  - **Updates to MitoDNA → Simply place updated module with most recent IbisTrack; integration is dynamic**
- **Provides functions for:**
  - **Analysis / QC of assay data and registration of profiles**
  - **Adding / removing databases, populations, sequence profiles, and base composition profiles**
  - **Automated conversion of sequence to mtDNA base count profiles**
  - **Population-based searching and reporting for mtDNA**
  - **Plate-based analysis reporting for mtDNA**

Data Processing



MassCollapse (raw processing)

Processor automation monitor

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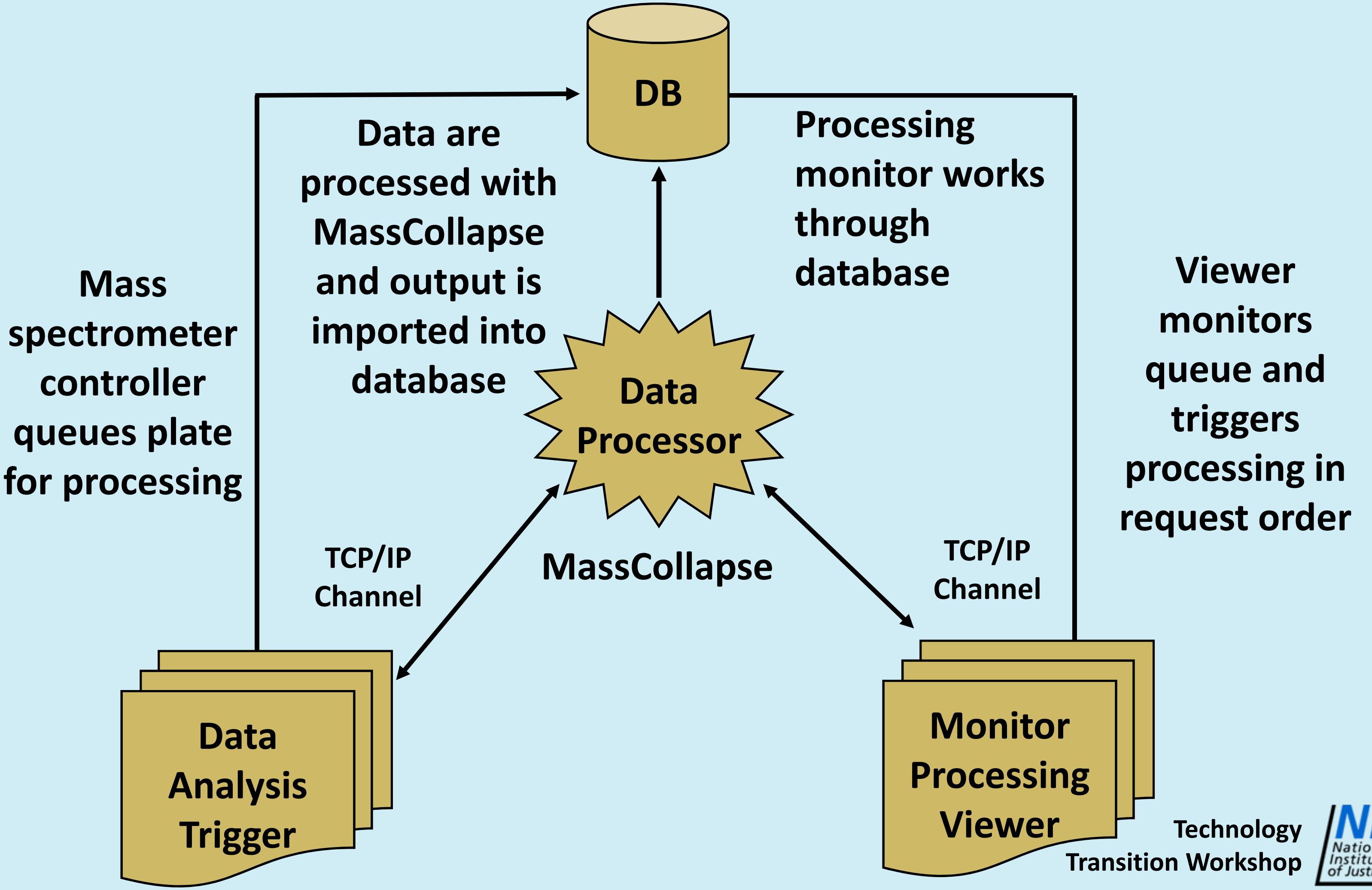
Forensic Data Processing Monitor



DB

Data are processed with MassCollapse and output is imported into database

Processing monitor works through database

Mass spectrometer controller queues plate for processing

Data Processor

Viewer monitors queue and triggers processing in request order

TCP/IP Channel

MassCollapse

TCP/IP Channel

Data Analysis Trigger

Monitor Processing Viewer

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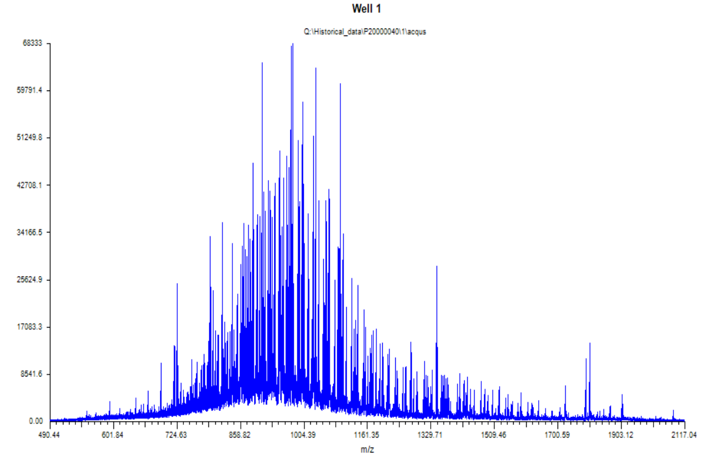
MassCollapse Deconvolution

Raw spectrum



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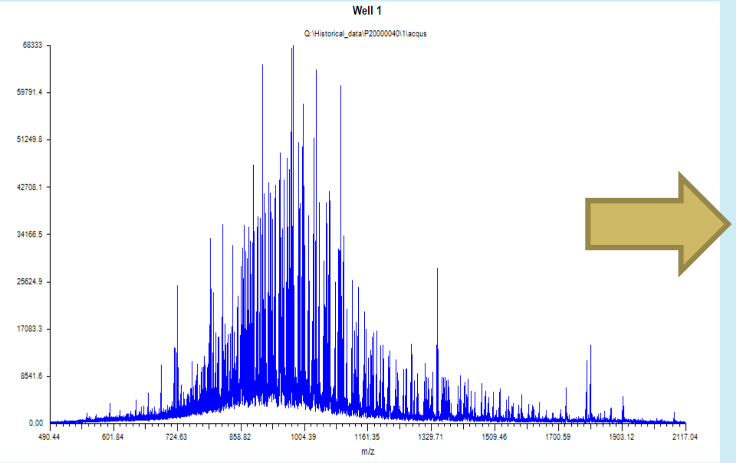
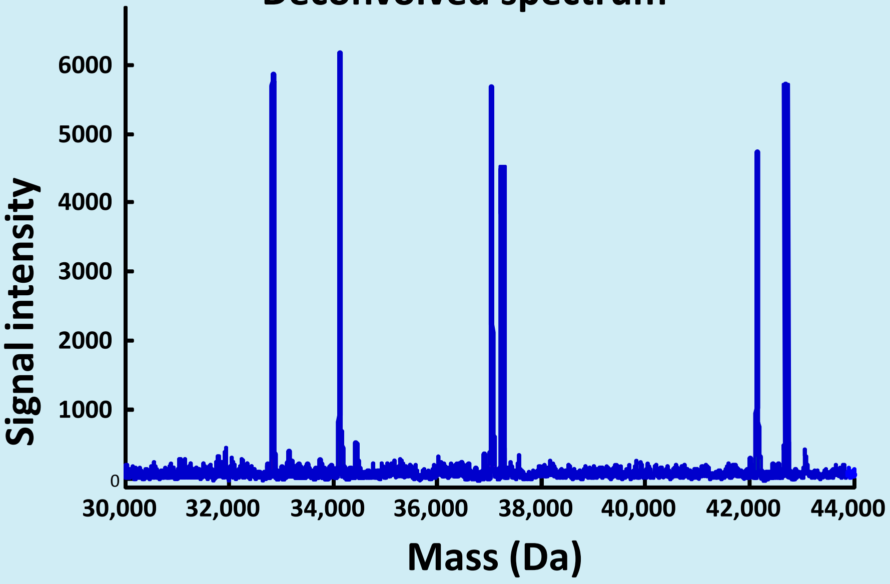
MassCollapse Deconvolution

Raw spectrum



Deconvolved spectrum



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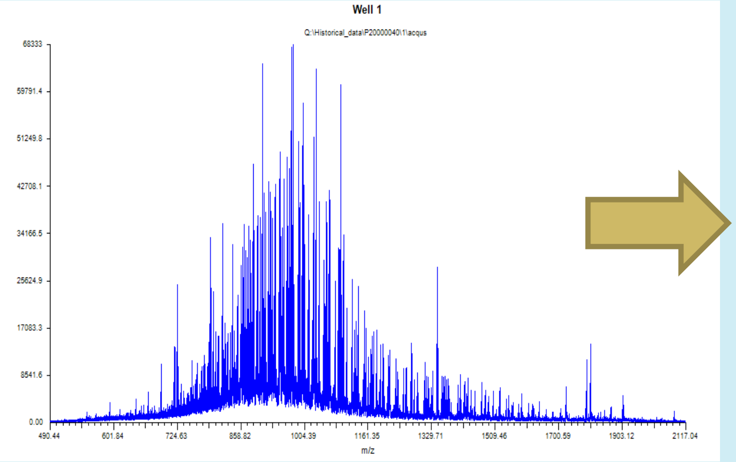
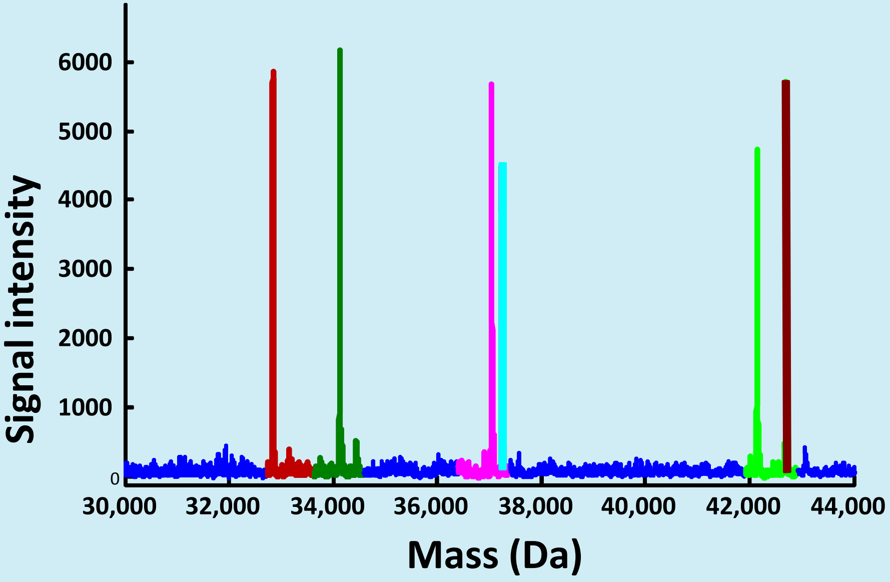
MassCollapse Deconvolution

Raw spectrum





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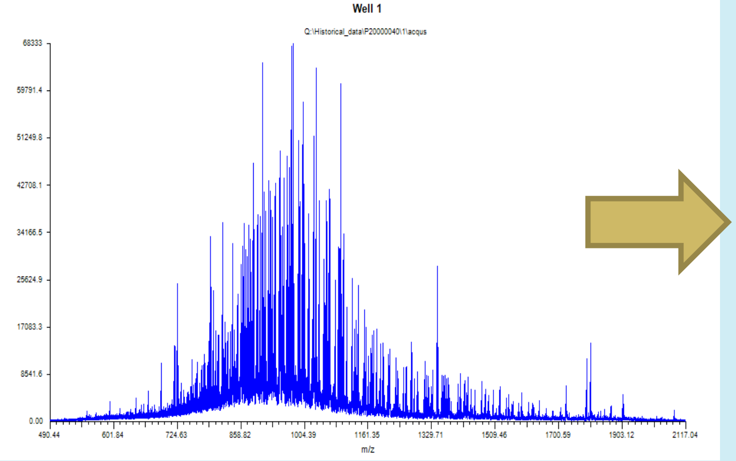
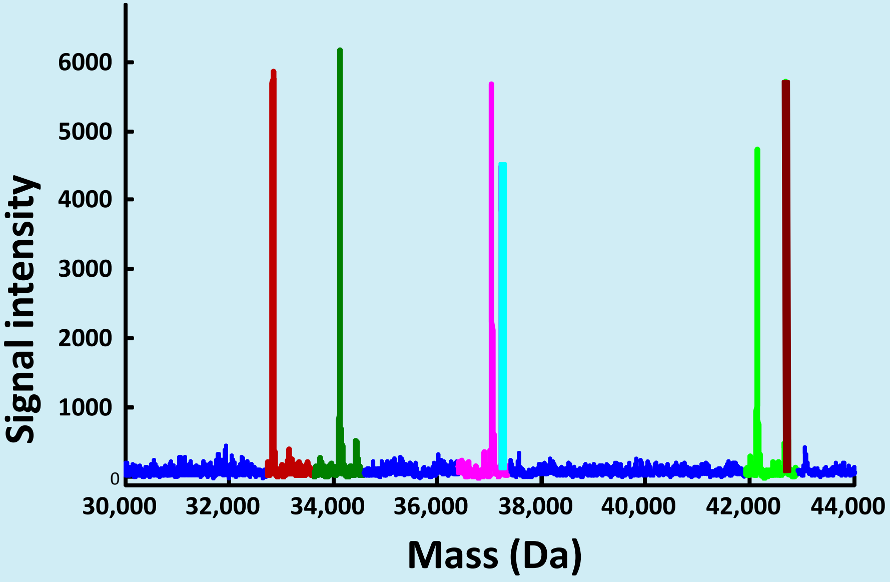
MassCollapse Deconvolution

Raw spectrum





List of masses and intensities

32855.1 Da
34143.8 Da
37058.2 Da
37254.2 Da
42162.1 Da
42710.3 Da

Collapsed data can also be interrogated for fit of base compositions given a model of the Ibis™ mass tag

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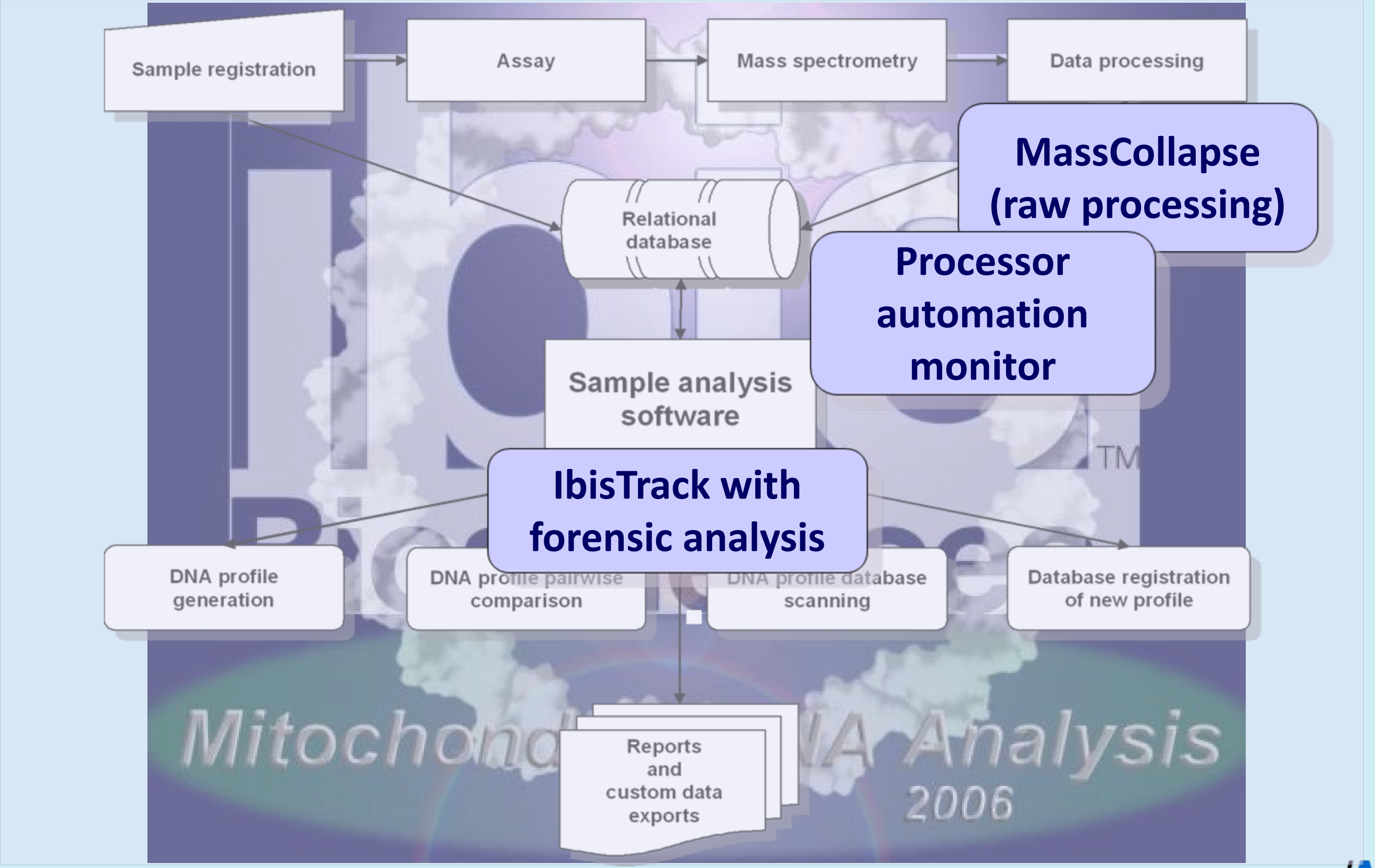
Forensic Analysis Software



MassCollapse (raw processing)

Processor automation monitor

IbisTrack with forensic analysis

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National Institute of Justice

Basic Application Interface

Welcome to *IbisTrack*

IbisTrack tracks samples and plates for the Ibis T5000 Biosensor System, from initiating an assay through viewing results.

Please select from one of the following common operations, or select from the tasks in the navigation pane to the left.

Casework
Register an assay for casework samples. The wizard associates user defined samples with assay plates as well as provides reports for sample layout on assay plates and robot instructions for setup from a 24 place tube rack. Prior to using the wizard, the user should associate the desired control layout with the appropriate plan.

Database Population
Register 9 assay plates for database samples. The wizard expects 91 samples arranged in a specified plate layout for setup. The wizard automatically distributes the 91 samples to 9 assay plates with associated controls.

Import
Import information for assay plates and assay configurations into the local kit inventory from files provided by Ibis Biosciences.

Analysis
Analyze and review processed results after running assay plates on the T5000.

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Main IbisTrack program menu

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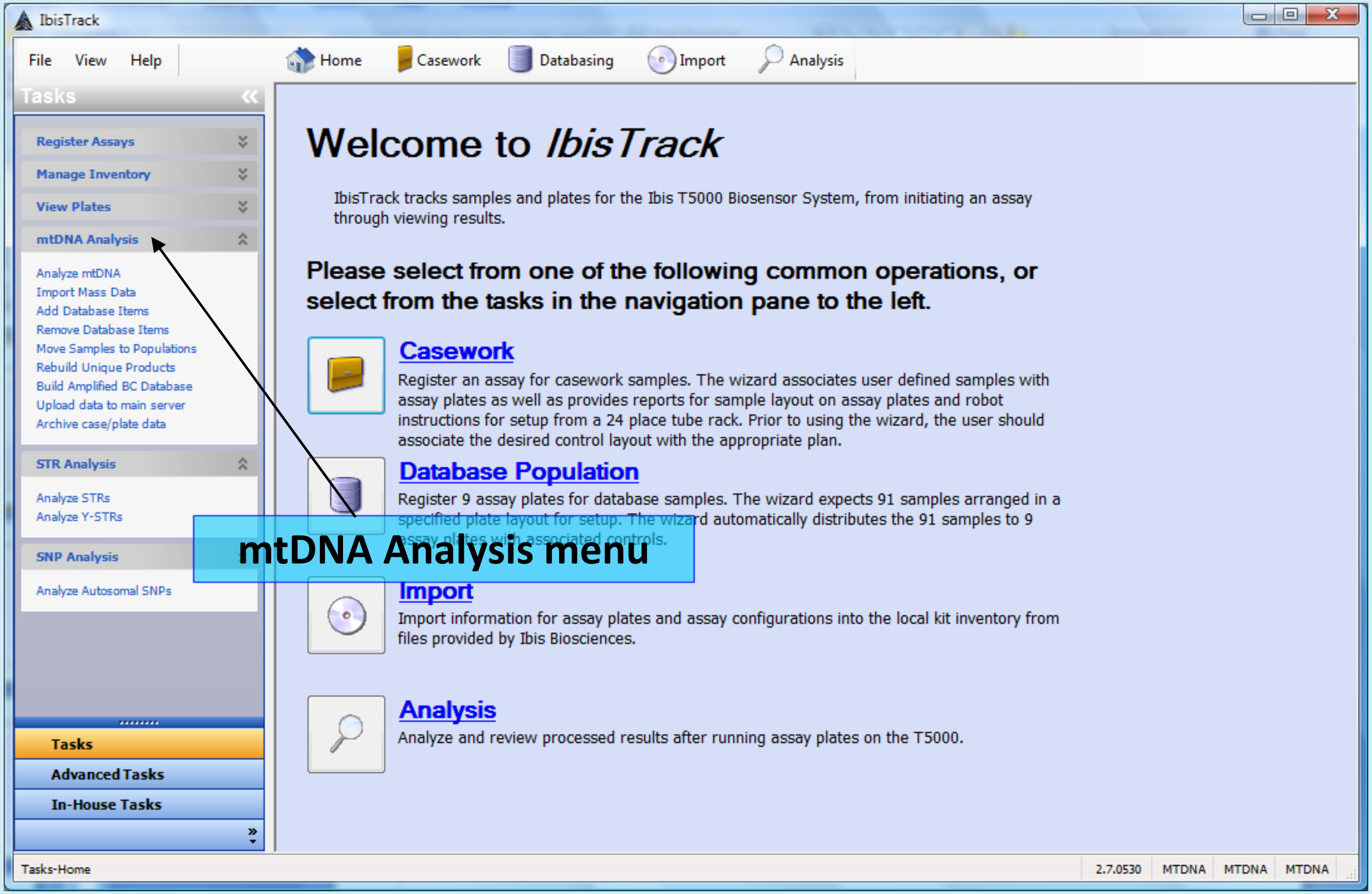
Basic Application Interface



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Analyze and review processed results after running assay plates on the T5000.

mtDNA Analysis menu

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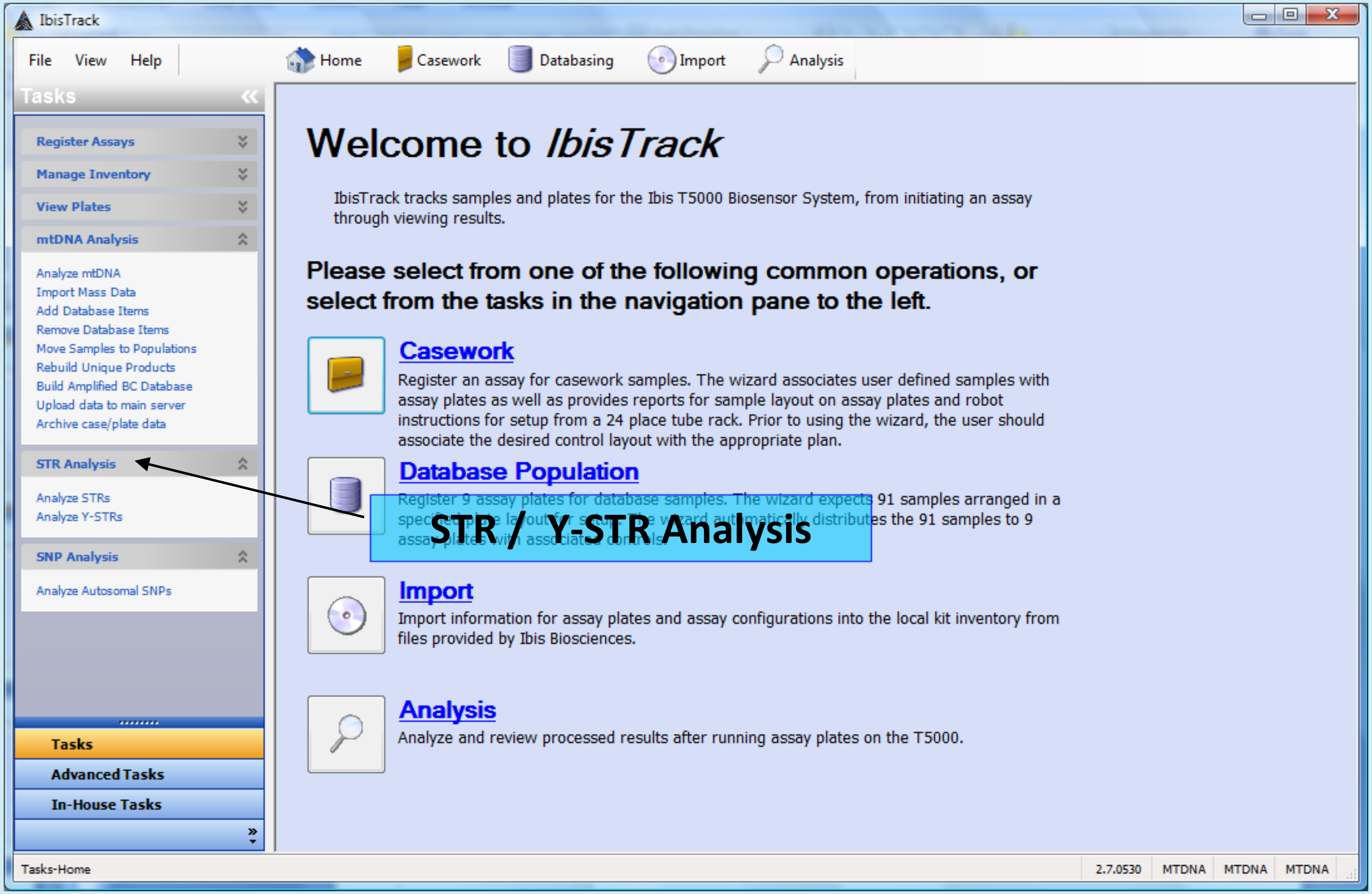
Basic Application Interface



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Analysis

Analyze and review processed results after running assay plates on the T5000.

STR / Y-STR Analysis

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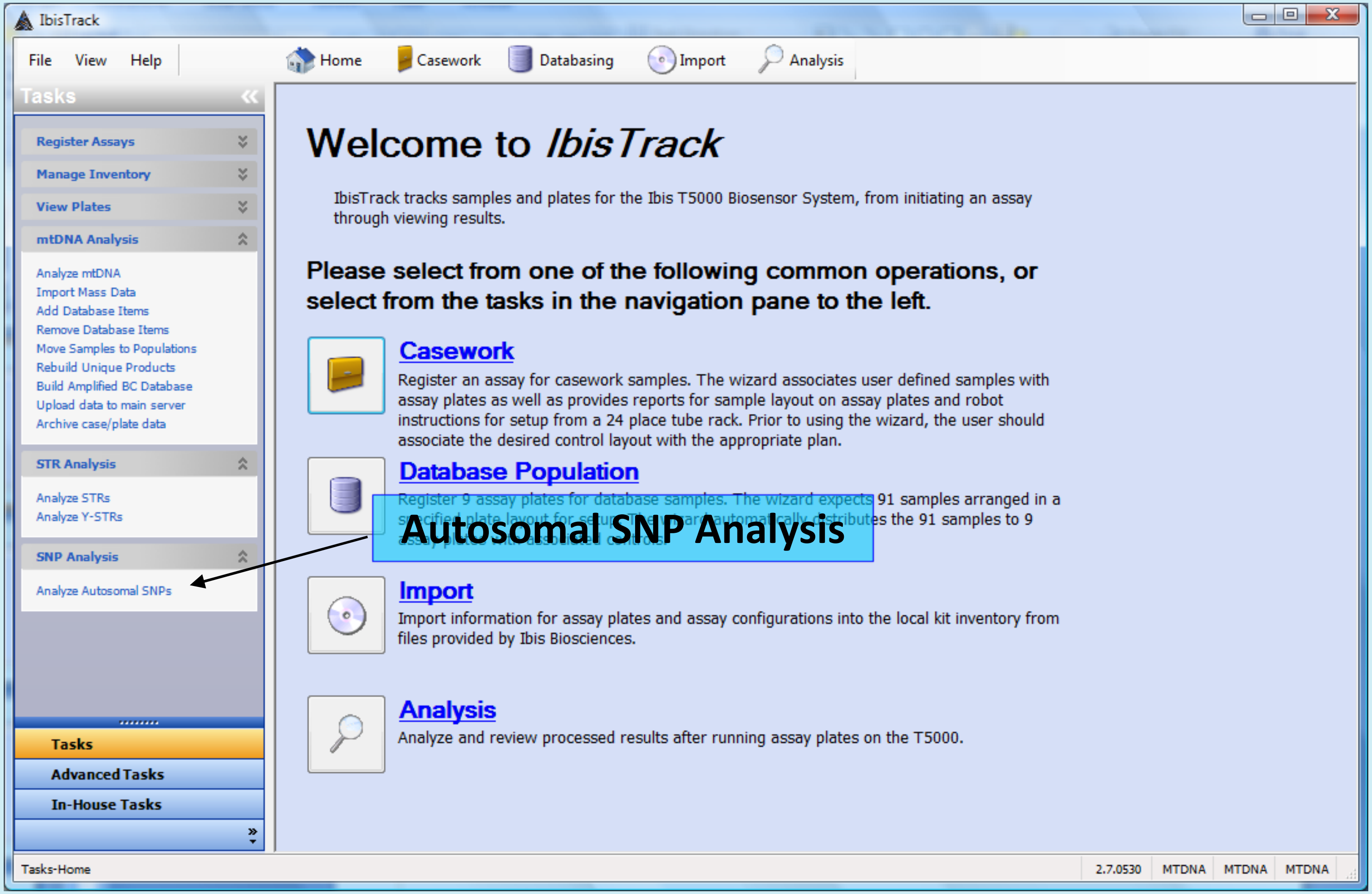
Basic Application Interface



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Analysis

Analyze and review processed results after running assay plates on the T5000.

Autosomal SNP Analysis

Basic Application Interface – mtDNA

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Analysis

Analyze and review processed results after running assay plates on the T5000.

Basic Application Interface – mtDNA

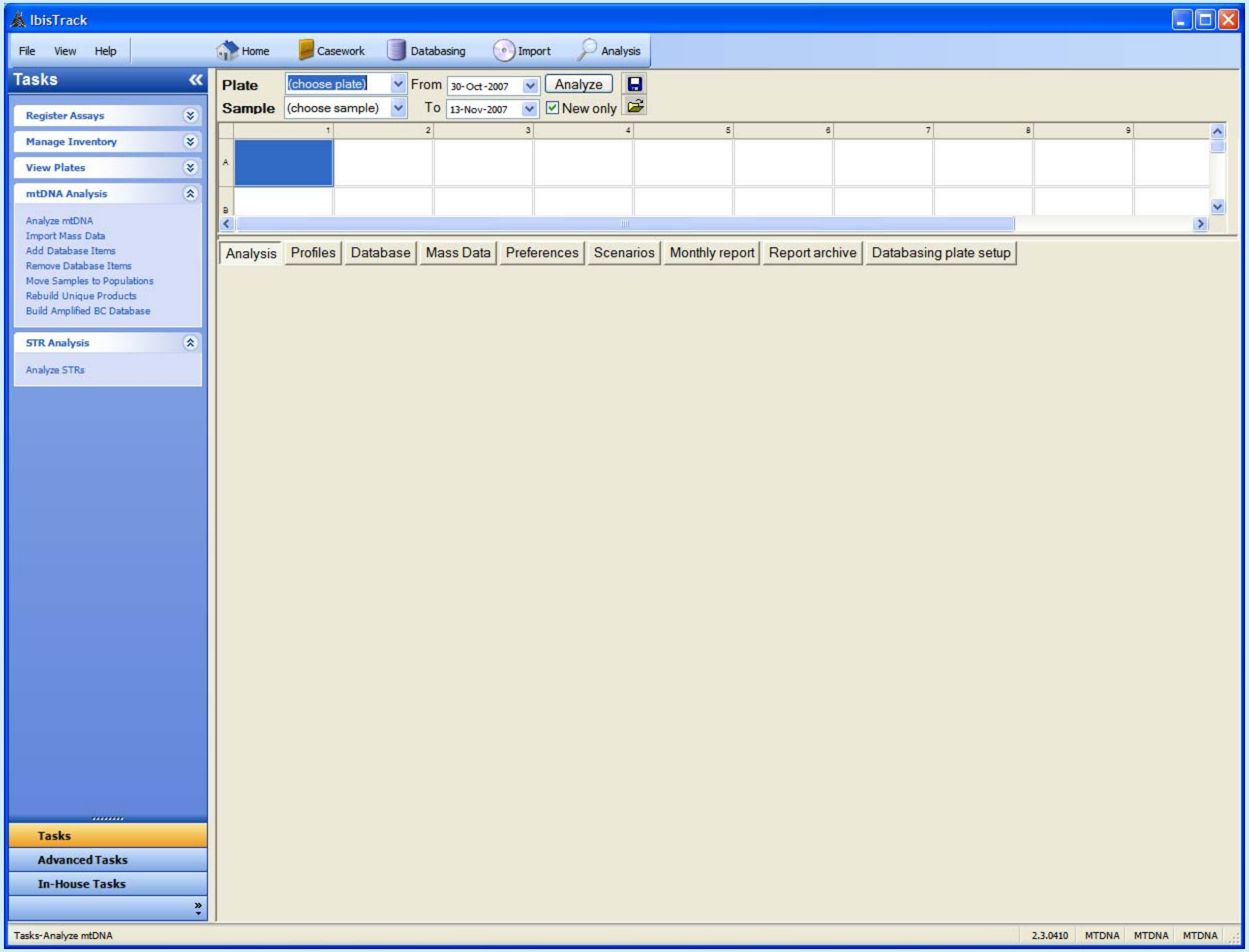

Plate (choose plate) From 30-Oct-2007

Sample (choose sample) To 13-Nov-2007 ☑ New only

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA MTDNA

Basic Application Interface – mtDNA

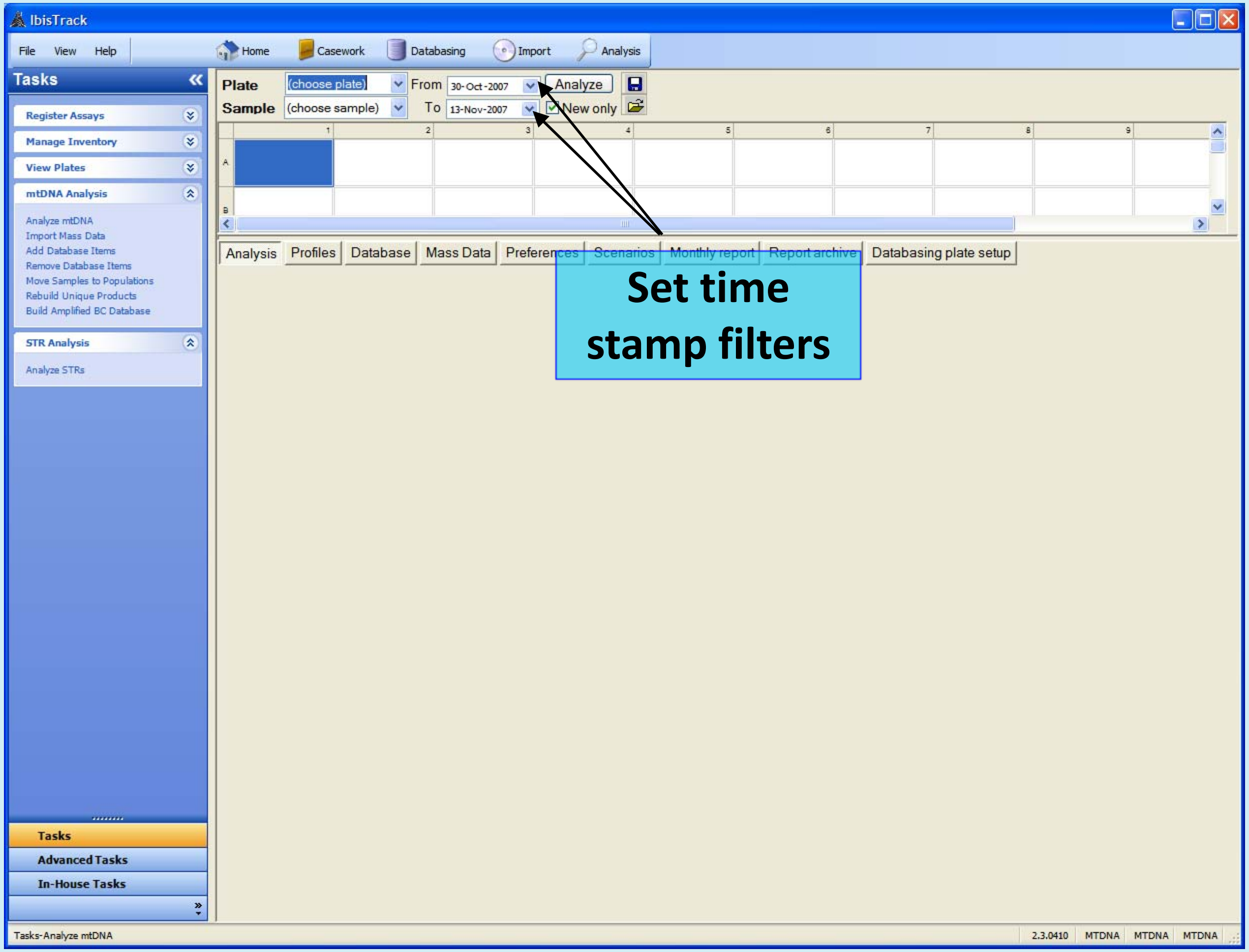

Plate (choose plate)
Sample (choose sample)
From 30-Oct-2007
To 13-Nov-2007
☑ New only

Set time stamp filters

Basic Application Interface – mtDNA

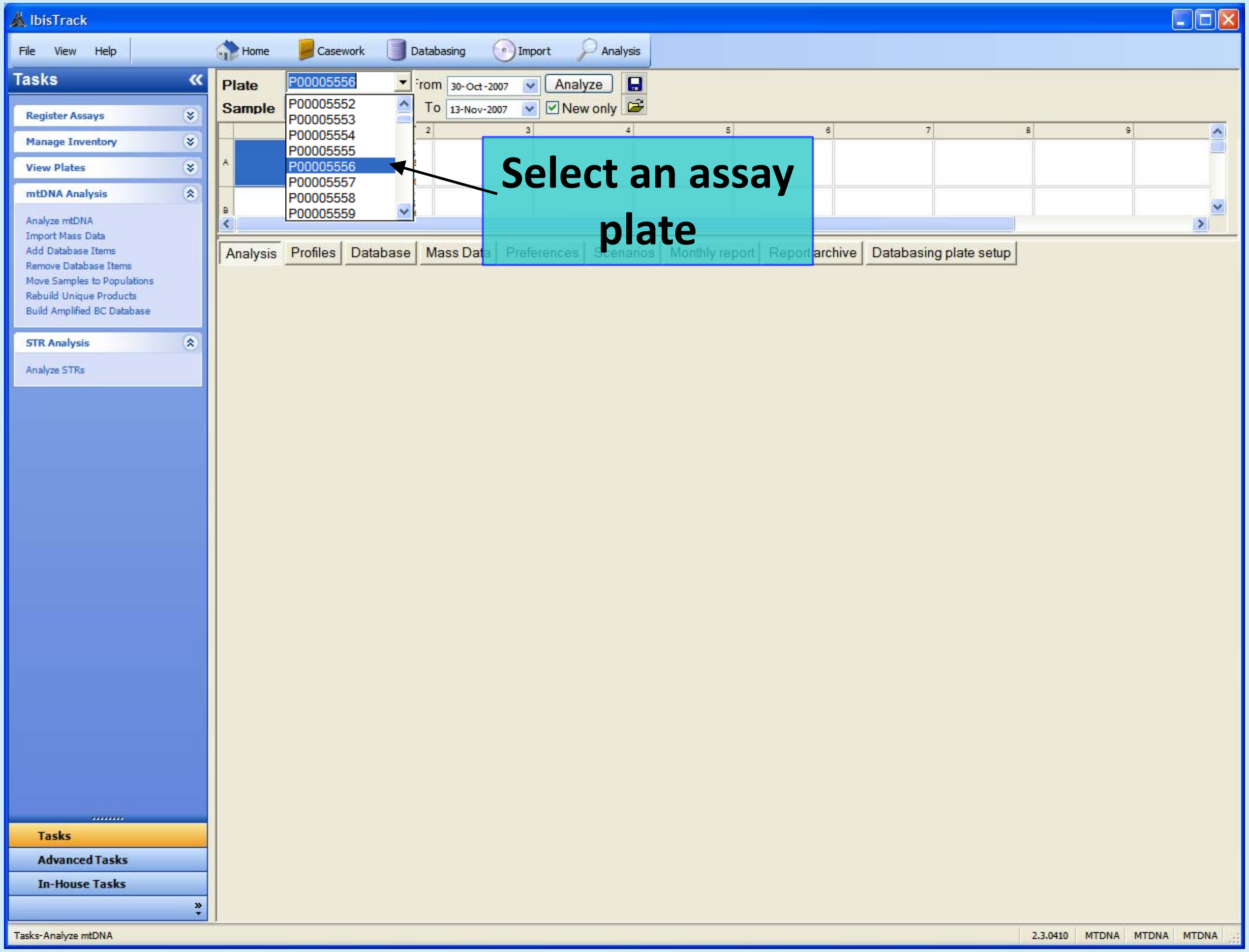

Plate: P00005556

Sample

P00005552
P00005553
P00005554
P00005555
P00005556
P00005557
P00005558
P00005559

From: 30-Oct-2007

To: 13-Nov-2007

☑ New only

Tasks-Analyze mtDNA | 2.3.0410 | MTDNA | MTDNA | MTDNA

Select an assay plate

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Basic Application Interface – mtDNA

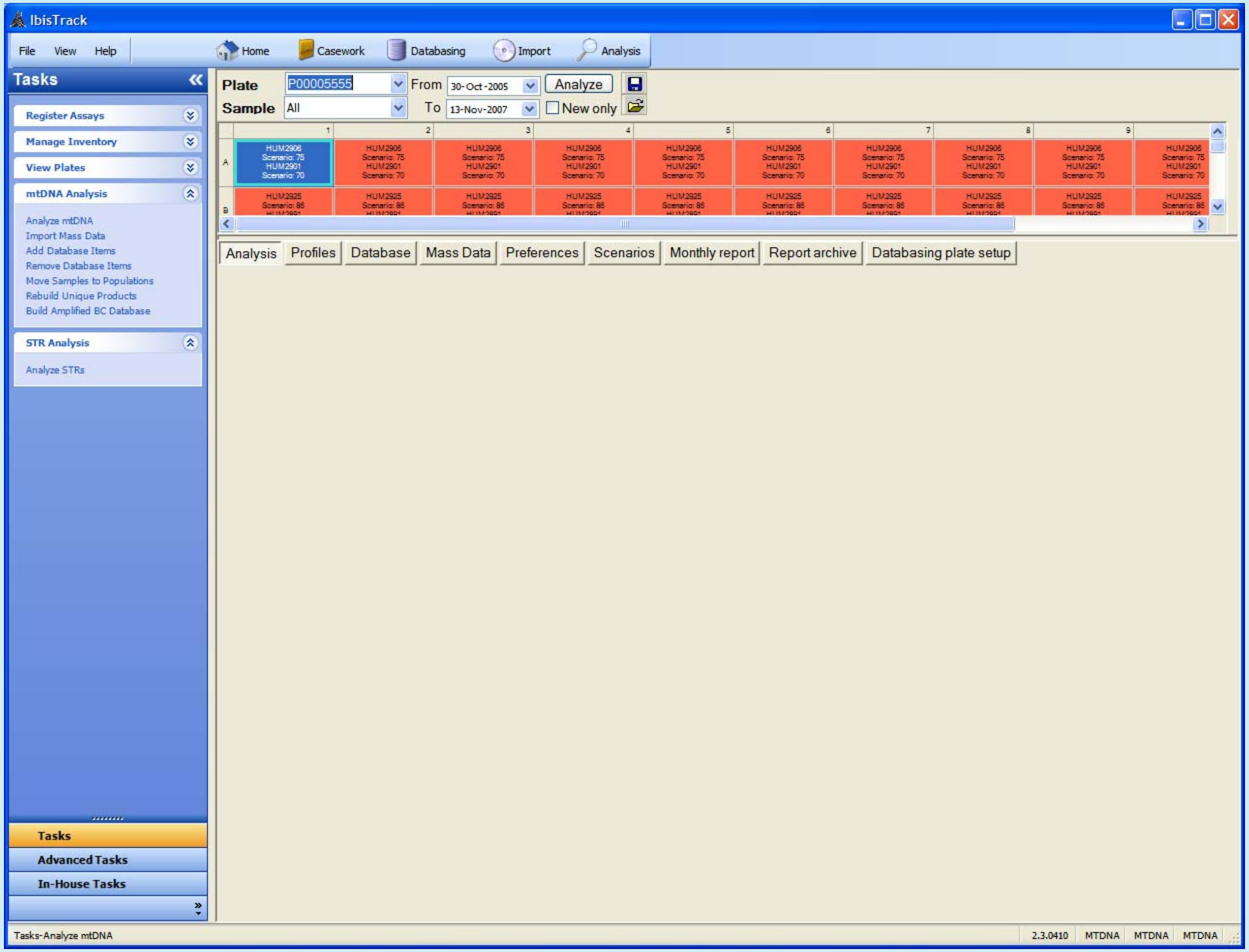

Plate P00005555 From 30-Oct-2005 Analyze

Sample All To 13-Nov-2007 ☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA MTDNA

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Basic Application Interface – mtDNA

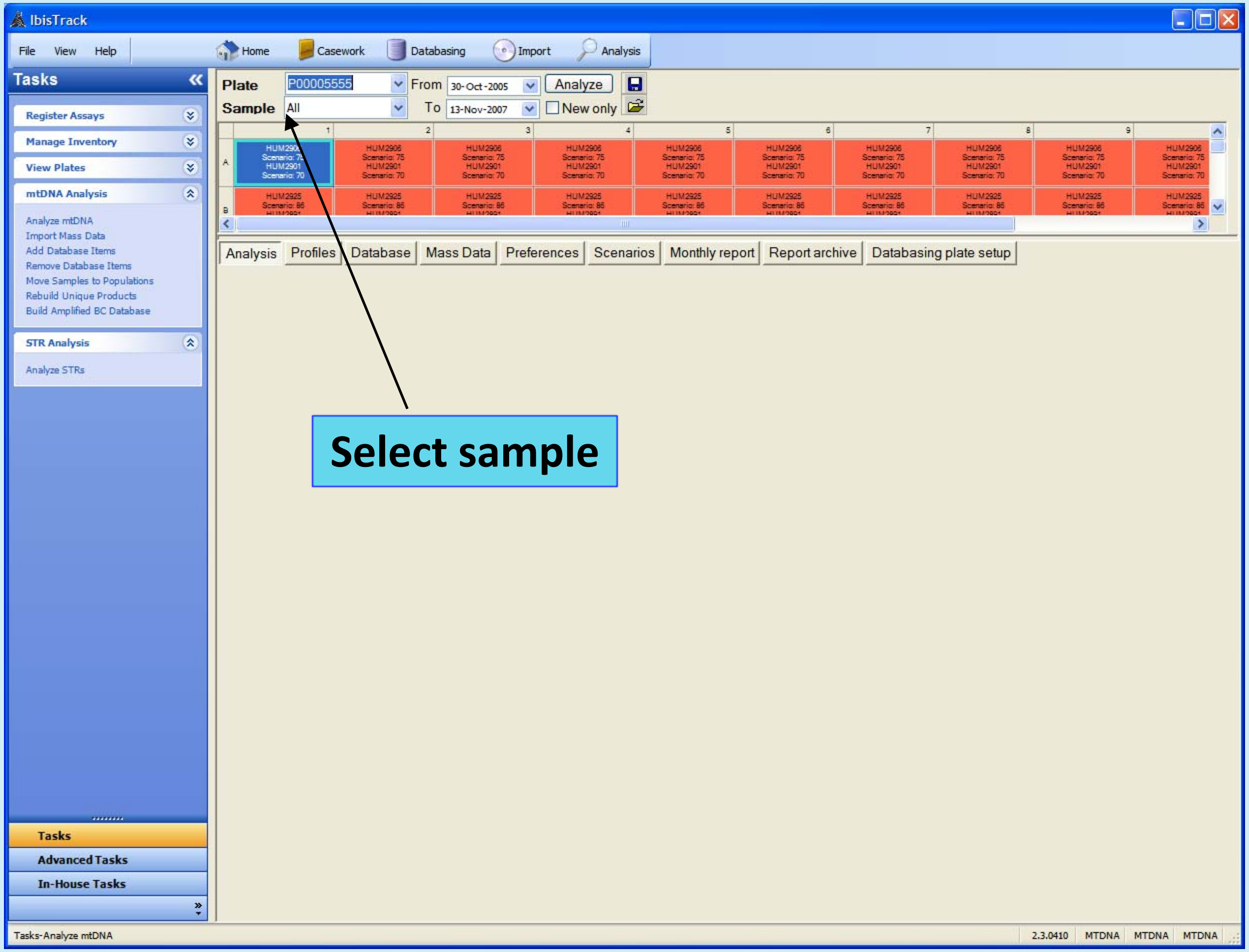

Plate P00005555 From 30-Oct-2005

Sample All To 13-Nov-2007 ☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM290 Scenario: 7 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA MTDNA

Select sample

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Basic Application Interface – mtDNA

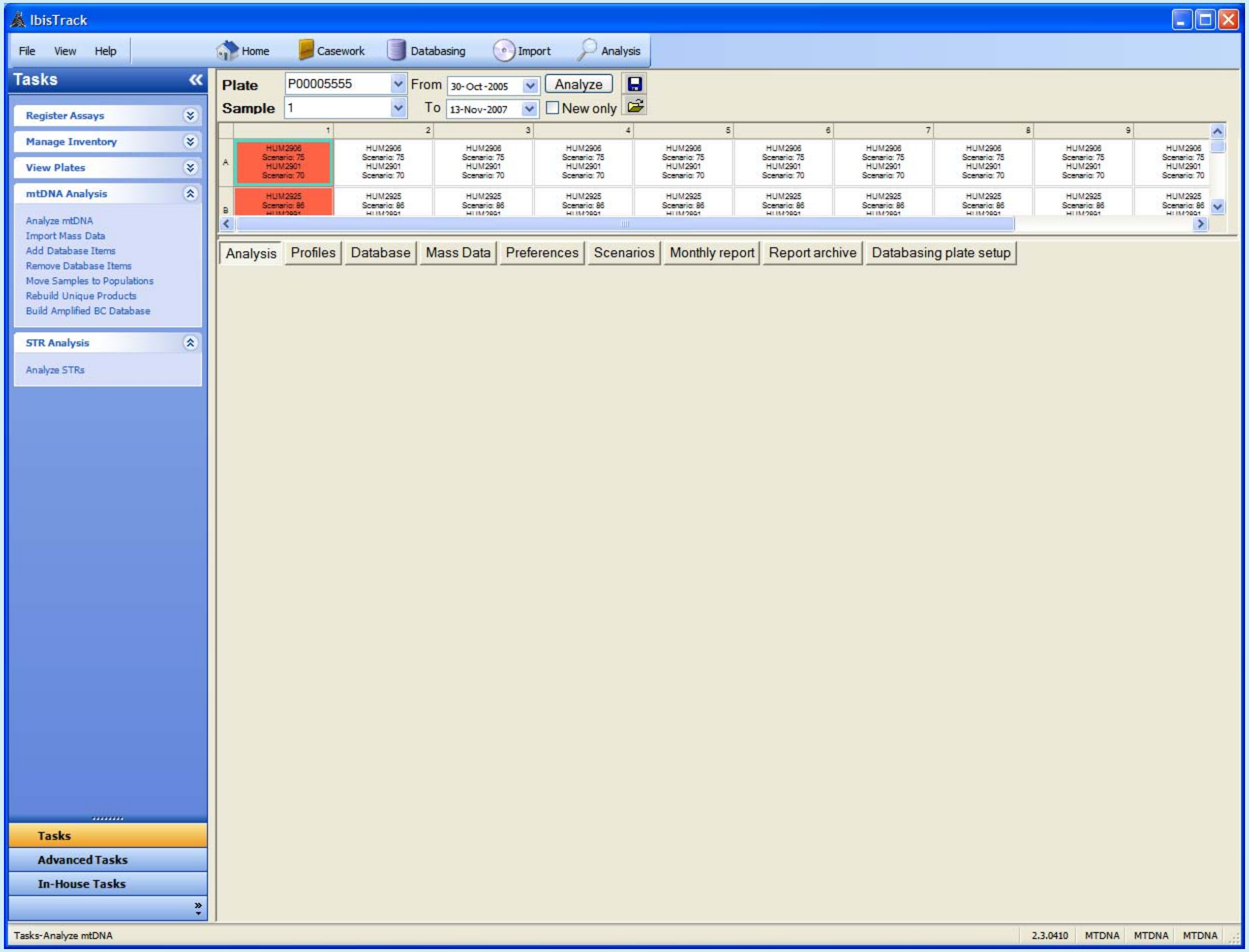

Plate P00005555

Sample 1

From 30-Oct-2005

To 13-Nov-2007

☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA | 2.3.0410 | MTDNA | MTDNA | MTDNA

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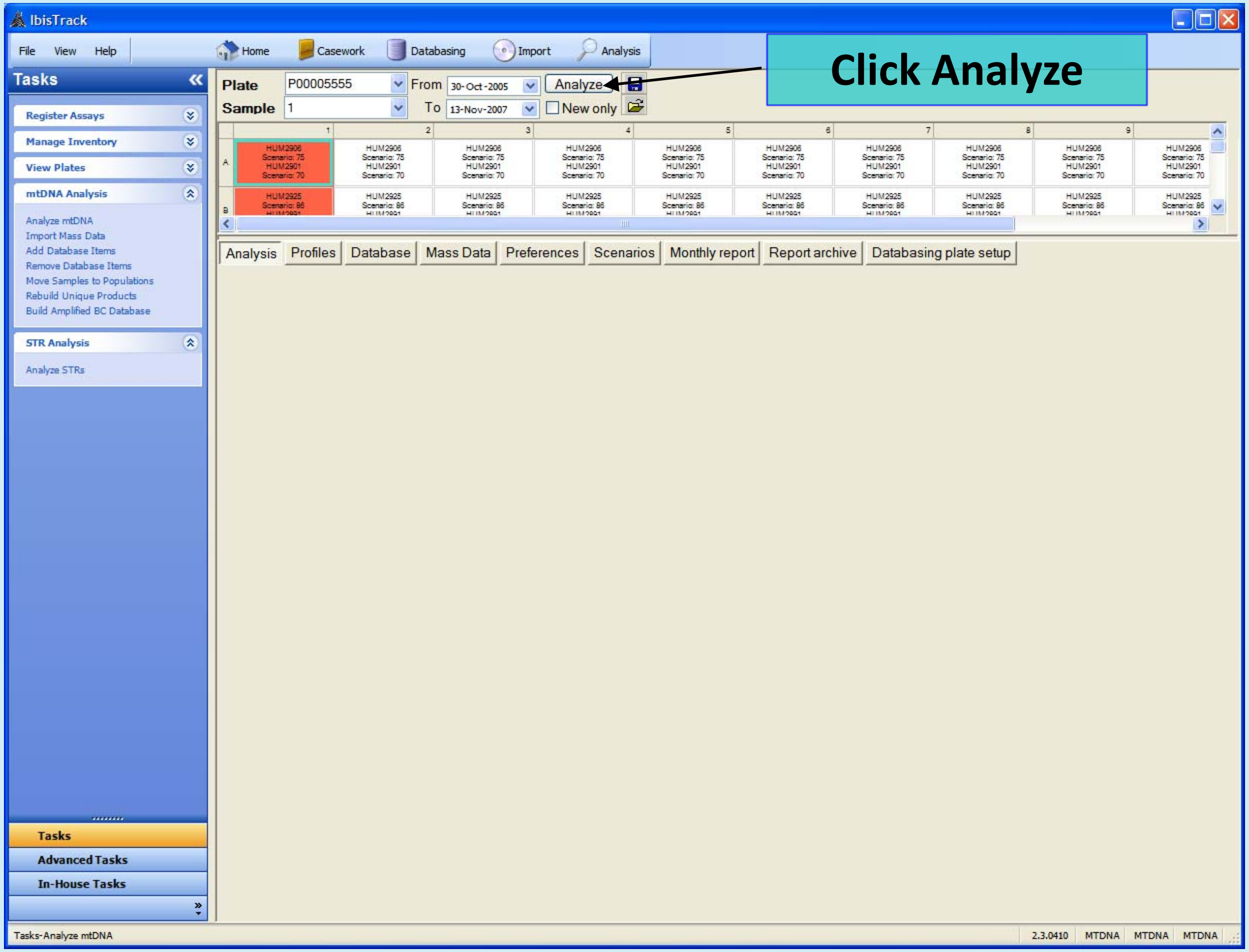
Basic Application Interface – mtDNA



Click Analyze

Plate P00005555 From 30-Oct-2005

Sample 1 To 13-Nov-2007 ☐ New only

	1	2	3	4	5	6	7	8	9
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
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Basic Application Interface – mtDNA

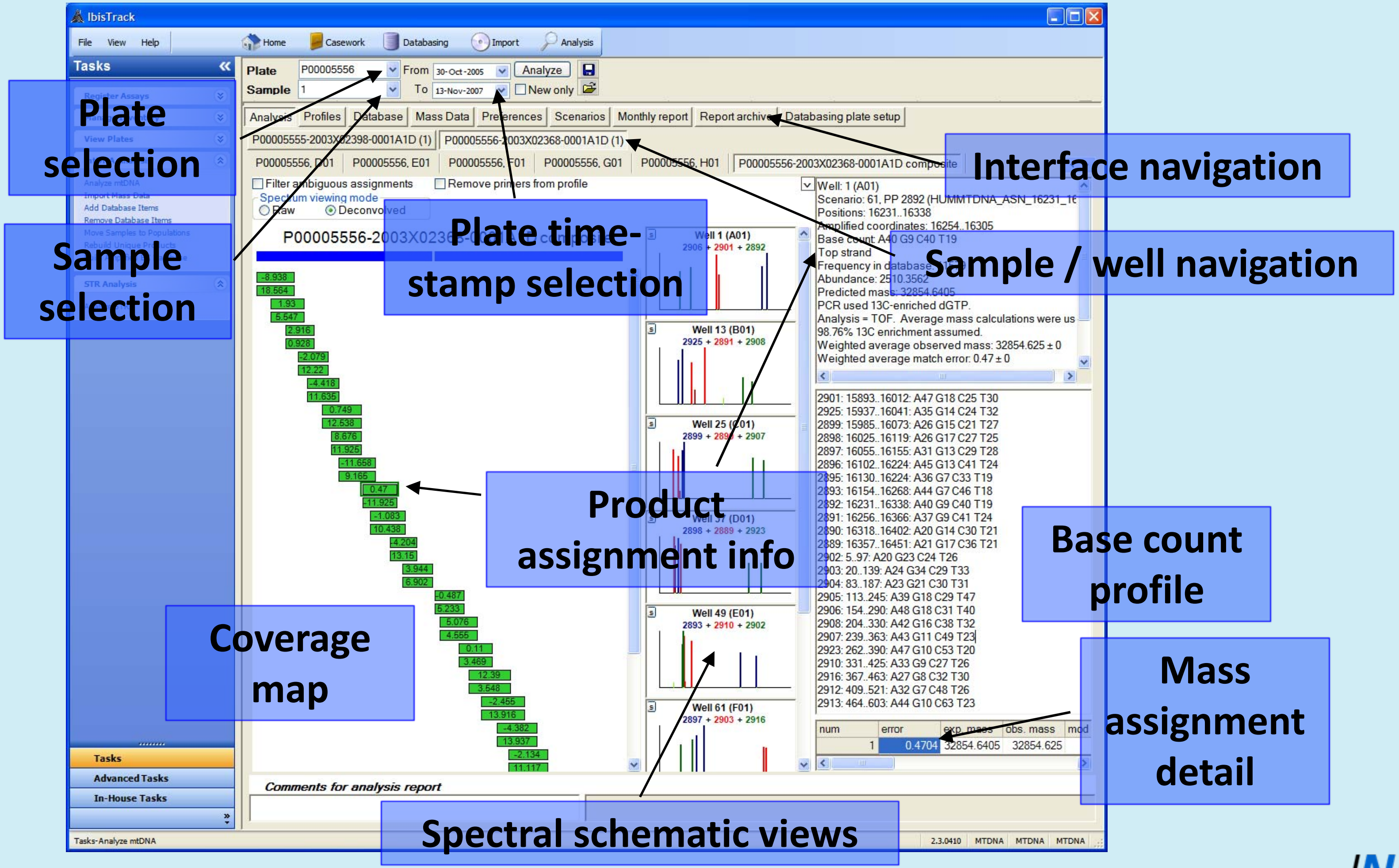

Plate: P00005556

Sample: 1

From: 30-Oct-2005

To: 13-Nov-2007

☐ New only

☐ Filter ambiguous assignments

☐ Remove primers from profile

Spectrum viewing mode: ○ Raw ● Deconvolved

Well: 1 (A01)
Scenario: 61, PP 2892 (HUMMTDNA_ASN_16231_16
Positions: 16231..16338
Amplified coordinates: 16254..16305
Base count: A40 G9 C40 T19
Top strand
Frequency in database: 1579
Abundance: 2510.3562
Predicted mass: 32854.6405
PCR used 13C-enriched dGTP.
Analysis = TOF. Average mass calculations were us
98.76% 13C enrichment assumed.
Weighted average observed mass: 32854.625 ± 0
Weighted average match error: 0.47 ± 0

2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C27 T25
2897: 16055..16155: A31 G13 C29 T28
2896: 16102..16224: A45 G13 C41 T24
2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A20 G23 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C30 T31
2905: 113..245: A39 G18 C29 T47
2906: 154..290: A48 G18 C31 T40
2908: 204..330: A42 G16 C38 T32
2907: 239..363: A43 G11 C49 T23
2923: 262..390: A47 G10 C53 T20
2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2912: 409..521: A32 G7 C48 T26
2913: 464..603: A44 G10 C63 T23

num	error	exp. mass	obs. mass	mod
1	0.4704	32854.6405	32854.625	

Plate selection

Sample selection

Plate time-stamp selection

Interface navigation

Sample / well navigation

Product assignment info

Base count profile

Coverage map

Mass assignment detail

Spectral schematic views

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Basic Application Interface – mtDNA

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Analysis

Analyze and review processed results after running assay plates on the T5000.

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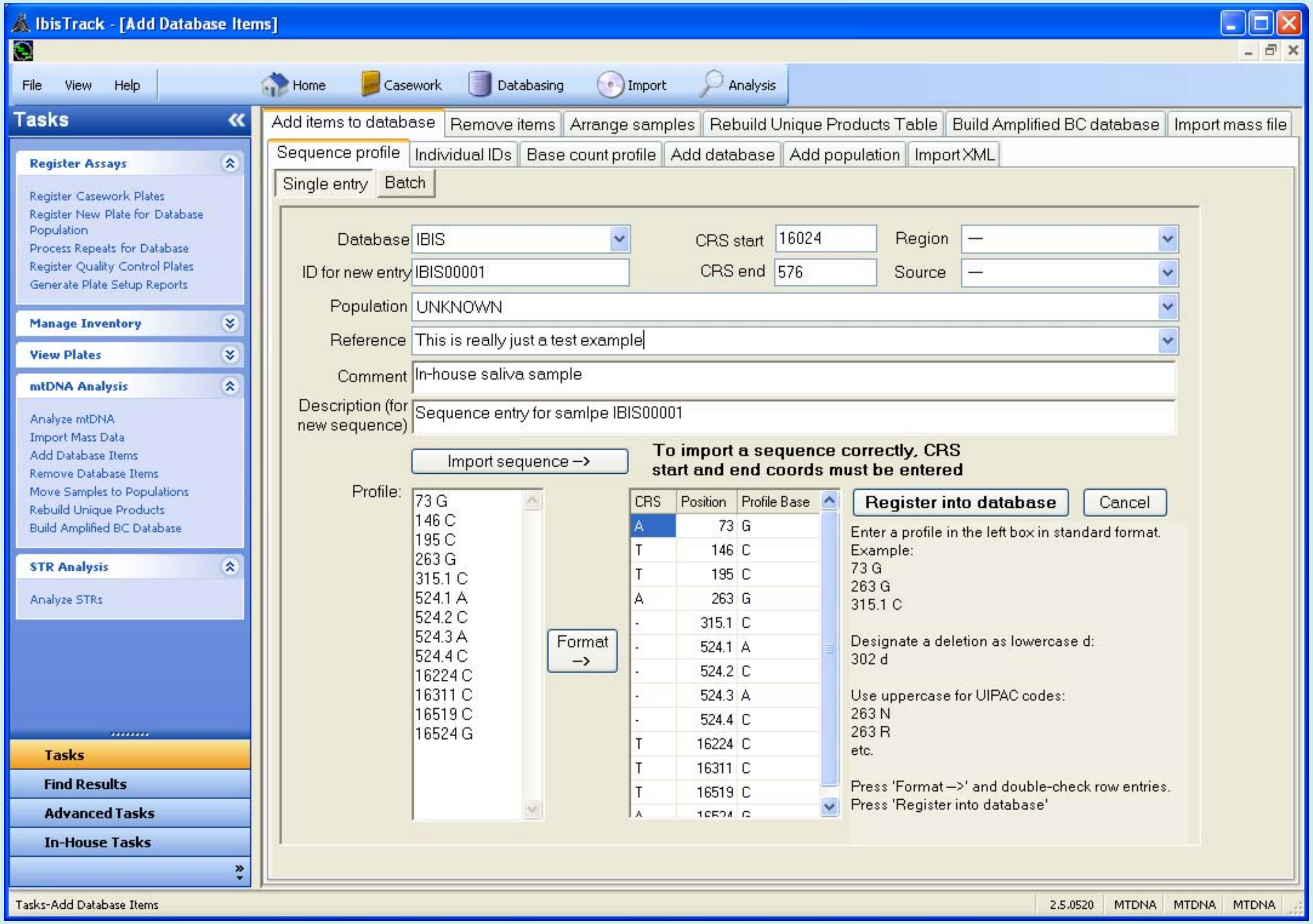
Basic Utilities Interface – mtDNA



Database: IBIS
ID for new entry: IBIS00001
Population: UNKNOWN
Reference: This is really just a test example
Comment: In-house saliva sample
Description (for new sequence): Sequence entry for samlpe IBIS00001
CRS start: 16024
CRS end: 576
Region: —
Source: —

To import a sequence correctly, CRS start and end coords must be entered

Profile:
73 G
146 C
195 C
263 G
315.1 C
524.1 A
524.2 C
524.3 A
524.4 C
16224 C
16311 C
16519 C
16524 G

CRS	Position	Profile Base
A	73	G
T	146	C
T	195	C
A	263	G
-	315.1	C
-	524.1	A
-	524.2	C
-	524.3	A
-	524.4	C
T	16224	C
T	16311	C
T	16519	C
A	16524	G

Enter a profile in the left box in standard format.
Example:
73 G
263 G
315.1 C

Designate a deletion as lowercase d:
302 d

Use uppercase for UIPAC codes:
263 N
263 R
etc.

Press 'Format –>' and double-check row entries.
Press 'Register into database'

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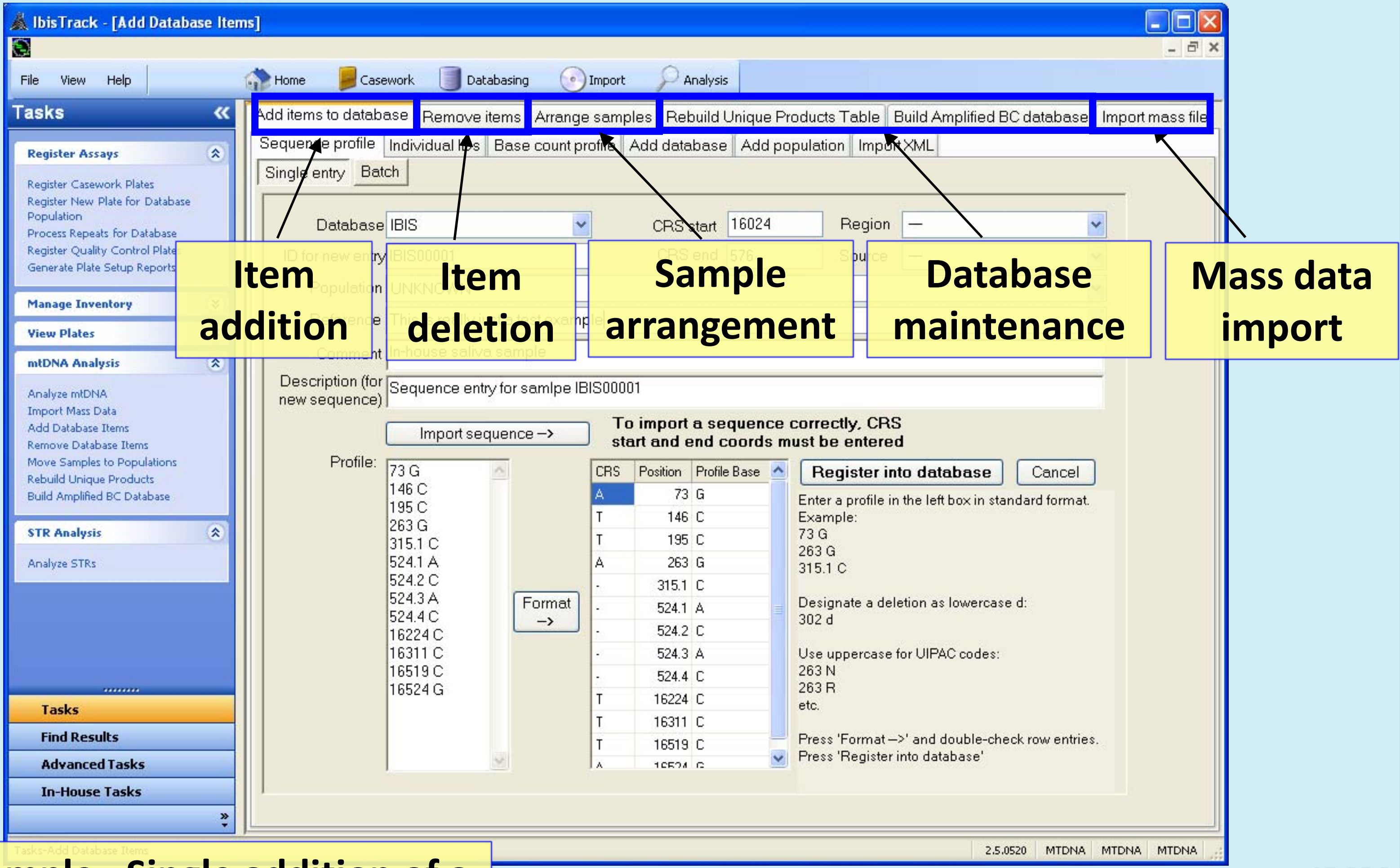
Basic Application Interface – mtDNA



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Region: —

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146 C
195 C
263 G
315.1 C
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524.2 C
524.3 A
524.4 C
16224 C
16311 C
16519 C
16524 G

CRS	Position	Profile Base
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T	146	C
T	195	C
A	263	G
-	315.1	C
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T	16519	C

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315.1 C

Designate a deletion as lowercase d:
302 d

Use uppercase for UIPAC codes:
263 N
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etc.

Press 'Format –>' and double-check row entries.
Press 'Register into database'

Item addition

Item deletion

Sample arrangement

Database maintenance

Mass data import

Example: Single addition of a sequence profile

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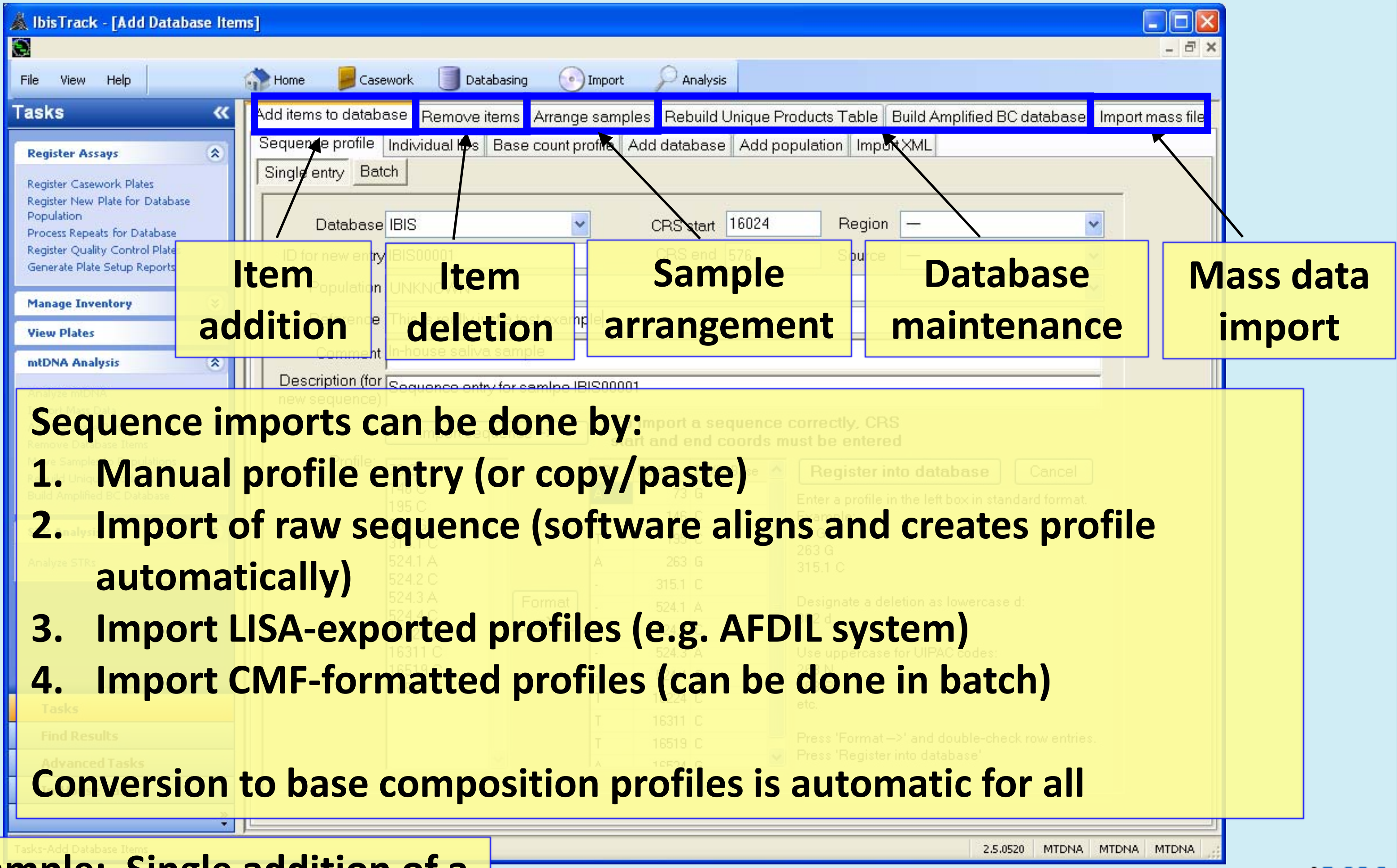
Basic Application Interface – mtDNA



Database: IBIS

CRS start: 16024

Region: —

ID for new entry: IBIS00001

Population: UNKNOWN

Comment: In-house saliva sample

Description (for new sequence): Sequence entry for sample IBIS00001

Item addition

Item deletion

Sample arrangement

Database maintenance

Mass data import

Sequence imports can be done by:

1. **Manual profile entry (or copy/paste)**
2. **Import of raw sequence (software aligns and creates profile automatically)**
3. **Import LISA-exported profiles (e.g. AFDIL system)**
4. **Import CMF-formatted profiles (can be done in batch)**

Conversion to base composition profiles is automatic for all

Example: Single addition of a sequence profile

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Sample Analysis – mtDNA

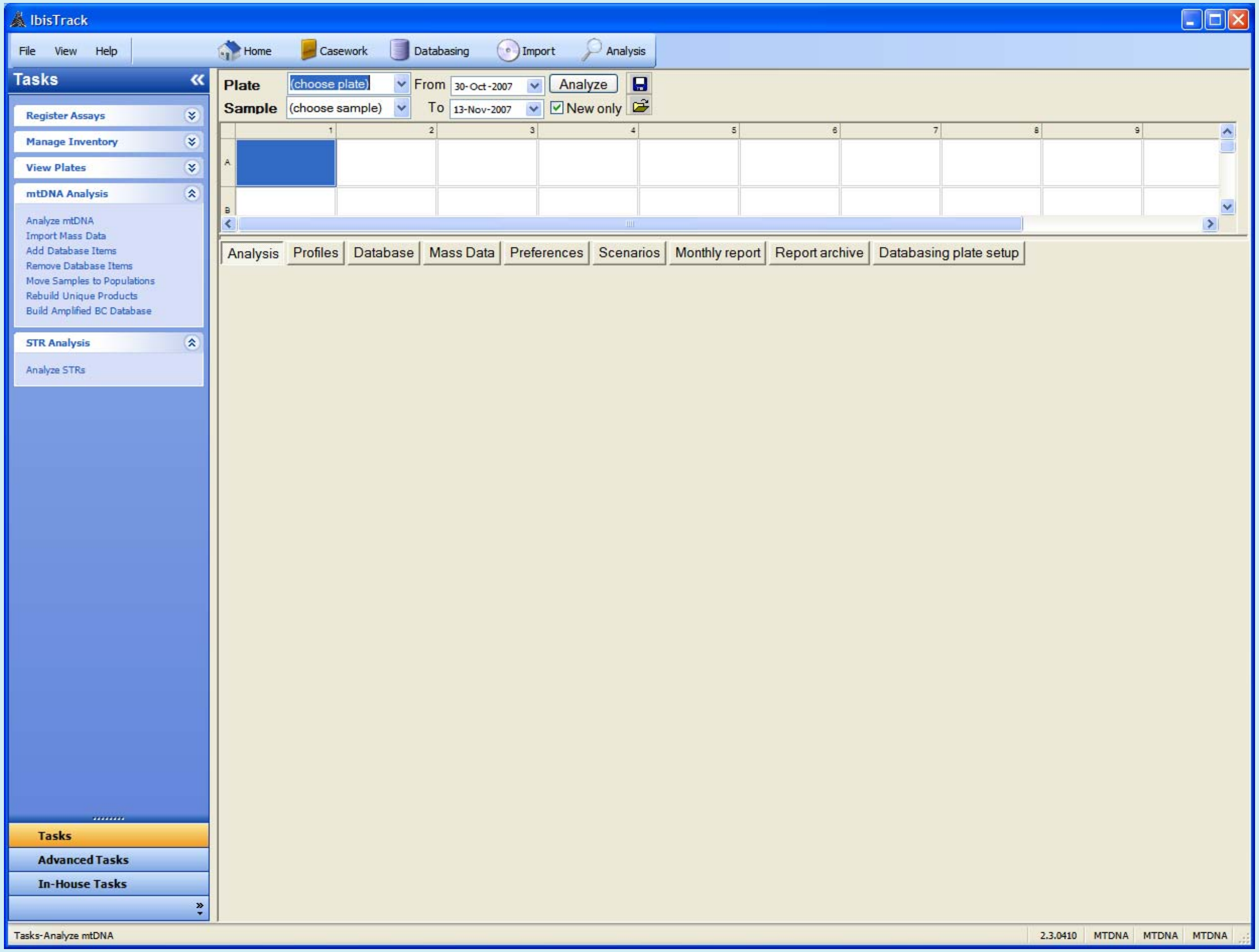

Plate (choose plate) From 30-Oct-2007

Sample (choose sample) To 13-Nov-2007 ☑ New only

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA MTDNA

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Sample Analysis – mtDNA

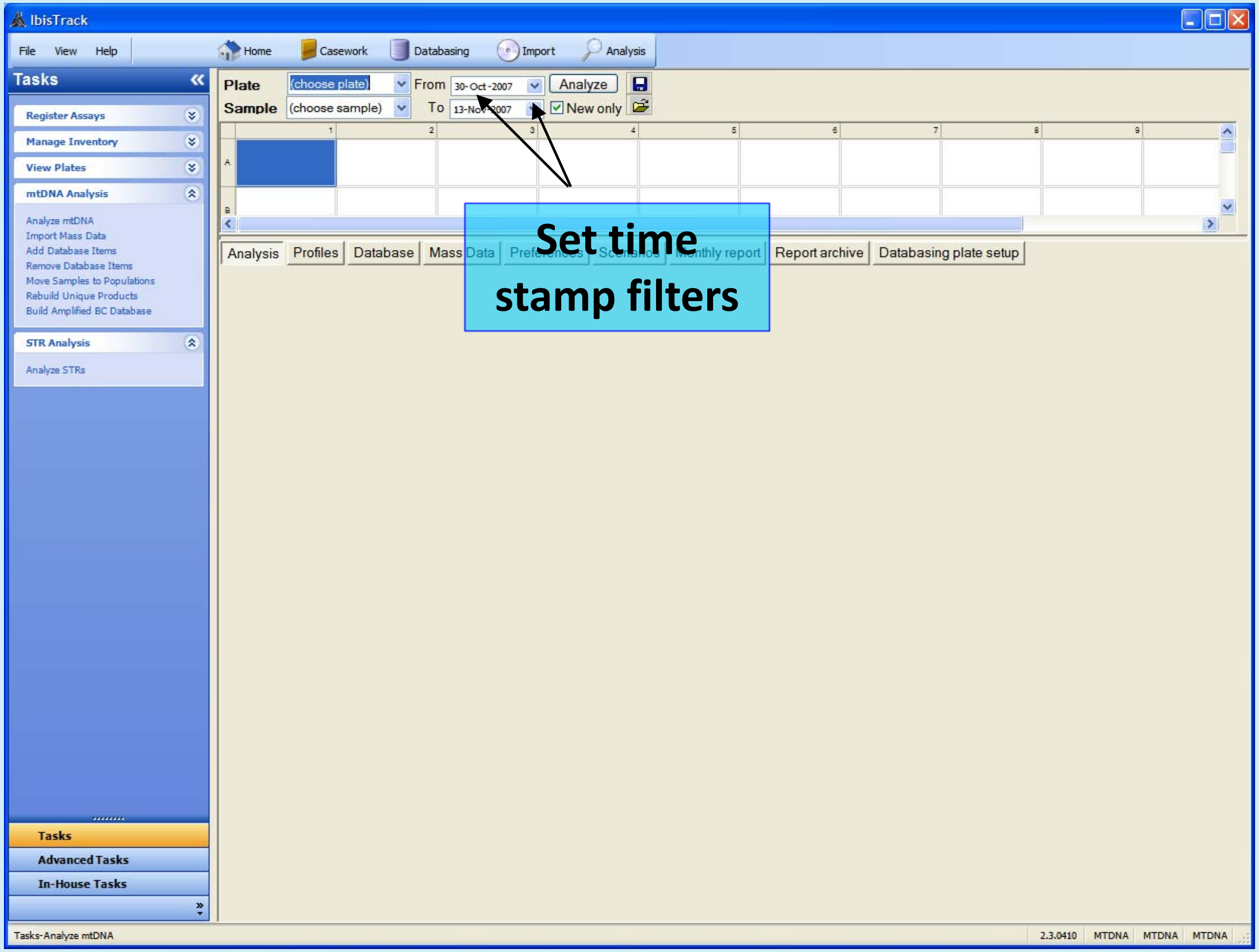

Plate (choose plate)
Sample (choose sample)
From 30-Oct-2007
To 13-Nov-2007
☑ New only

Tasks-Analyze mtDNA | 2.3.0410 | MTDNA | MTDNA | MTDNA

Set time stamp filters

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Sample Analysis – mtDNA

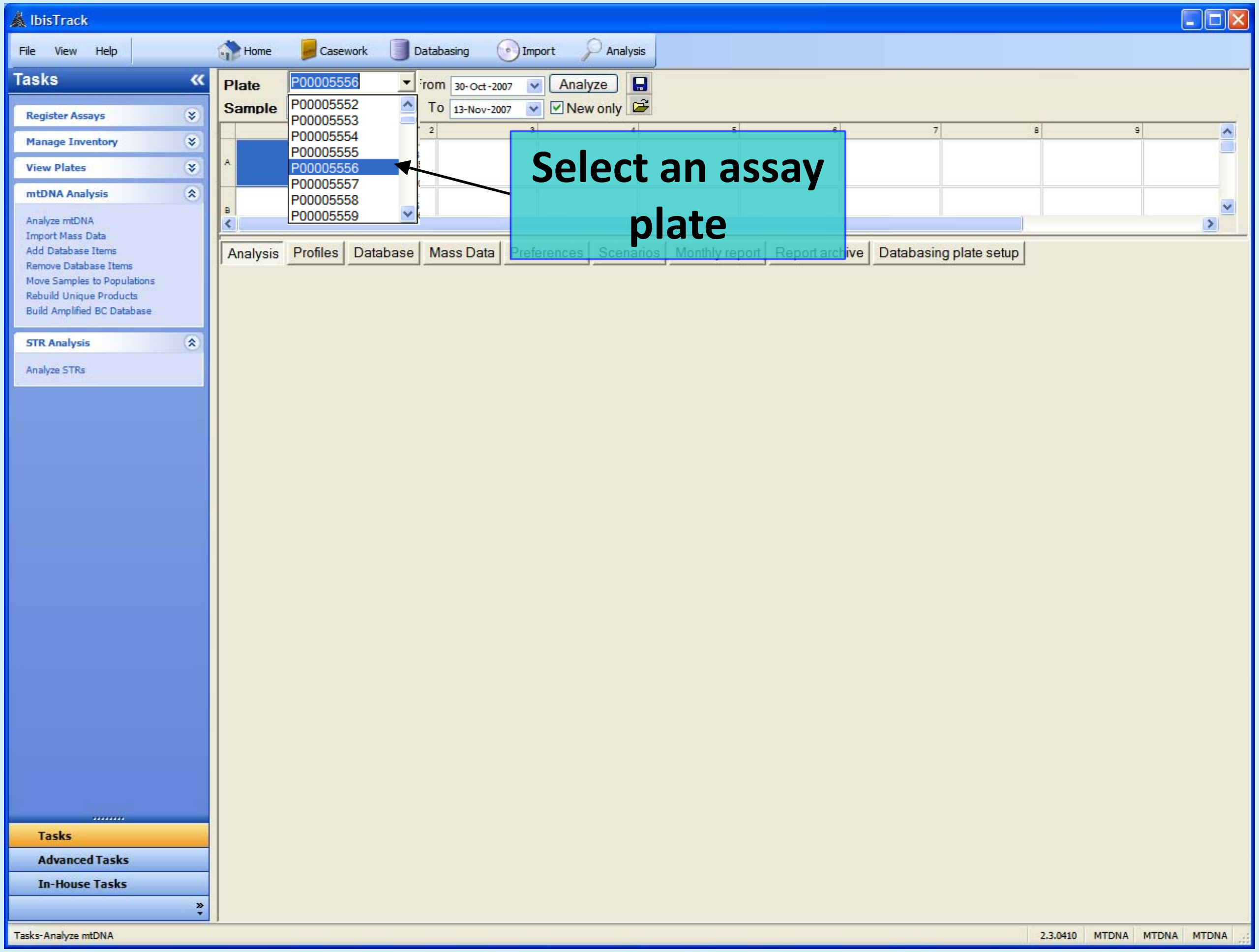

Plate P00005556

Sample

From 30-Oct-2007

To 13-Nov-2007

☑ New only

Select an assay plate

Sample Analysis – mtDNA

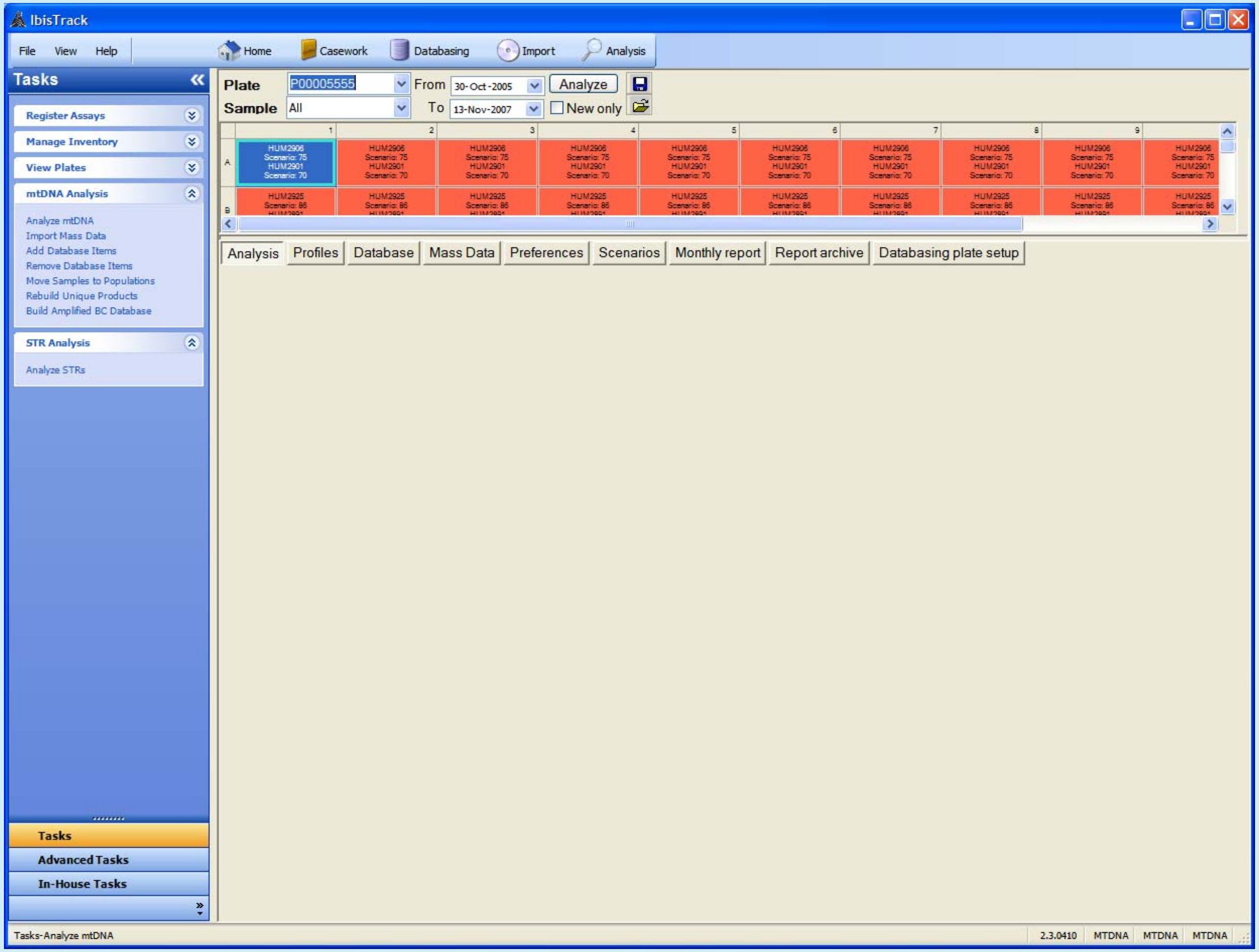

Plate P00005555
Sample All
From 30-Oct-2005
To 13-Nov-2007
☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA | 2.3.0410 | MTDNA | MTDNA | MTDNA

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Sample Analysis – mtDNA

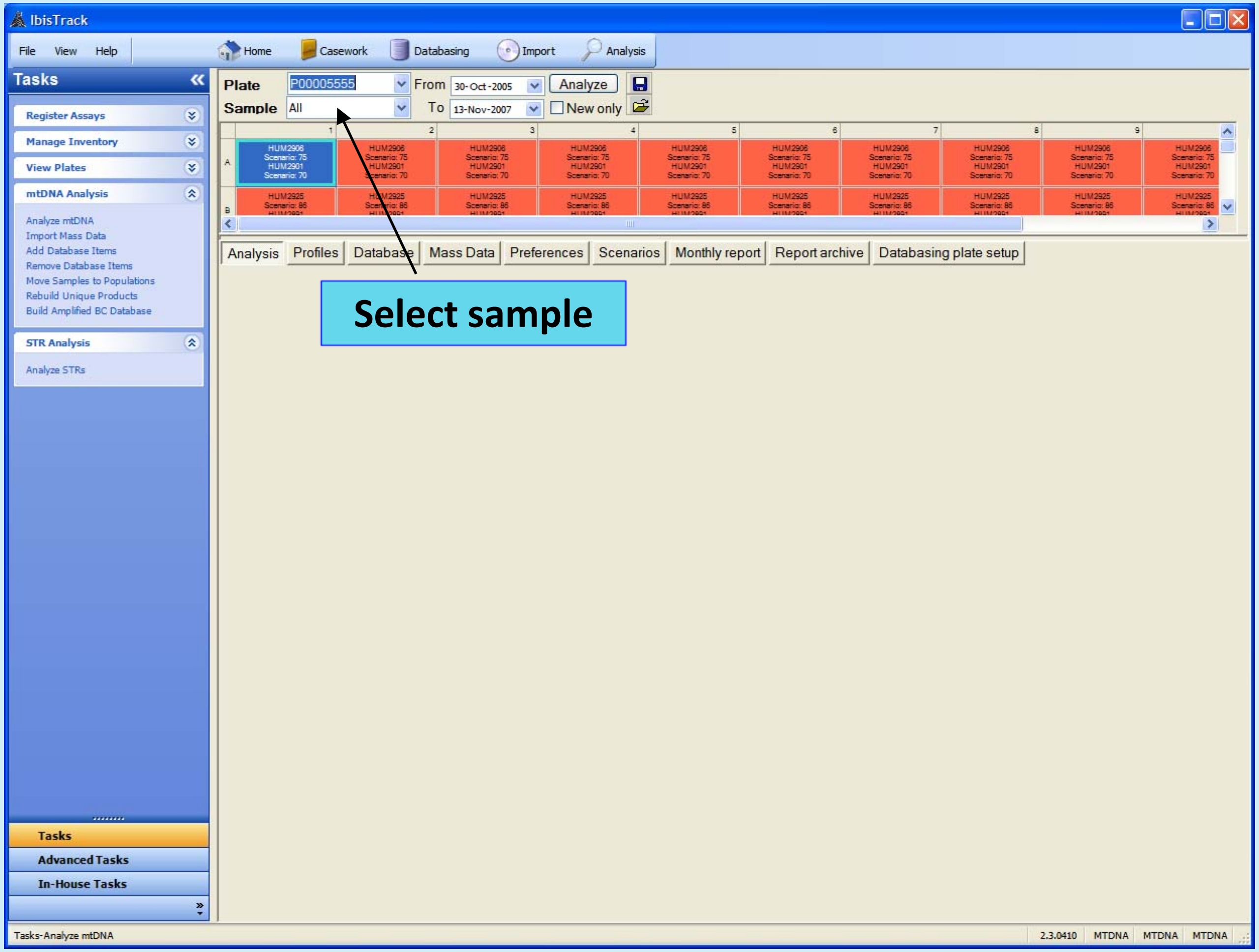

Plate: P00005555
Sample: All
From: 30-Oct-2005
To: 13-Nov-2007
☐ New only

Tasks-Analyze mtDNA | 2.3.0410 | MTDNA | MTDNA | MTDNA

Select sample

Sample Analysis – mtDNA

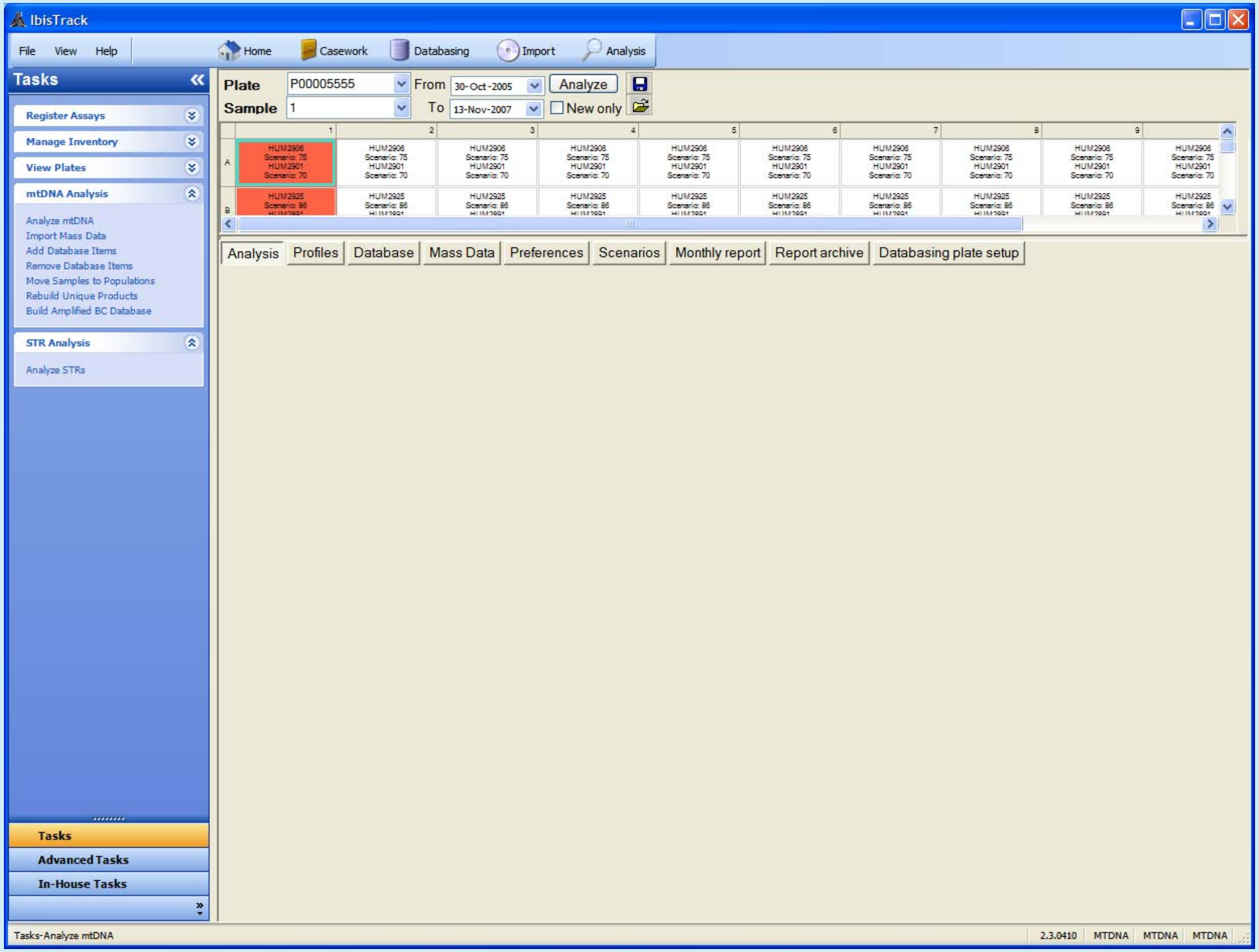

Plate P00005555
Sample 1
From 30-Oct-2005
To 13-Nov-2007
☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA
2.3.0410 MTDNA MTDNA MTDNA

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Sample Analysis – mtDNA

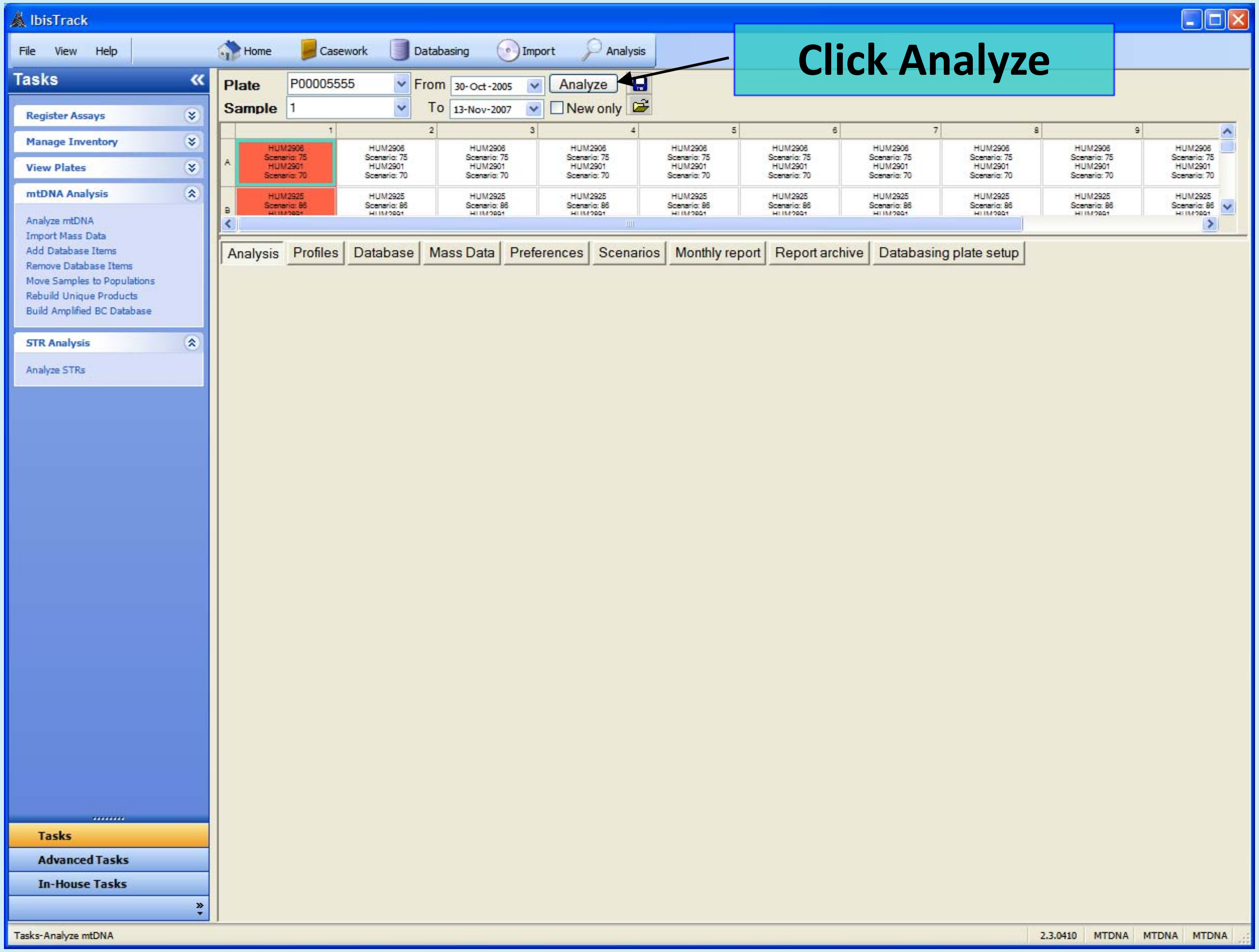

Plate P00005555 From 30-Oct-2005

Sample 1 To 13-Nov-2007 ☐ New only

	1	2	3	4	5	6	7	8	9	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70
B	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86	HUM2925 Scenario: 86

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA MTDNA

Click Analyze

Single Sample Analysis – mtDNA

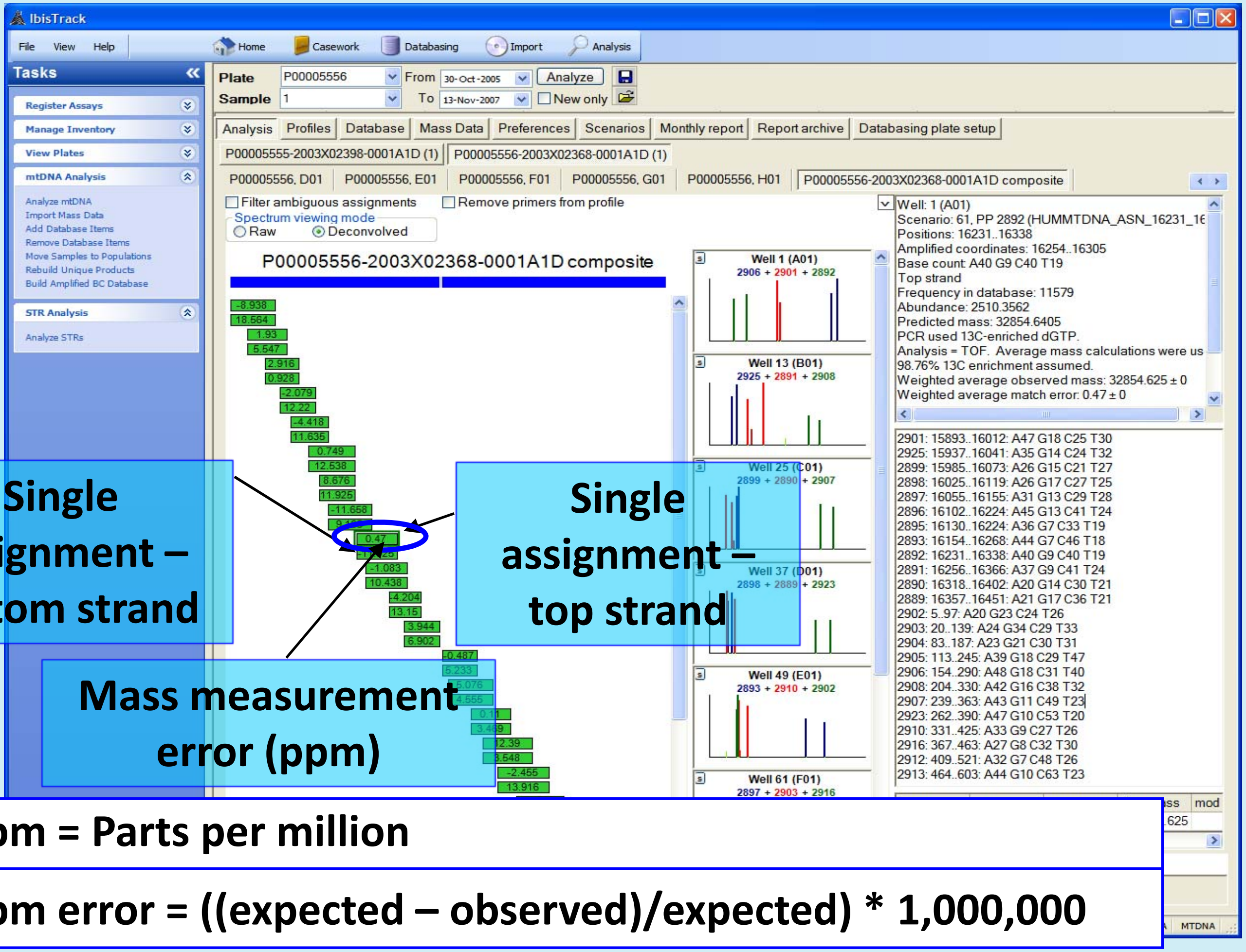

Plate P00005556 From 30-Oct-2005
Sample 1 To 13-Nov-2007

Well: 1 (A01)
Scenario: 61, PP 2892 (HUMMTDNA_ASN_16231_16
Positions: 16231..16338
Amplified coordinates: 16254..16305
Base count: A40 G9 C40 T19
Top strand
Frequency in database: 11579
Abundance: 2510.3562
Predicted mass: 32854.6405
PCR used 13C-enriched dGTP.
Analysis = TOF. Average mass calculations were us
98.76% 13C enrichment assumed.
Weighted average observed mass: 32854.625 ± 0
Weighted average match error: 0.47 ± 0

2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C27 T25
2897: 16055..16155: A31 G13 C29 T28
2896: 16102..16224: A45 G13 C41 T24
2895: 16130..16224: A36 G7 C33 T19
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A20 G23 C24 T26
2903: 20..139: A24 G34 C29 T33
2904: 83..187: A23 G21 C30 T31
2905: 113..245: A39 G18 C29 T47
2906: 154..290: A48 G18 C31 T40
2908: 204..330: A42 G16 C38 T32
2907: 239..363: A43 G11 C49 T23
2923: 262..390: A47 G10 C53 T20
2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2912: 409..521: A32 G7 C48 T26
2913: 464..603: A44 G10 C63 T23

Single assignment – bottom strand

Single assignment – top strand

Mass measurement error (ppm)

ppm = Parts per million

ppm error = ((expected – observed)/expected) * 1,000,000

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Single Sample Analysis – mtDNA

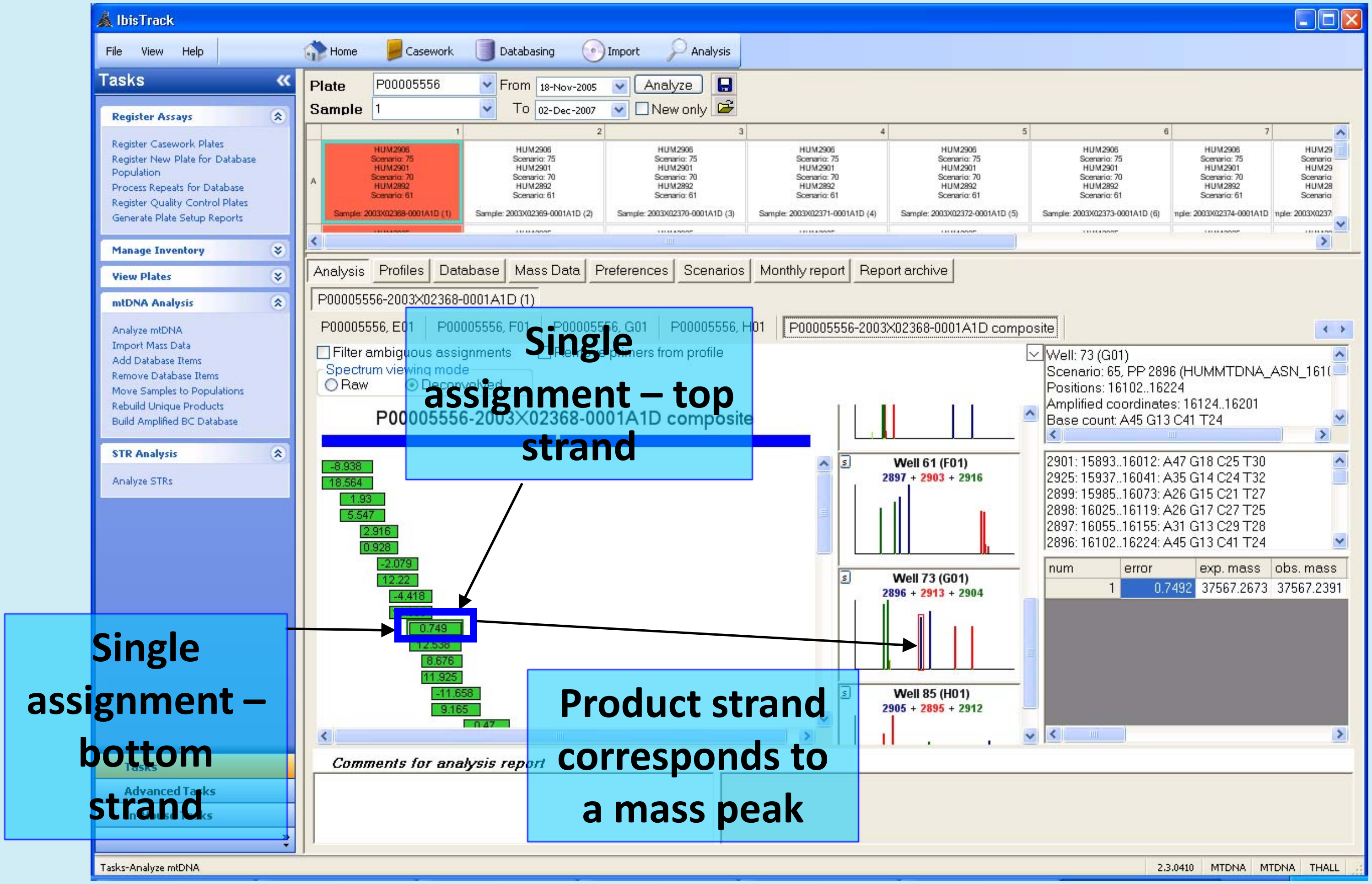

Plate P00005556 From 18-Nov-2005
Sample 1 To 02-Dec-2007
☐ New only

	1	2	3	4	5	6	7
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02368-0001A1D (1)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02369-0001A1D (2)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02370-0001A1D (3)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02371-0001A1D (4)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02372-0001A1D (5)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 Sample: 2003X02373-0001A1D (6)	HUM2906 Scenario: 75 HUM2901 Scenario: 70 HUM2892 Scenario: 61 nple: 2003X02374-0001A1D

P00005556-2003X02368-0001A1D (1)

P00005556, E01 | P00005556, F01 | P00005556, G01 | P00005556, H01 | P00005556-2003X02368-0001A1D composite

☐ Filter ambiguous assignments ☐ Remove primers from profile

Spectrum viewing mode ○ Raw ● Deconvoluted

P00005556-2003X02368-0001A1D composite

-8.938
18.564
1.93
5.547
2.916
0.928
-2.079
12.22
-4.418
0.749
12.538
8.676
11.925
-11.658
9.165
0.47

Well 61 (F01)
2897 + 2903 + 2916

Well 73 (G01)
2896 + 2913 + 2904

Well 85 (H01)
2905 + 2895 + 2912

Well: 73 (G01)
Scenario: 65, PP 2896 (HUMMTDNA_ASN_161(
Positions: 16102..16224
Amplified coordinates: 16124..16201
Base count: A45 G13 C41 T24

2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C27 T25
2897: 16055..16155: A31 G13 C29 T28
2896: 16102..16224: A45 G13 C41 T24

num	error	exp. mass	obs. mass
1	0.7492	37567.2673	37567.2391

Single assignment – top strand

Single assignment – bottom strand

Product strand corresponds to a mass peak

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Single Sample Analysis – mtDNA

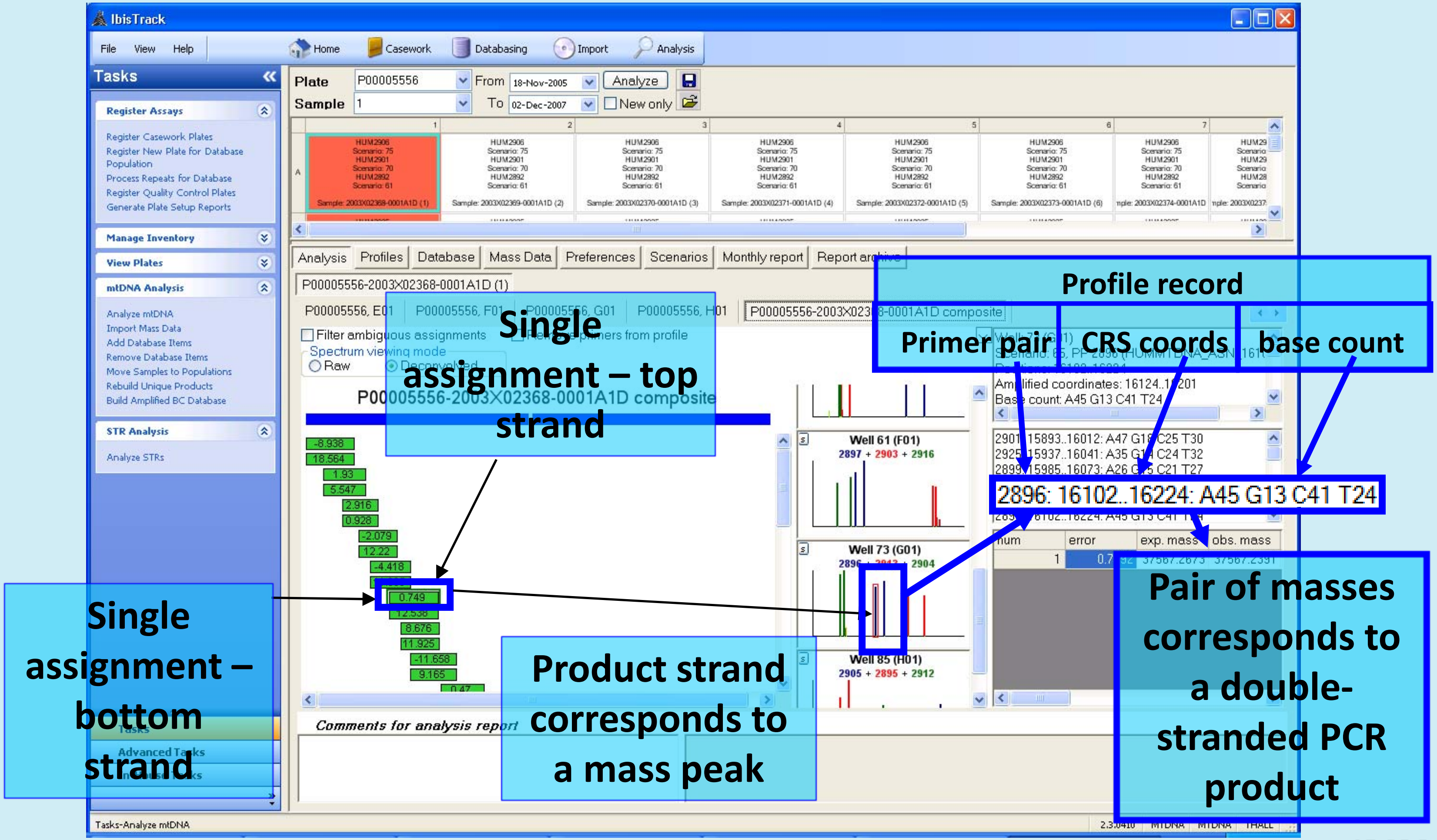


Plate P00005556 From 18-Nov-2005

Sample 1 To 02-Dec-2007 ☐ New only

P00005556-2003X02368-0001A1D (1)

P00005556, E01 P00005556, F01 P00005556, G01 P00005556, H01 P00005556-2003X02368-0001A1D composite

☐ Filter ambiguous assignments

Spectrum viewing mode ○ Raw ● Deconvolved

P00005556-2003X02368-0001A1D composite

Well 61 (F01) 2897 + 2903 + 2916

Well 73 (G01) 2896 + 2913 + 2904

Well 85 (H01) 2905 + 2895 + 2912

Amplified coordinates: 16124..16201

Base count: A45 G13 C41 T24

2901: 15893..16012: A47 G18 C25 T30

2925: 15937..16041: A35 G14 C24 T32

2899: 15985..16073: A26 G15 C21 T27

2896: 16102..16224: A45 G13 C41 T24

Profile record

Primer pair

CRS coords

base count

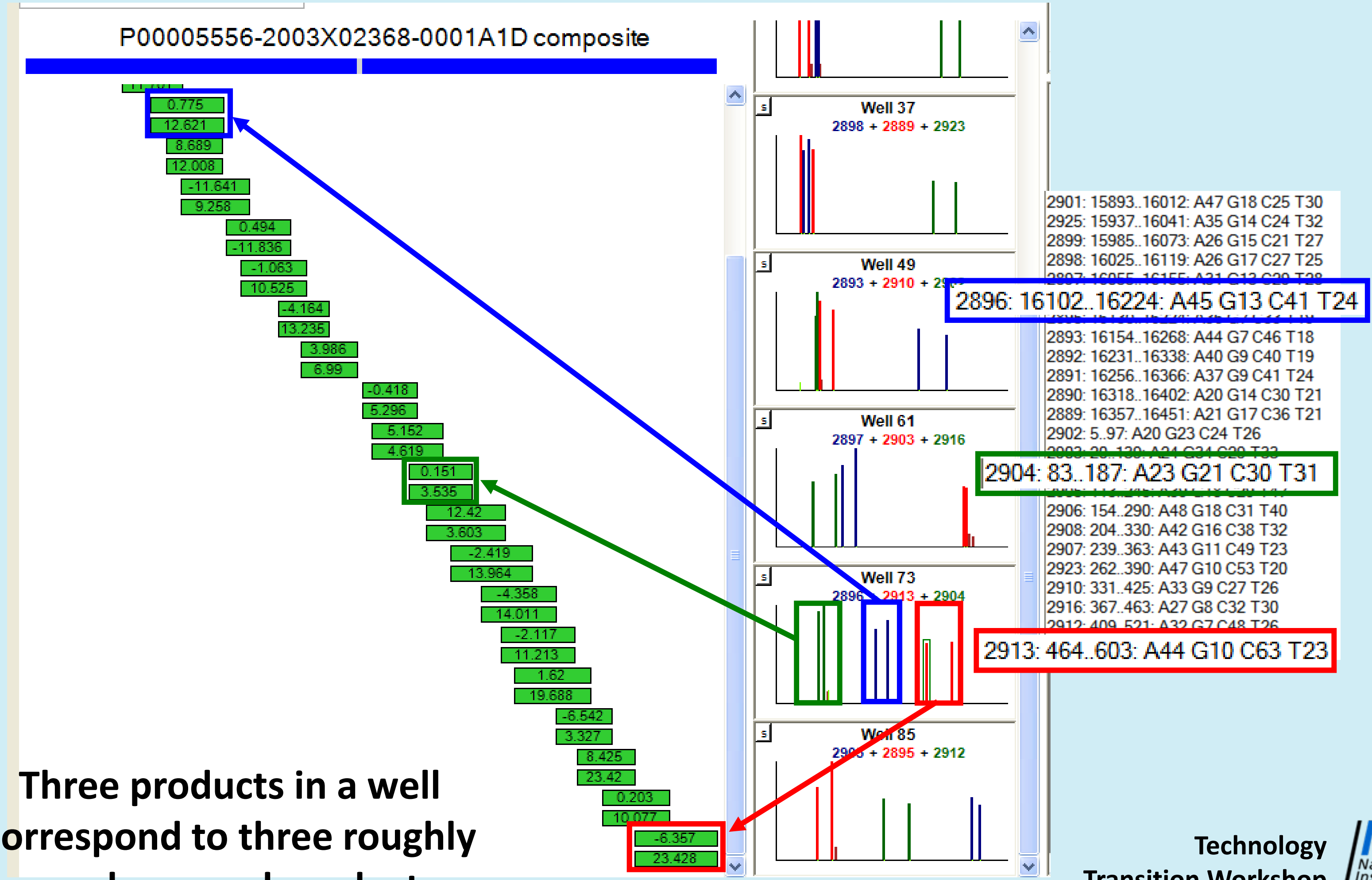
Single assignment – top strand

Single assignment – bottom strand

Product strand corresponds to a mass peak

Pair of masses corresponds to a double-stranded PCR product

Single Sample Analysis – mtDNA



2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C27 T25
2896: 16102..16224: A45 G13 C41 T24
2893: 16154..16268: A44 G7 C46 T18
2892: 16231..16338: A40 G9 C40 T19
2891: 16256..16366: A37 G9 C41 T24
2890: 16318..16402: A20 G14 C30 T21
2889: 16357..16451: A21 G17 C36 T21
2902: 5..97: A20 G23 C24 T26
2904: 83..187: A23 G21 C30 T31
2906: 154..290: A48 G18 C31 T40
2908: 204..330: A42 G16 C38 T32
2907: 239..363: A43 G11 C49 T23
2923: 262..390: A47 G10 C53 T20
2910: 331..425: A33 G9 C27 T26
2916: 367..463: A27 G8 C32 T30
2913: 464..603: A44 G10 C63 T23

Three products in a well correspond to three roughly evenly-spaced products

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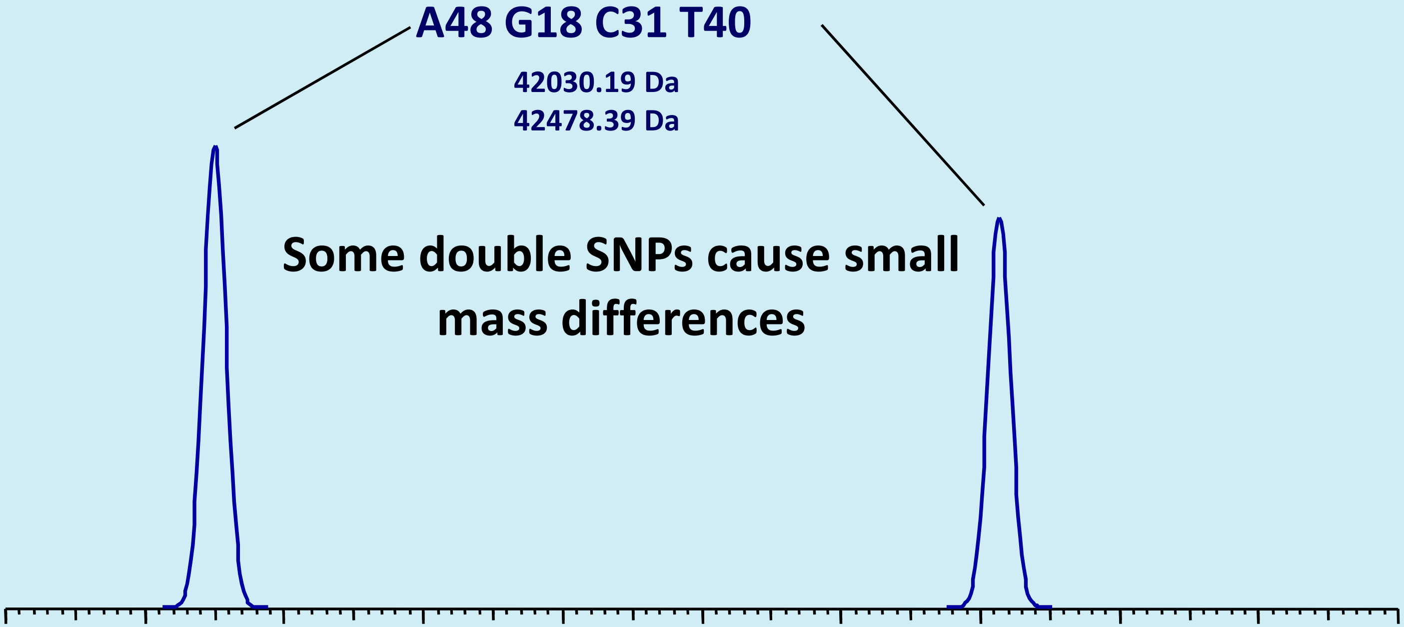
Use of a Mass Tag

High mass precision and mass tag combine to provide unambiguous base compositions in routine operation



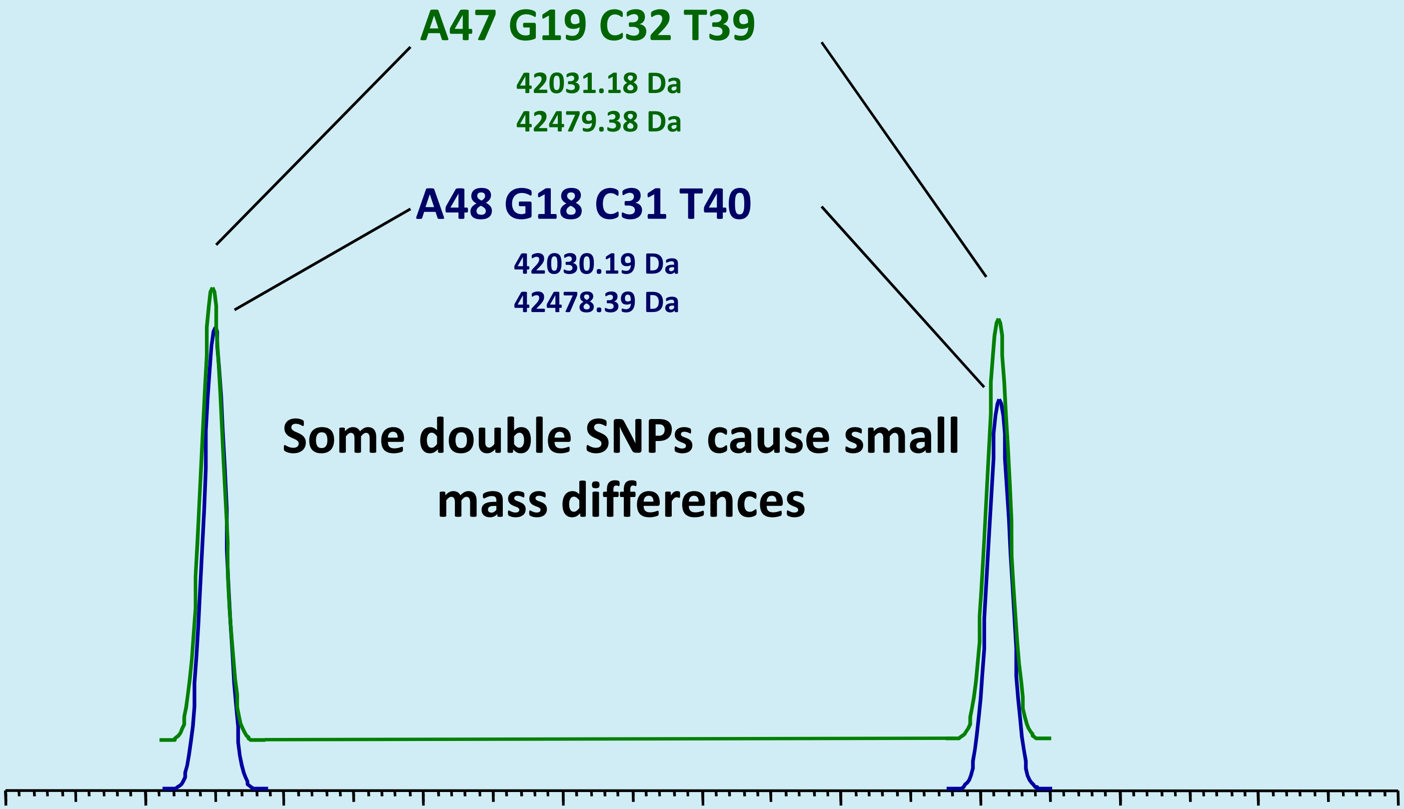
A48 G18 C31 T40

42030.19 Da
42478.39 Da

Some double SNPs cause small mass differences

Use of a Mass Tag

<u>Without mass tag:</u> Product strands differ by 1 Da for two products that differ by a G→A and C→T SNP at the same time



A47 G19 C32 T39

42031.18 Da
42479.38 Da

A48 G18 C31 T40

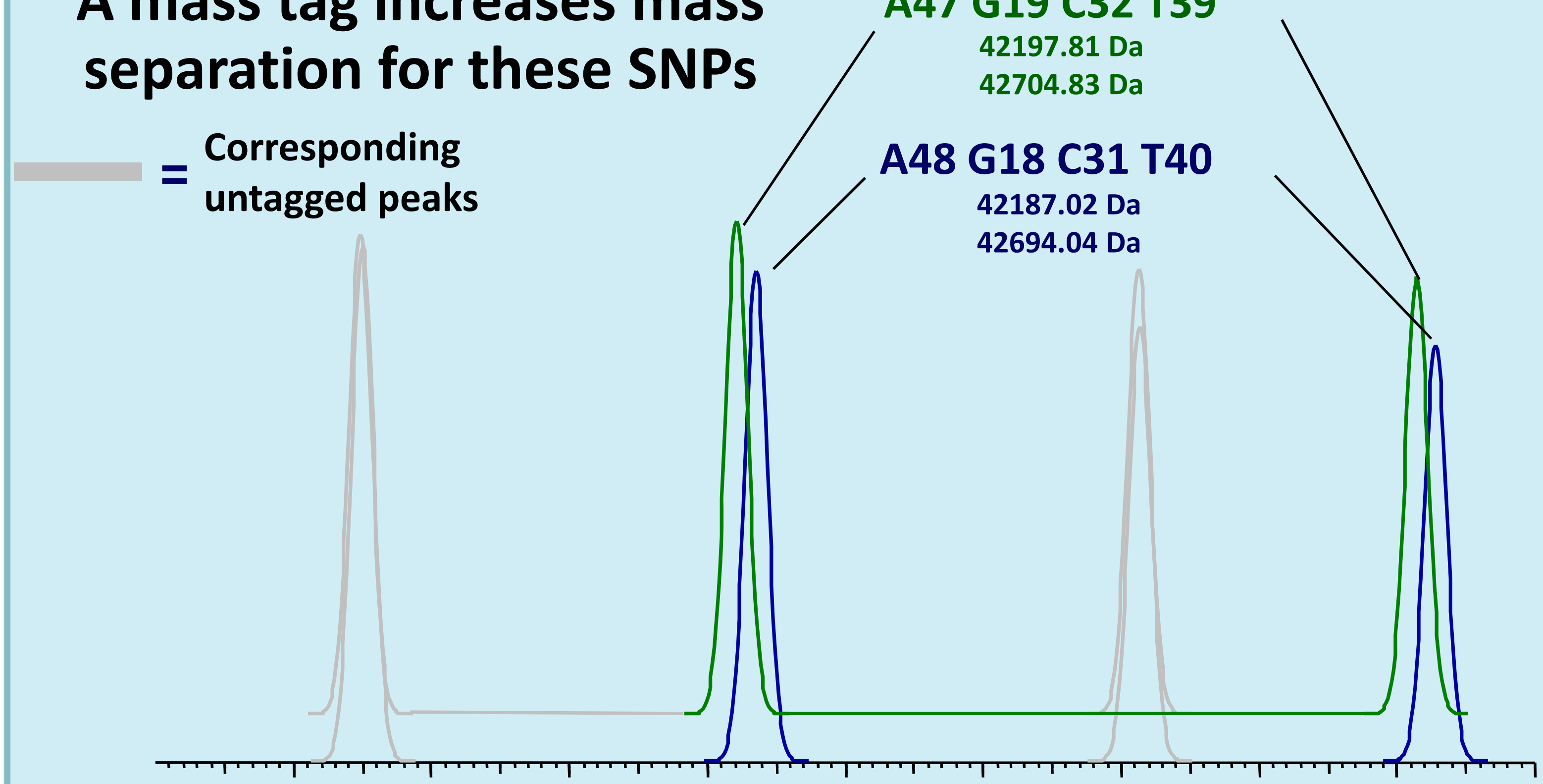
42030.19 Da
42478.39 Da

Some double SNPs cause small mass differences

Use of a Mass Tag

__With mass tag:__ With ^{13}C-dGTP, the mass separation increases to ~10 Da for each strand; this is about 300 ppm (we generally see errors ~10 ppm)

A mass tag increases mass separation for these SNPs



▬ = Corresponding untagged peaks

A47 G19 C32 T39
42197.81 Da
42704.83 Da

A48 G18 C31 T40
42187.02 Da
42694.04 Da

Real Data with Mass Tag

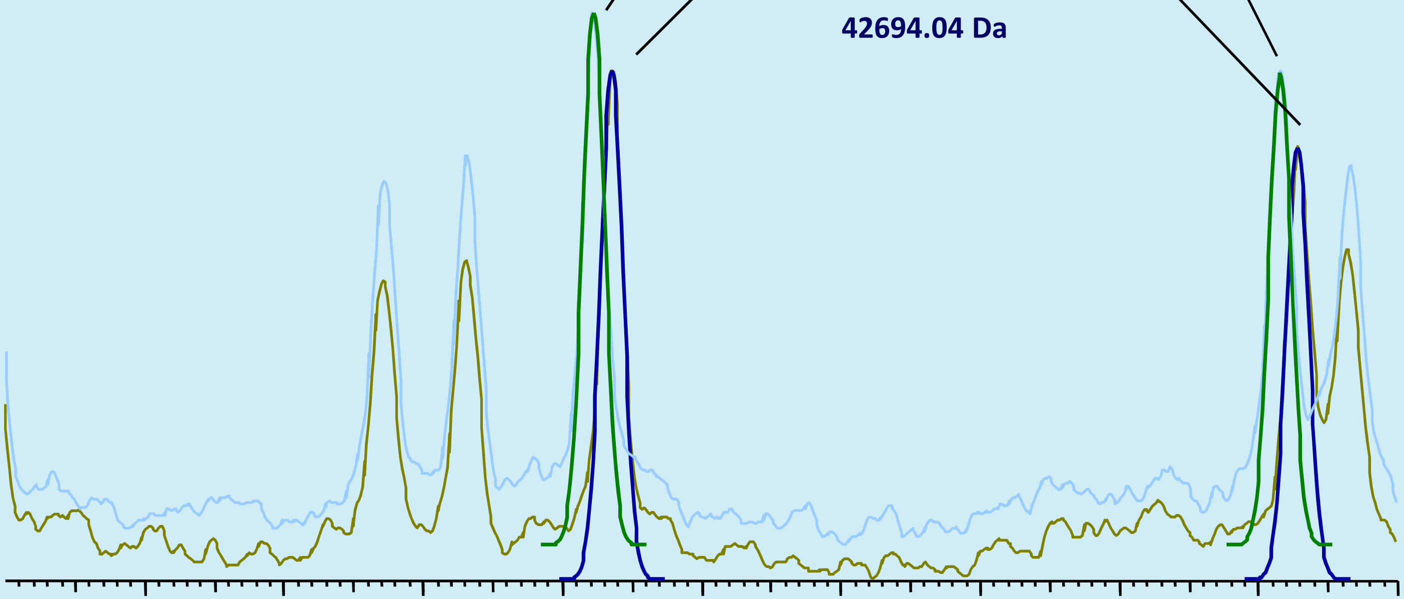
Primer pair 2906 product from two representative samples (data for primer pair 2906 are overlayed)

Well A05: P00005582

A47 G19 C32 T39
42197.81 Da
42704.83 Da

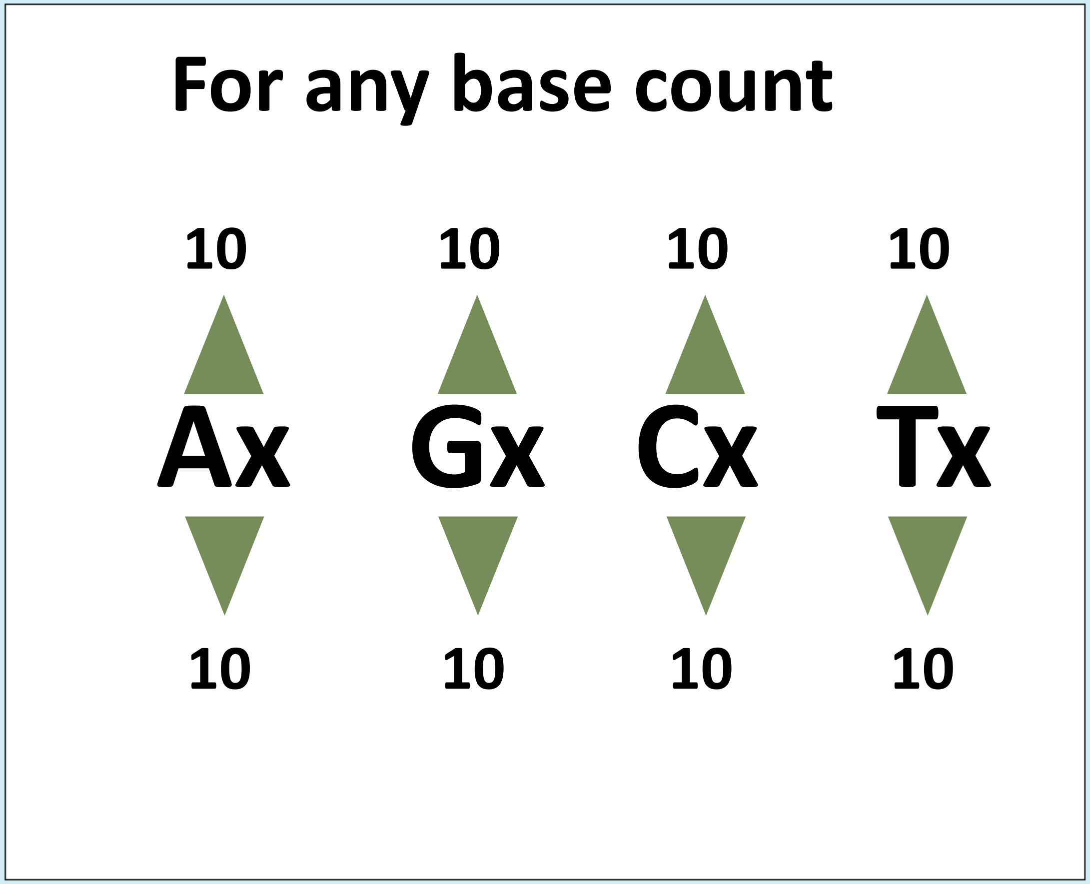
Well A01: P00005553

A48 G18 C31 T40
42187.02 Da
42694.04 Da



What Does Mass Tagging Mean?

For <u>all</u> combinations of base differences plus or minus 10 A, G, C, T …



For any base count

10	10	10	10
▲	▲	▲	▲
Ax	**Gx**	**Cx**	**Tx**
▼	▼	▼	▼
10	10	10	10

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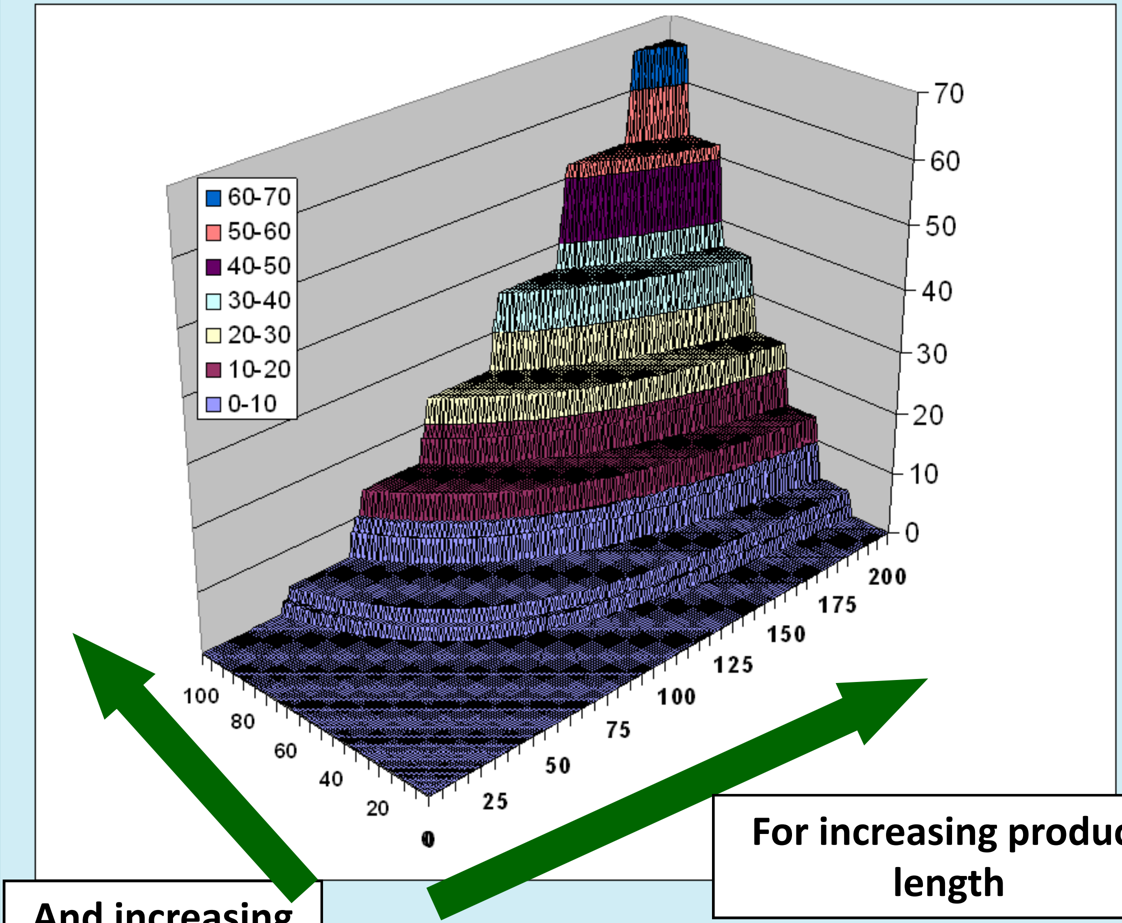
What Does Mass Tagging Mean?



For increasing product length

And increasing measurement error

For all combinations of base differences plus or minus 10 A, G, C, T …

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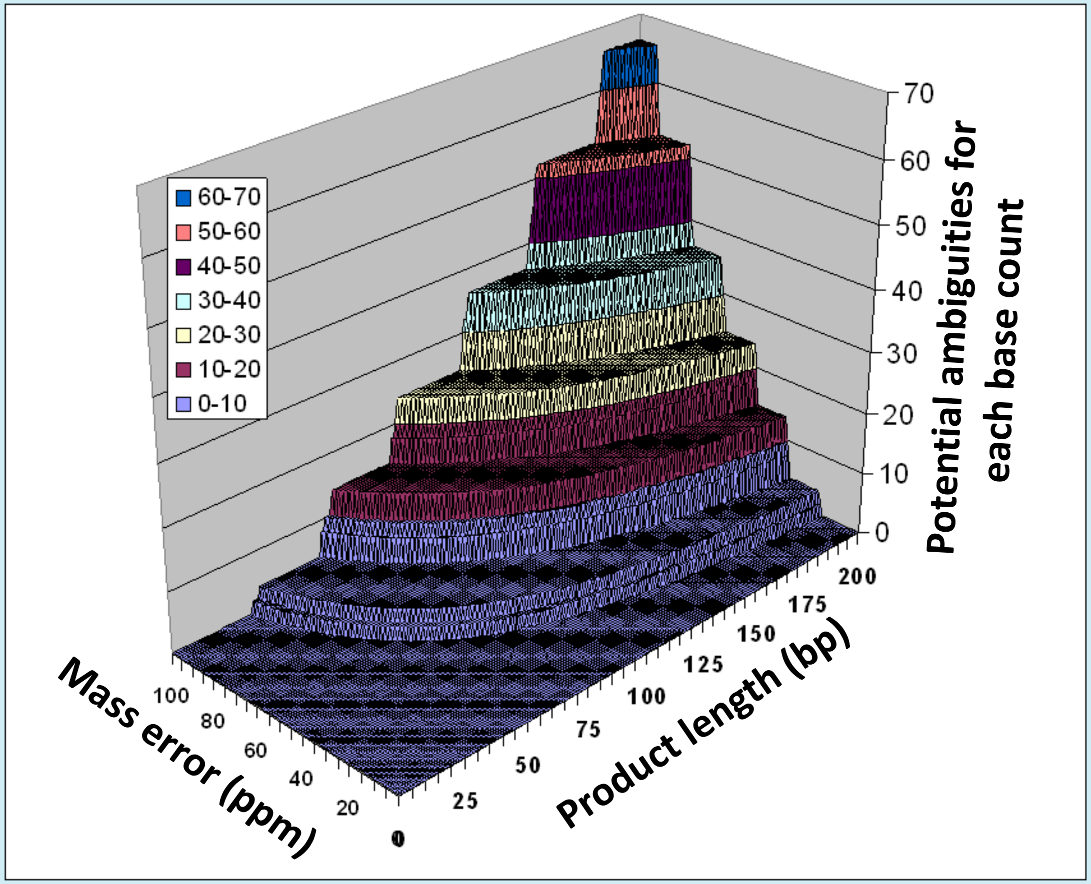
What Does Mass Tagging Mean?

Potential assignment ambiguities increase dramatically with measurement error and product size



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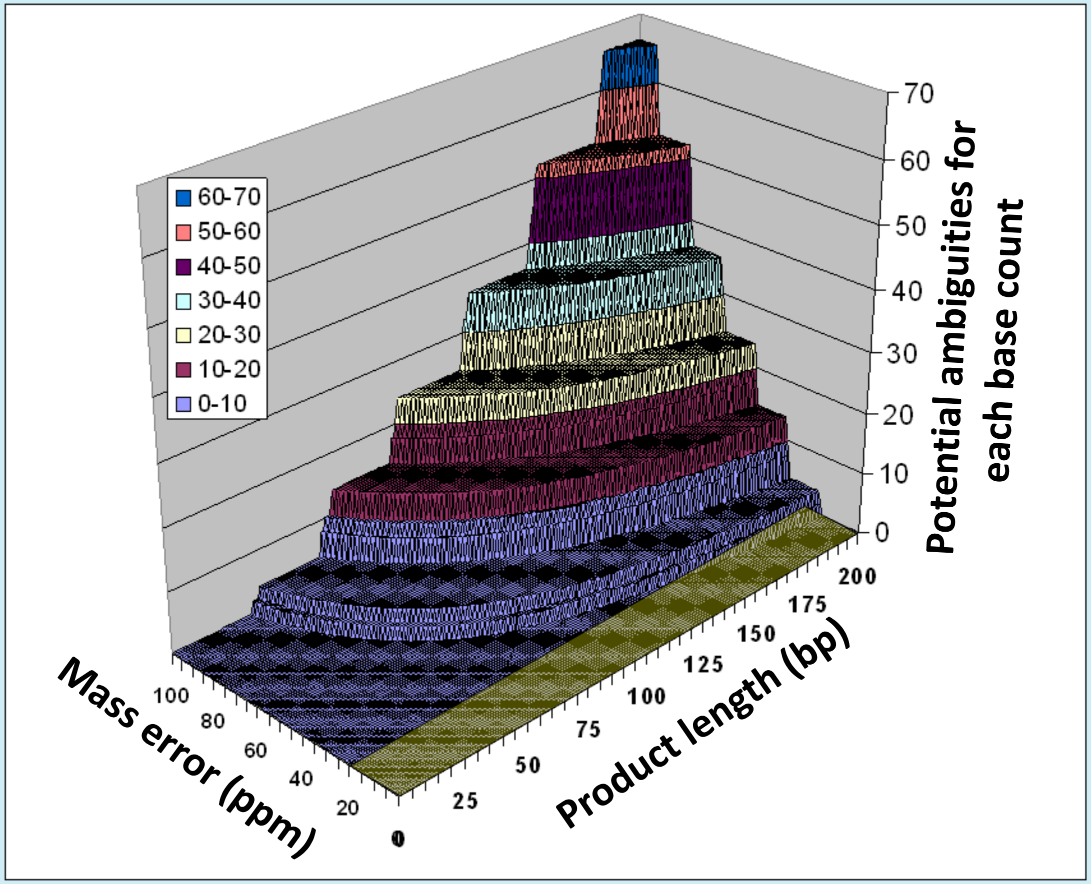
What Does Mass Tagging Mean?

Ambiguous assignments not a problem in our normal working range of mass measurement

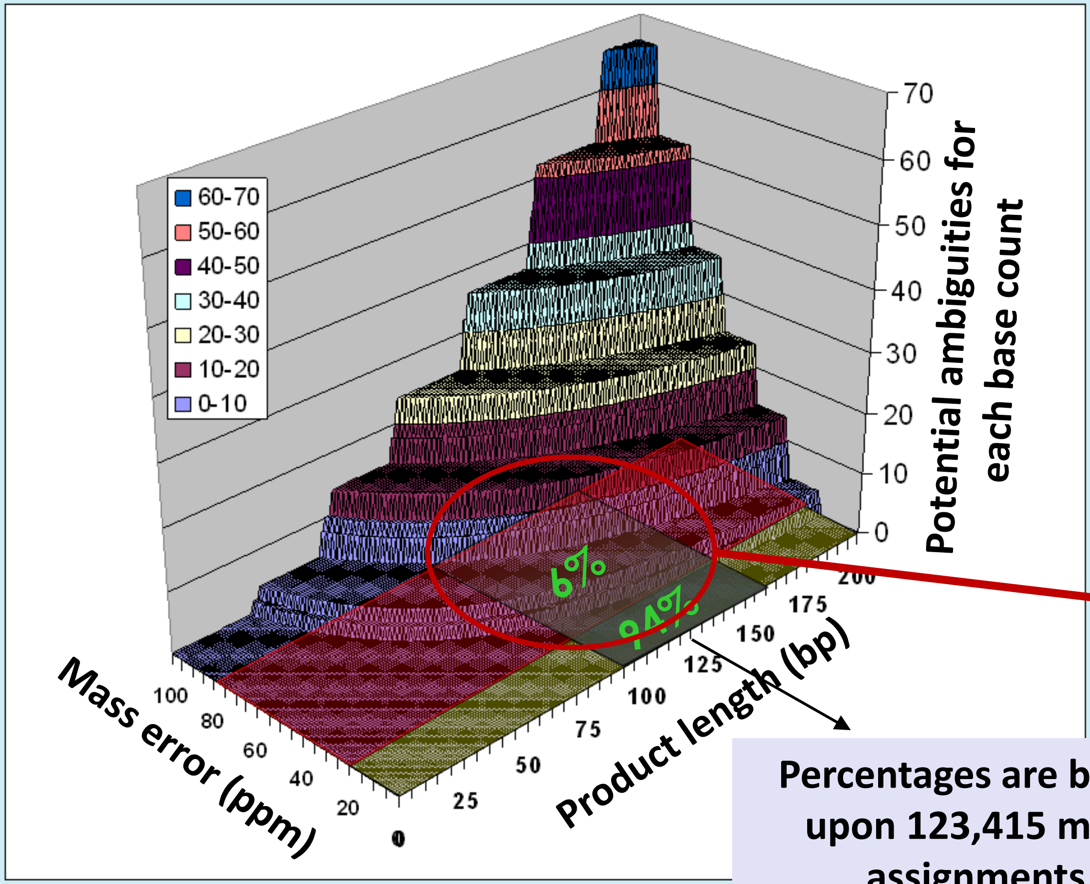


🟨 **Working range for TOF (<20 ppm)**

What Does Mass Tagging Mean?

Most assignments would be fine with natural nucleotides.

Some assignments fall outside of “normal” range.



🟨 **Working range for TOF (<20 ppm)**

Which 6% of measurements are a problem?

Percentages are based upon 123,415 mass assignments

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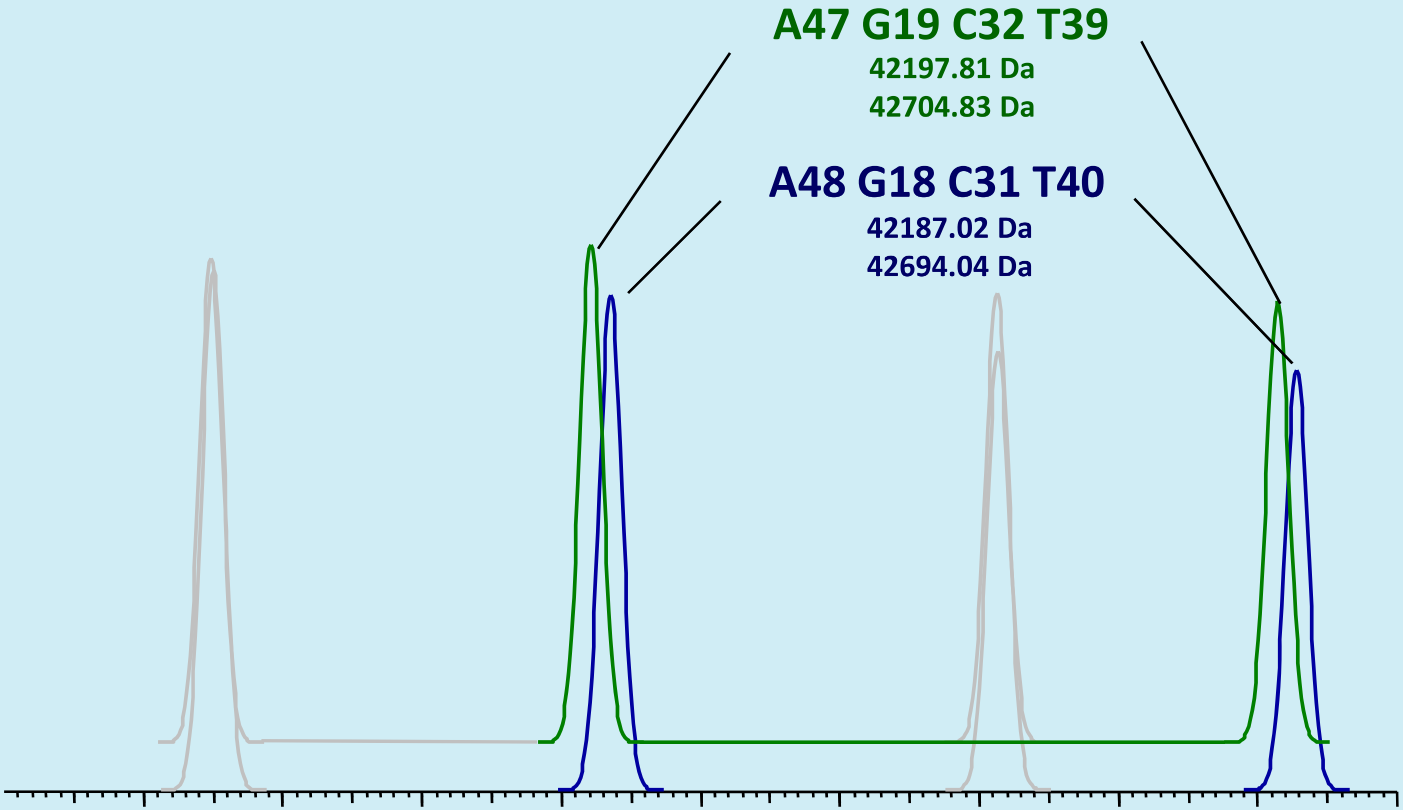
Mass Tag Changes Rules

With ^{13}C-dGTP, the mass separation increases to about 10 Da for each strand; this is about 300 ppm (we generally see errors about 10 ppm)



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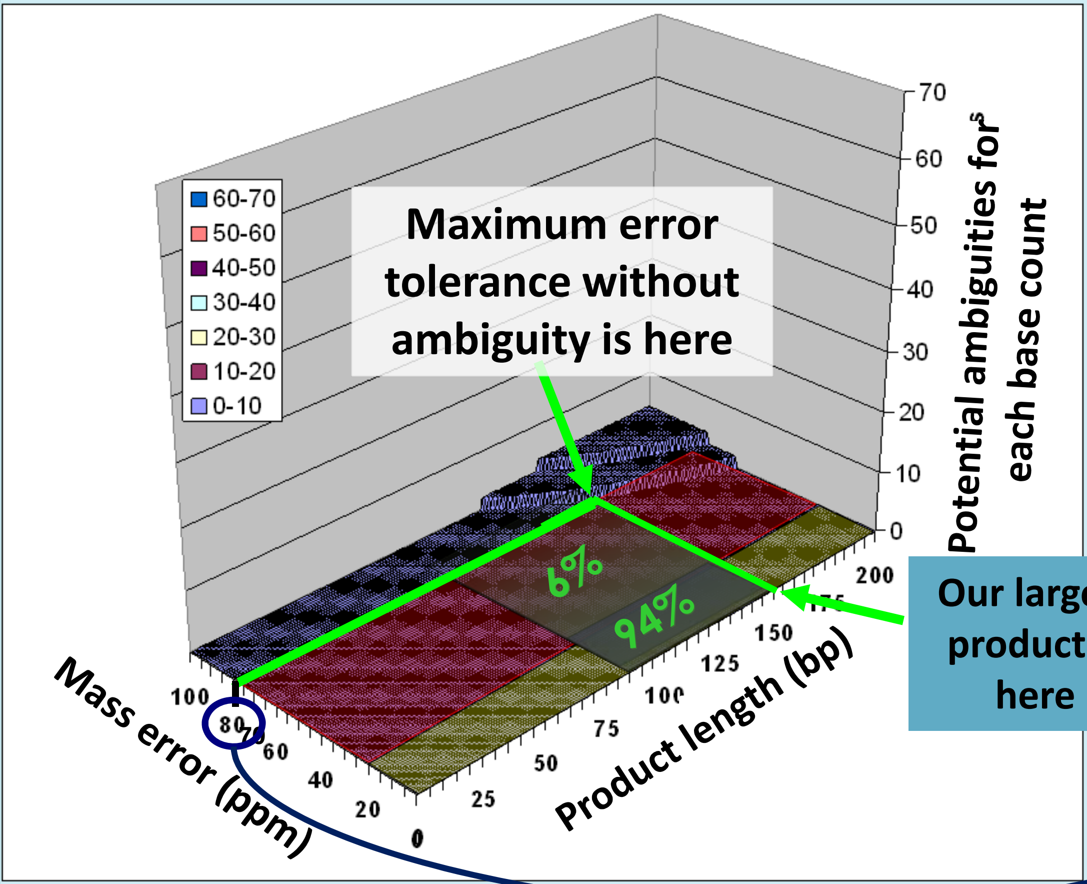
What Does Mass Tagging Mean?

Using ^{13}C-dGTP eliminates ambiguity with a wide margin around our working zone



Maximum error tolerance without ambiguity is here

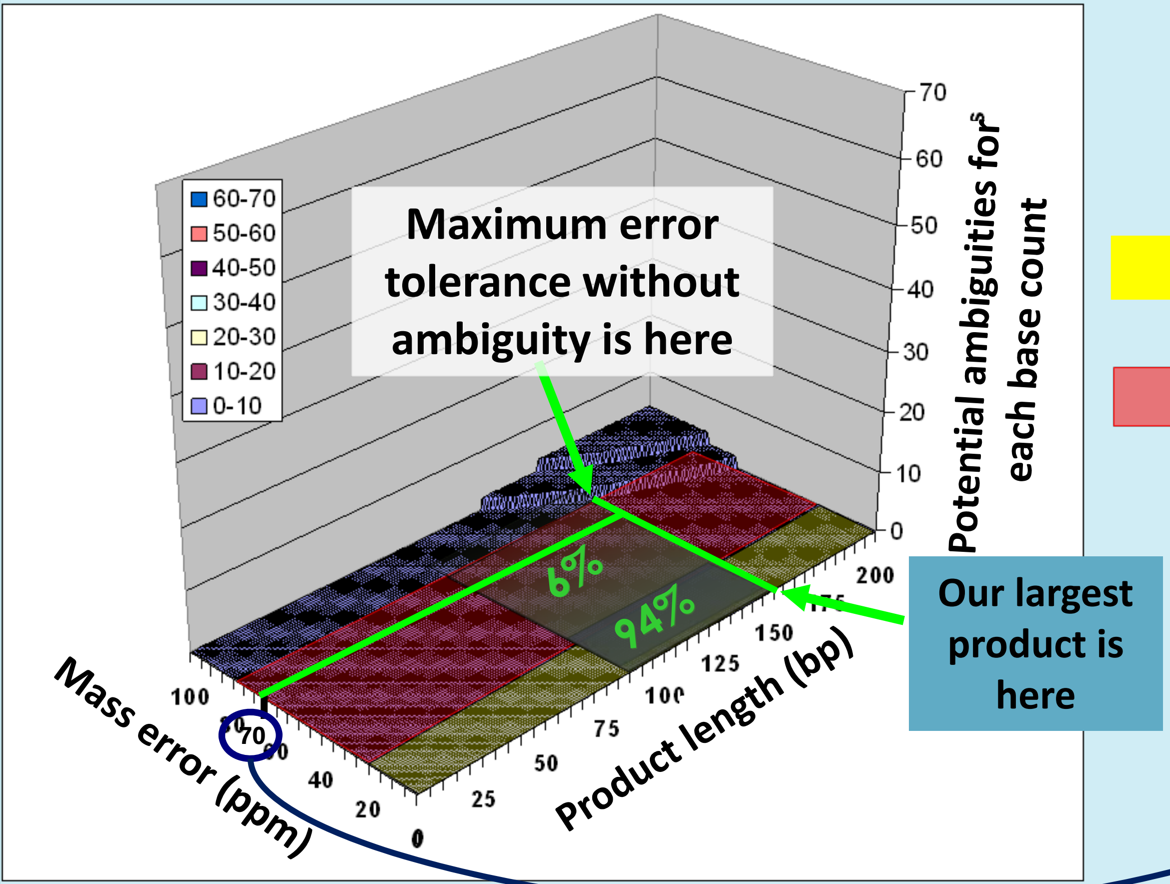
Our largest product is here

🟨 **Working range for TOF (<20 ppm)**

🟥 **80 ppm measurement error maximum in this assay**

What Does Mass Tagging Mean?

Using ^{13}C-dGTP eliminates ambiguity with a wide margin around our working zone



Maximum error tolerance without ambiguity is here

Our largest product is here

🟨 **Working range for TOF (<20 ppm)**

🟥 **80 ppm measurement error maximum in this assay**

Threshold at 70 ppm to be conservative

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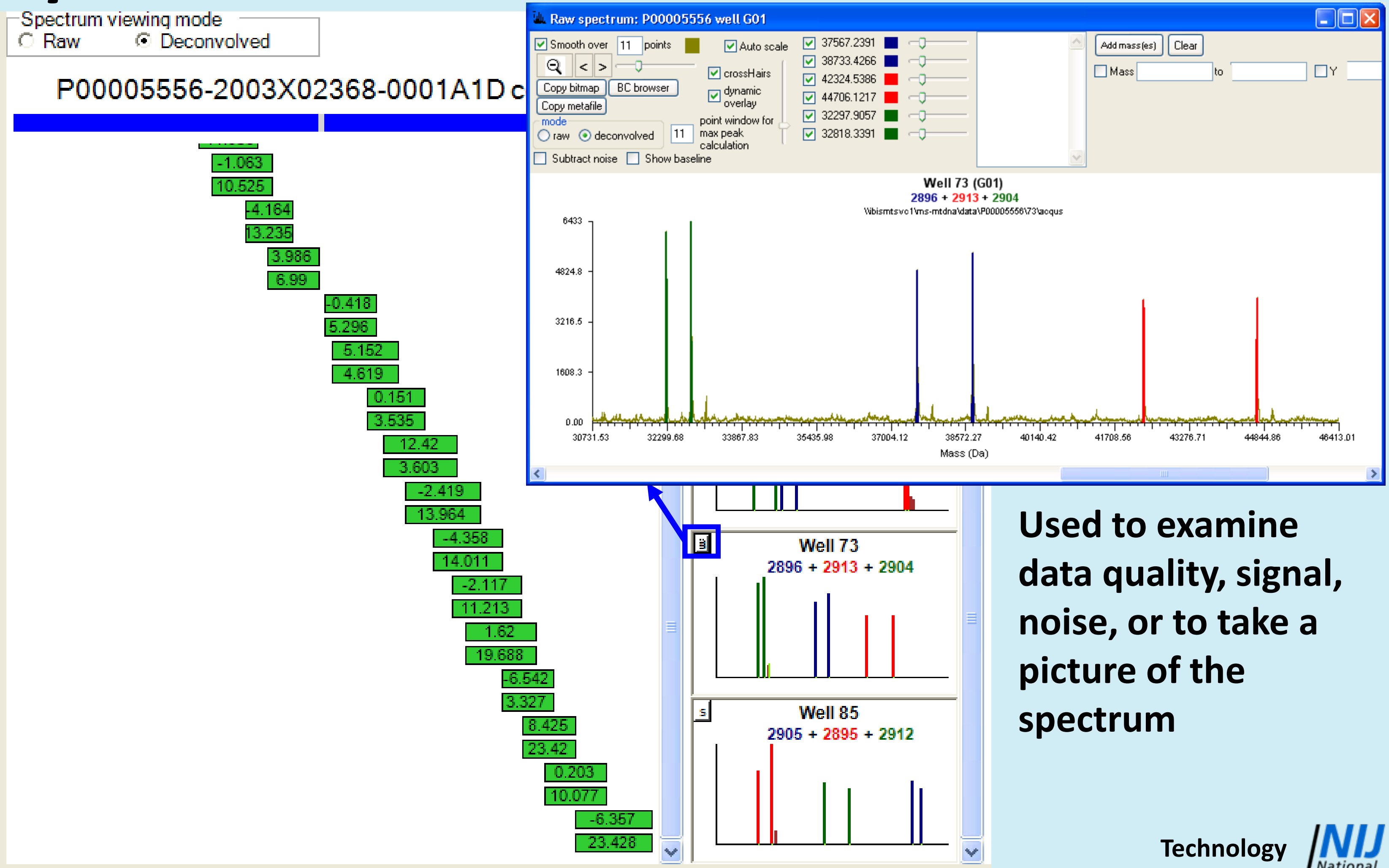
Spectral Viewer



Spectrum viewing mode
○ Raw ● Deconvolved

P00005556-2003X02368-0001A1D c

-1.063
10.525
-4.164
13.235
3.986
6.99
-0.418
5.296
5.152
4.619
0.151
3.535
12.42
3.603
-2.419
13.964
-4.358
14.011
-2.117
11.213
1.62
19.688
-6.542
3.327
8.425
23.42
0.203
10.077
-6.357
23.428

Raw spectrum: P00005556 well G01

☑ Smooth over 11 points
☑ Auto scale
☑ crossHairs
☑ dynamic overlay
mode: ○ raw ● deconvolved
11 point window for max peak calculation
☐ Subtract noise ☐ Show baseline

☑ 37567.2391 🟦
☑ 38733.4266 🟦
☑ 42324.5386 🟥
☑ 44706.1217 🟥
☑ 32297.9057 🟩
☑ 32818.3391 🟩

☐ Mass to ☐ Y

Well 73 (G01)
2896 + 2913 + 2904
\\ibismtsvc1\ms-mtdna\data\P00005556\73\acqus

Well 73
2896 + 2913 + 2904

Well 85
2905 + 2895 + 2912

Used to examine data quality, signal, noise, or to take a picture of the spectrum

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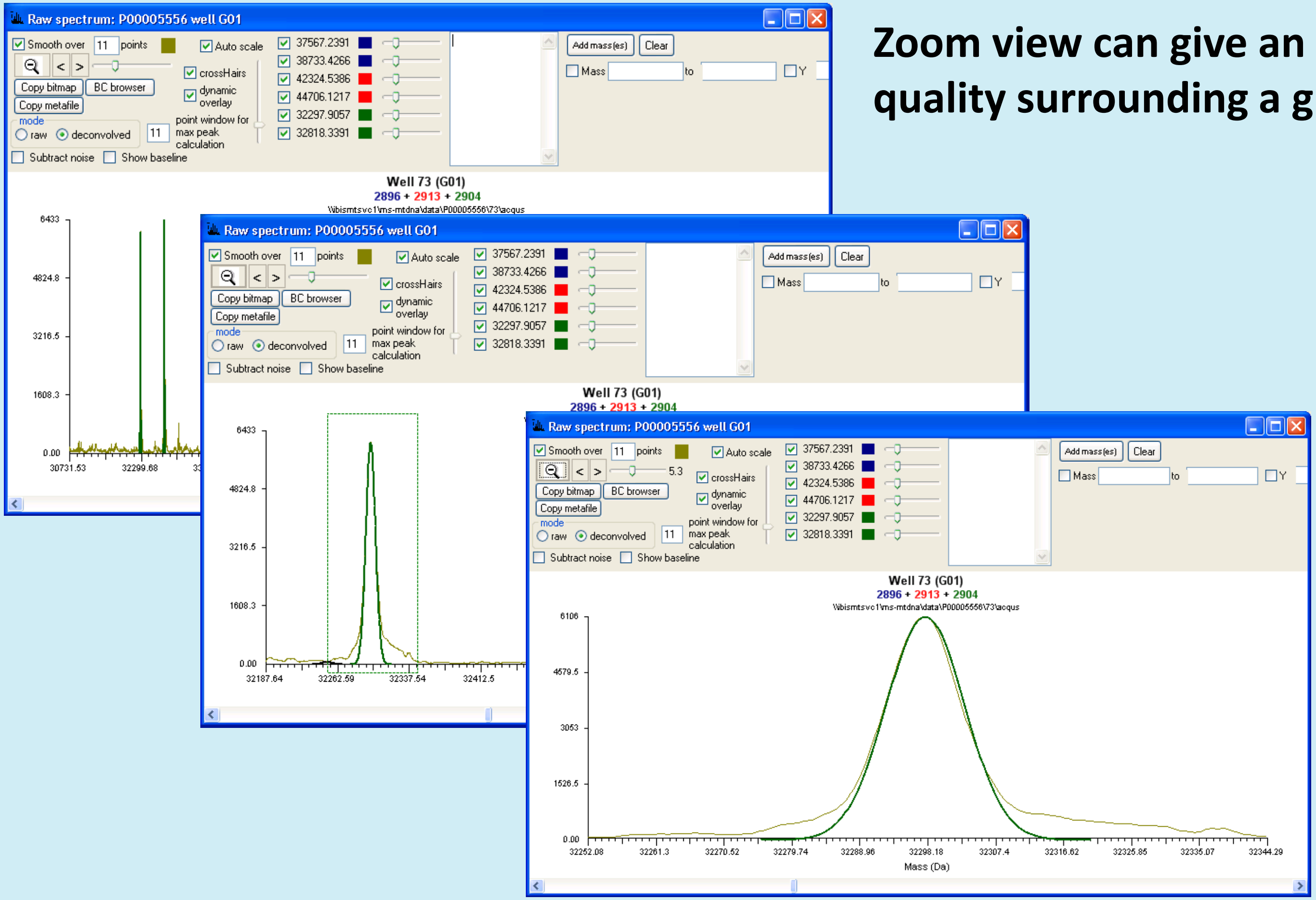
Spectral Viewer – Deconvolved



Raw spectrum: P00005556 well G01

☑ Smooth over 11 points

☑ Auto scale

☑ crossHairs

☑ dynamic overlay

point window for max peak calculation: 11

mode: ○ raw ● deconvolved

☐ Subtract noise ☐ Show baseline

☑ 37567.2391 🟦

☑ 38733.4266 🟦

☑ 42324.5386 🟥

☑ 44706.1217 🟥

☑ 32297.9057 🟩

☑ 32818.3391 🟩

☐ Mass ___ to ___ ☐ Y

Well 73 (G01)

2896 + 2913 + 2904

\\bismtsvc1\ms-mtdna\data\P00005556\73\acqus

Zoom view can give an idea of data quality surrounding a given peak

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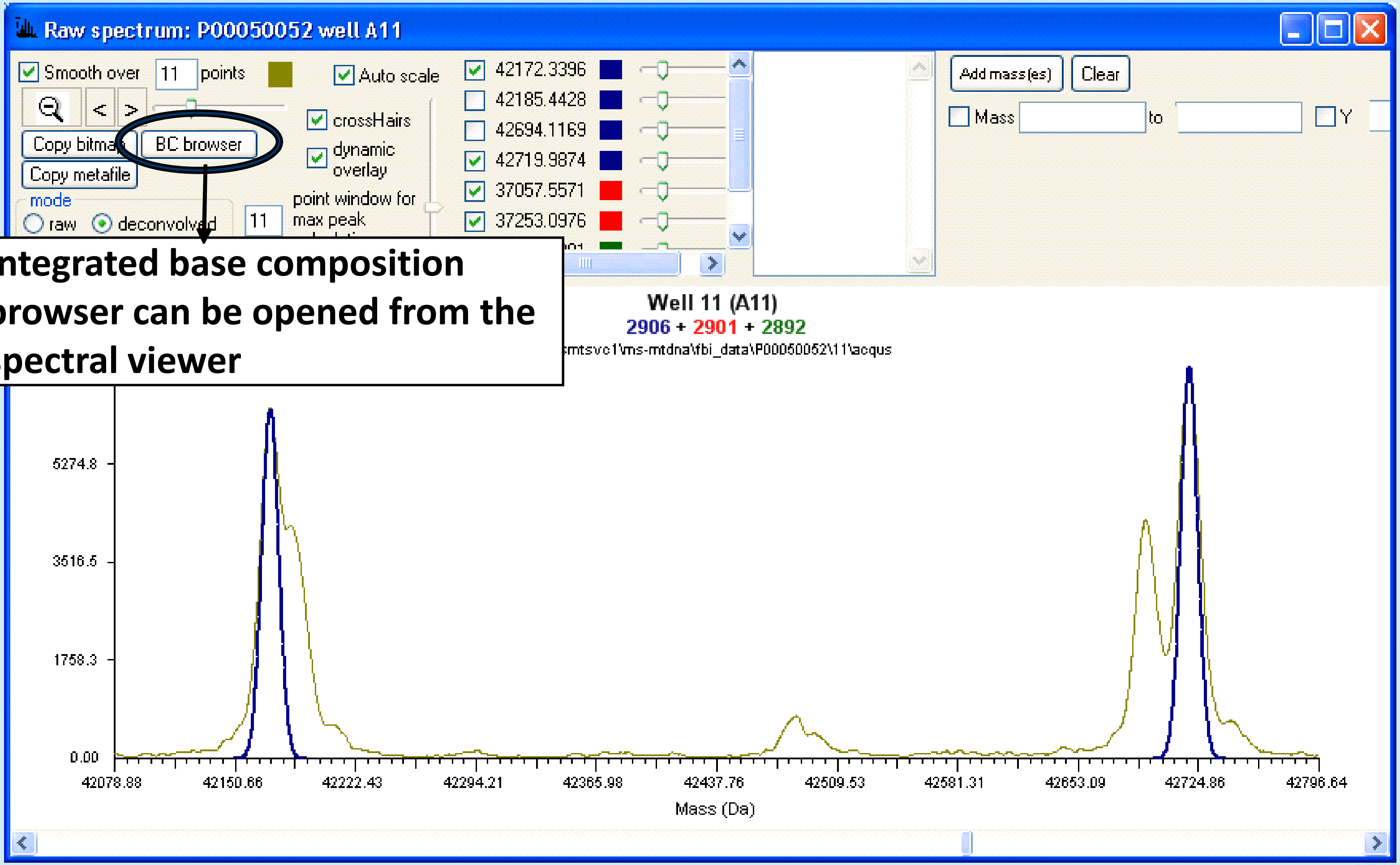
Base Composition Browser



☑ Smooth over 11 points

☑ Auto scale

☑ crossHairs

☑ dynamic overlay

mode ○ raw ● deconvolved

11 point window for max peak

☑ 42172.3396
☐ 42185.4428
☐ 42694.1169
☑ 42719.9874
☑ 37057.5571
☑ 37253.0976

Well 11 (A11)
2906 + 2901 + 2892
mtsvc1\ms-mtdna\fbi_data\P00050052\11\acqus

Integrated base composition browser can be opened from the spectral viewer

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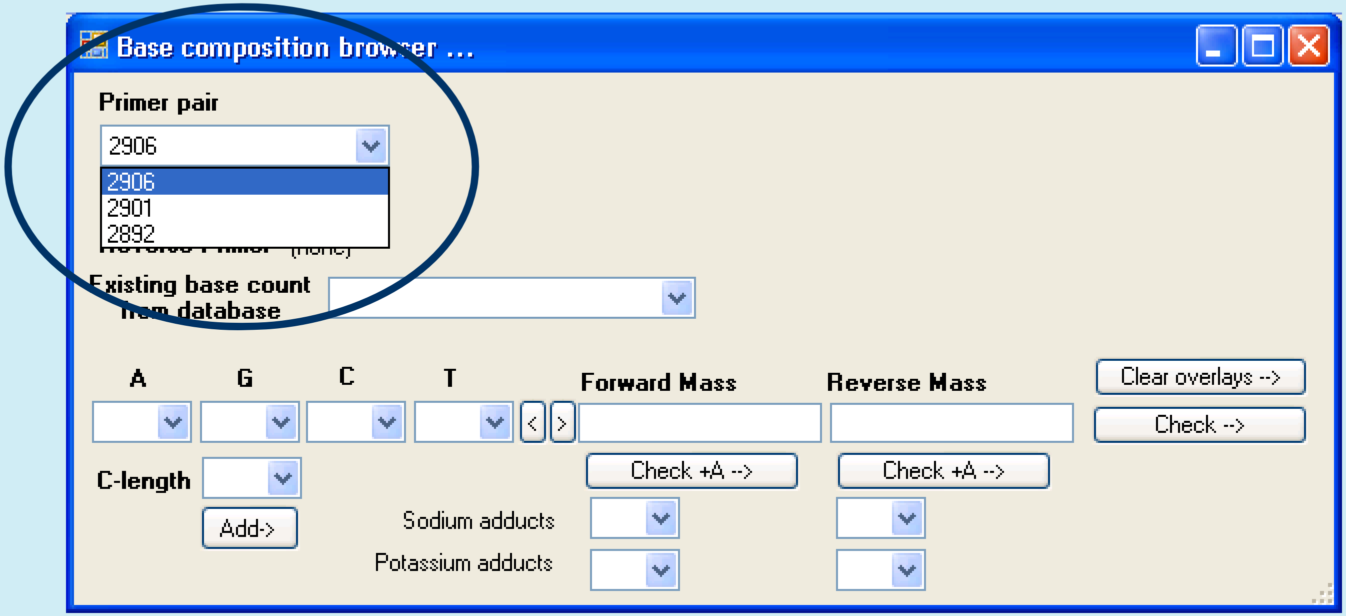
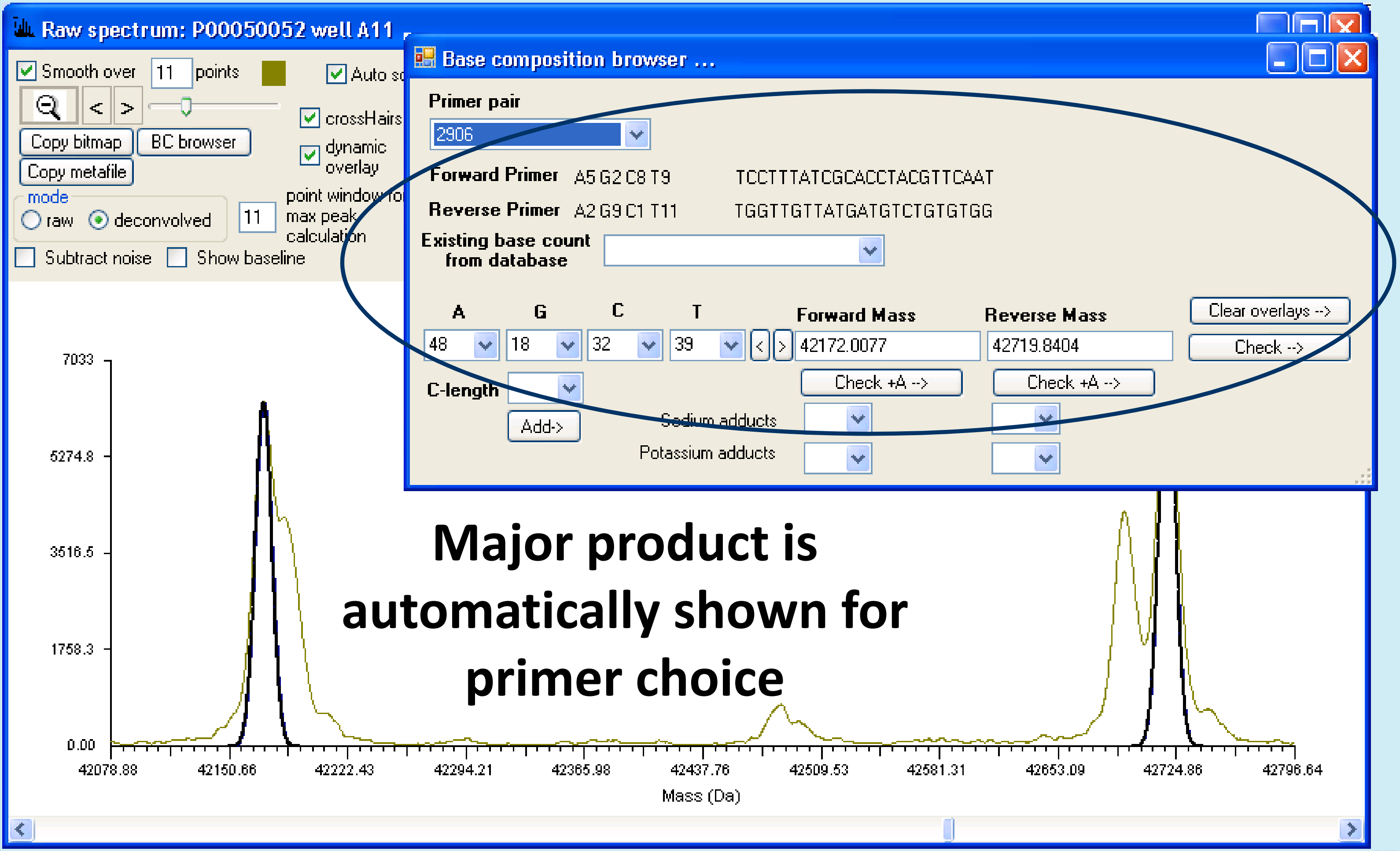
Base Composition Browser

Primer pairs in current well are integrated to this view



Primer pair: 2906

Base Composition Browser



Raw spectrum: P00050052 well A11

☑ Smooth over 11 points

☑ Auto sc

☑ crossHairs

☑ dynamic overlay

mode: ○ raw ● deconvolved

11 point window for max peak calculation

☐ Subtract noise ☐ Show baseline

Base composition browser ...

Primer pair: 2906

Forward Primer A5 G2 C8 T9 TCCTTTATCGCACCTACGTTCAAT

Reverse Primer A2 G9 C1 T11 TGGTTGTTATGATGTCTGTGTGG

Existing base count from database

A	G	C	T	Forward Mass	Reverse Mass
48	18	32	39	42172.0077	42719.8404

C-length

Sodium adducts

Potassium adducts

Major product is automatically shown for primer choice

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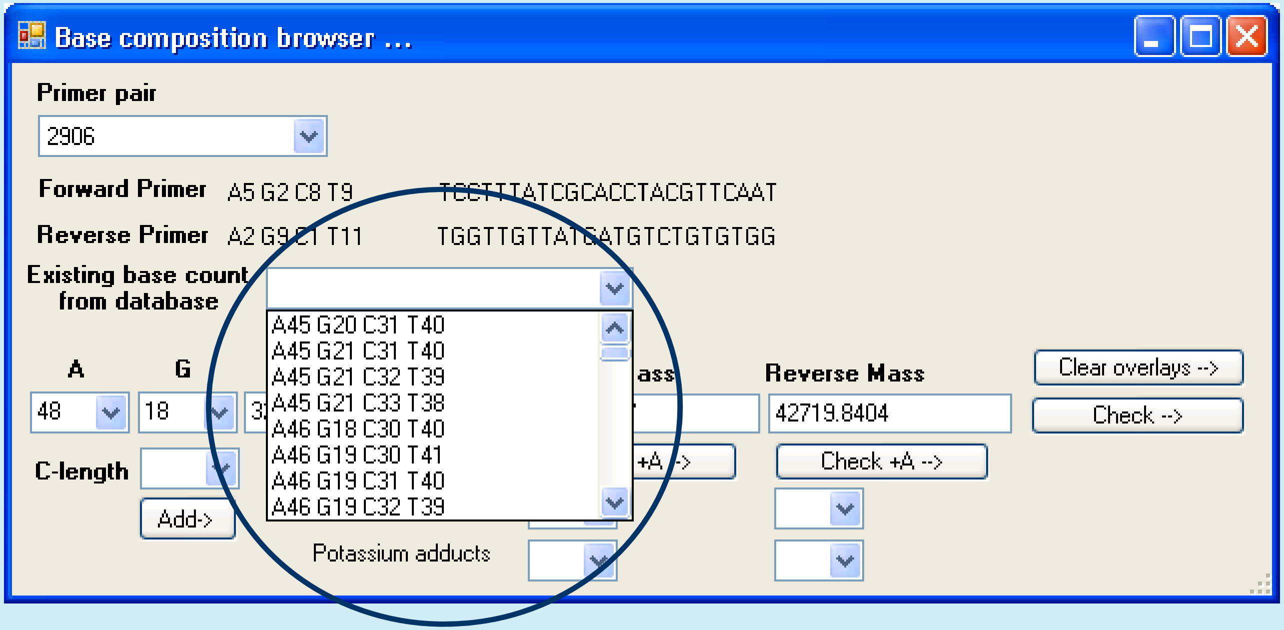
Base Composition Browser



Primer pair: 2906

Forward Primer: A5 G2 C8 T9 TCCTTTATCGCACCTACGTTCAAT

Reverse Primer: A2 G9 C1 T11 TGGTTGTTATGATGTCTGTGTGG

A45 G20 C31 T40
A45 G21 C31 T40
A45 G21 C32 T39
A45 G21 C33 T38
A46 G18 C30 T40
A46 G19 C30 T41
A46 G19 C31 T40
A46 G19 C32 T39

A: 48

G: 18

Reverse Mass: 42719.8404

All known products for selected primer pair are in this list

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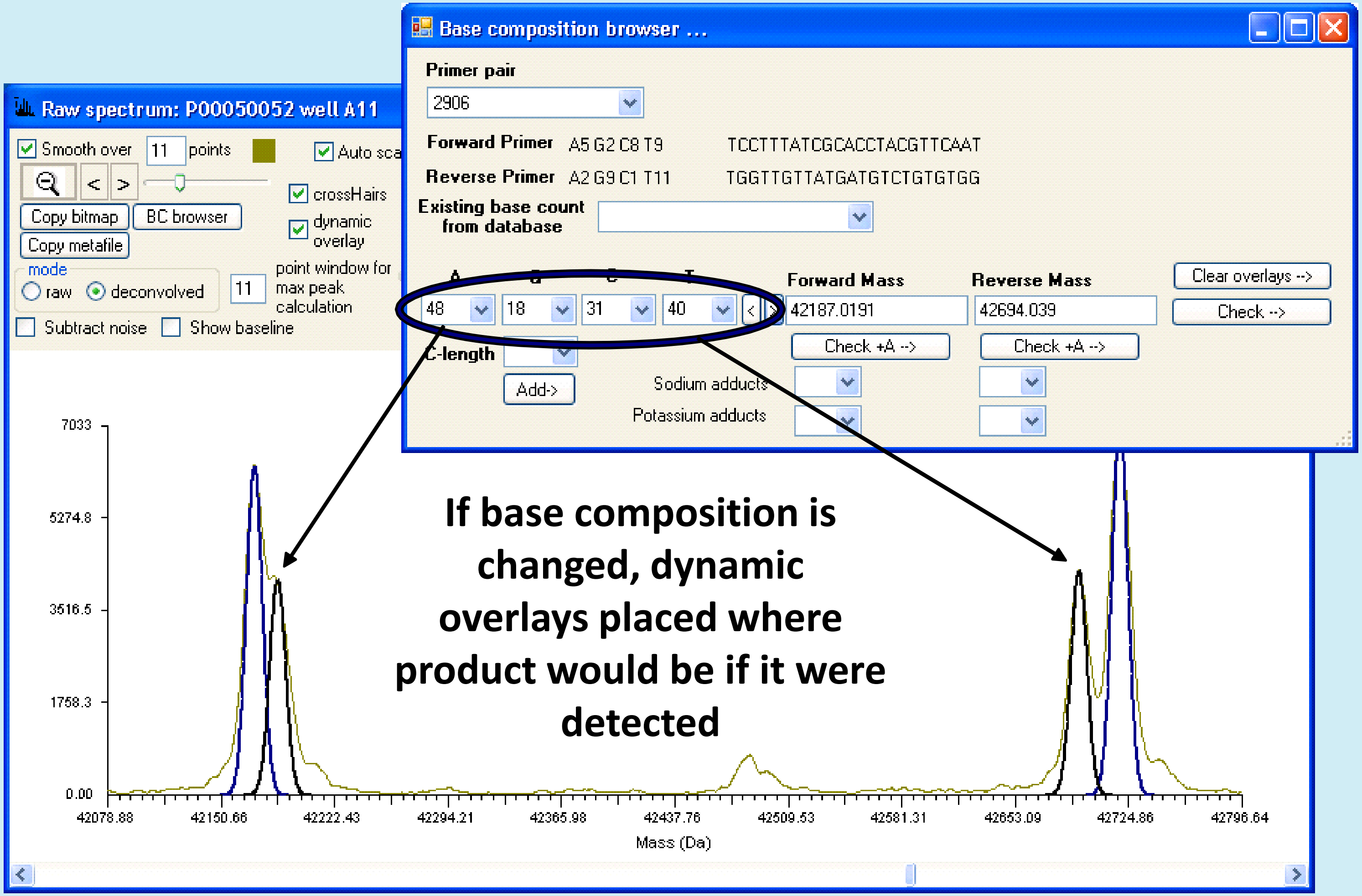
Base Composition Browser



Raw spectrum: P00050052 well A11

☑ Smooth over 11 points

☑ Auto sca

☑ crossHairs

☑ dynamic overlay

mode: ○ raw ● deconvolved

11 point window for max peak calculation

☐ Subtract noise ☐ Show baseline

Base composition browser ...

Primer pair: 2906

Forward Primer A5 G2 C8 T9 TCCTTTATCGCACCTACGTTCAAT

Reverse Primer A2 G9 C1 T11 TGGTTGTTATGATGTCTGTGTGG

Existing base count from database:

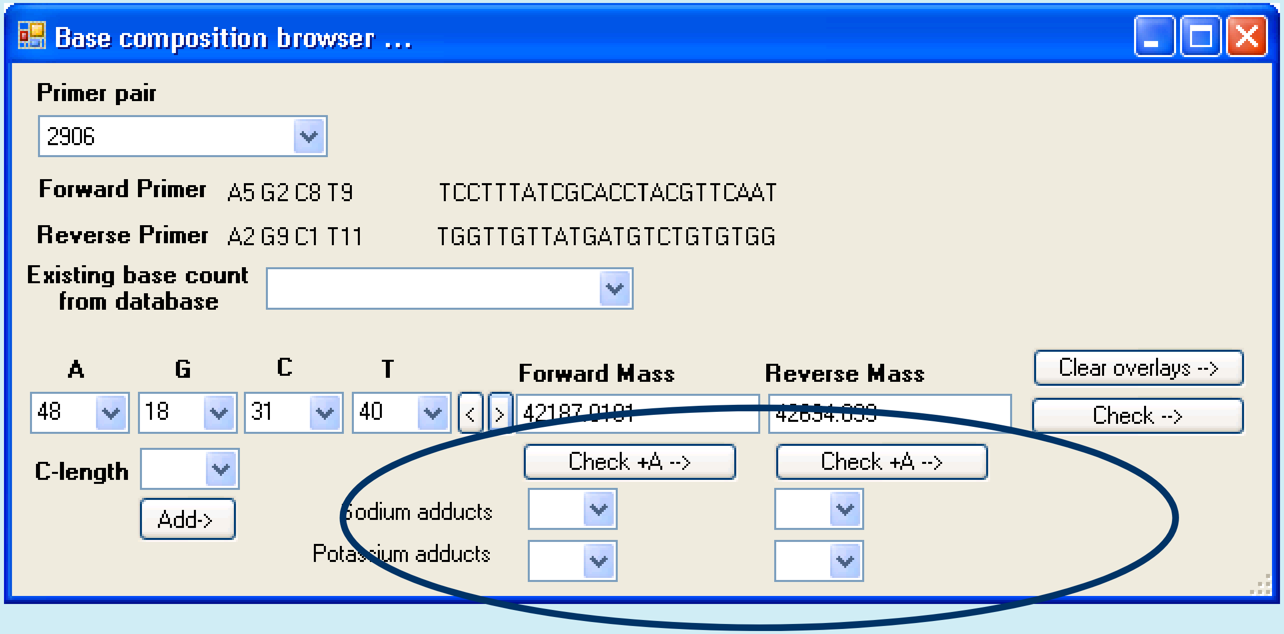
A: 48 G: 18 C: 31 T: 40

Forward Mass: 42187.0191

Reverse Mass: 42694.039

If base composition is changed, dynamic overlays placed where product would be if it were detected

Base Composition Browser



Base composition browser ...

Primer pair: 2906

Forward Primer: A5 G2 C8 T9 TCCTTTATCGCACCTACGTTCAAT

Reverse Primer: A2 G9 C1 T11 TGGTTGTTATGATGTCTGTGTGG

Existing base count from database:

A	G	C	T	Forward Mass	Reverse Mass
48	18	31	40	42187.0101	42654.039

C-length:

Adenylation products, sodium adducts, and potassium adducts can also be looked for here

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NIJ National Institute of Justice

Search Database – mtDNA

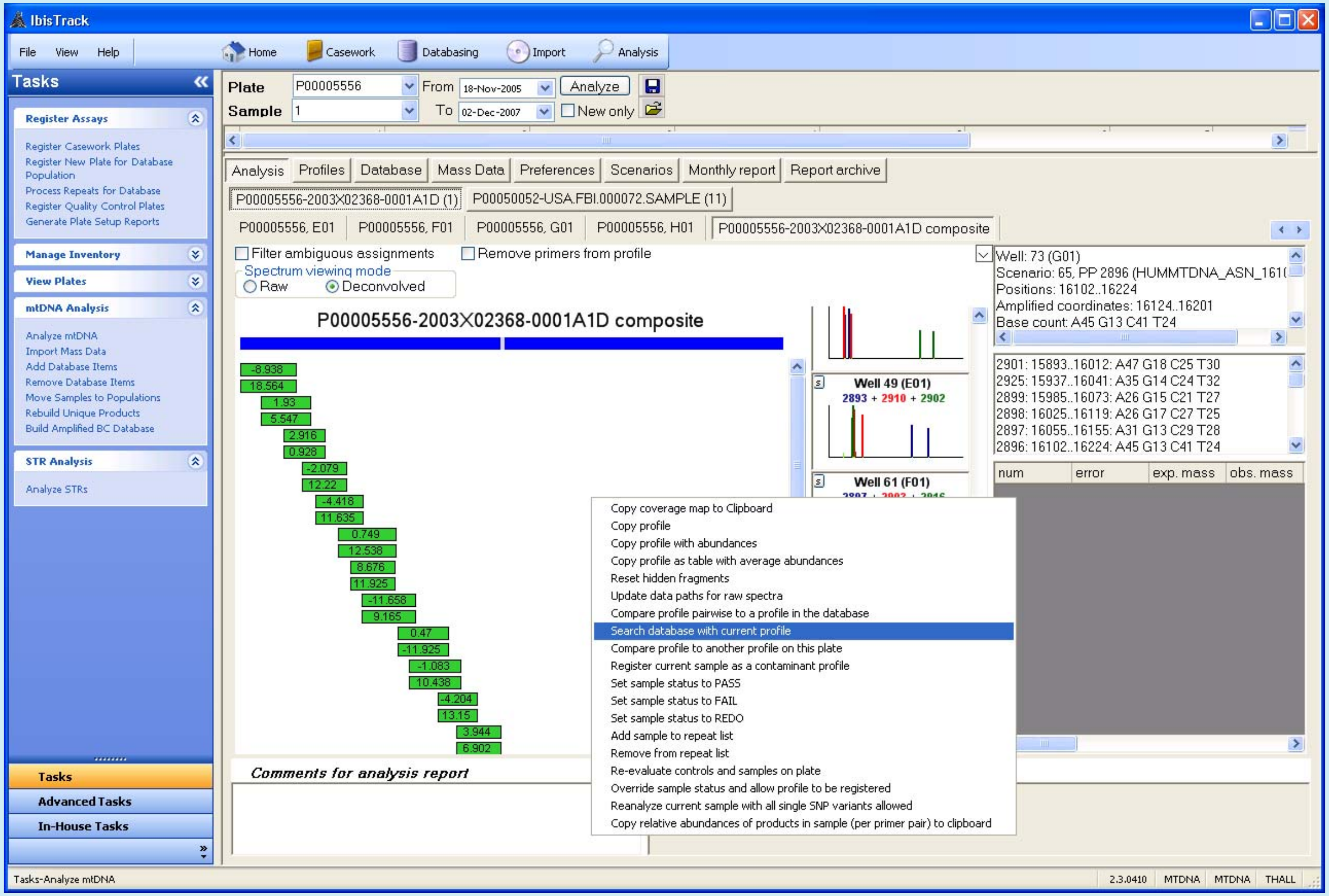


Plate P00005556 From 18-Nov-2005

Sample 1 To 02-Dec-2007 ☐ New only

☐ Filter ambiguous assignments ☐ Remove primers from profile

Spectrum viewing mode ○ Raw ● Deconvolved

P00005556-2003X02368-0001A1D composite

-8.938
18.564
1.93
5.547
2.916
0.928
-2.079
12.22
-4.418
11.635
0.749
12.538
8.676
11.925
-11.658
9.165
0.47
-11.925
-1.083
10.438
-4.204
13.15
3.944
6.902

Well 49 (E01)
2893 + 2910 + 2902

Well 61 (F01)

Well: 73 (G01)
Scenario: 65, PP 2896 (HUMMTDNA_ASN_161(
Positions: 16102..16224
Amplified coordinates: 16124..16201
Base count: A45 G13 C41 T24

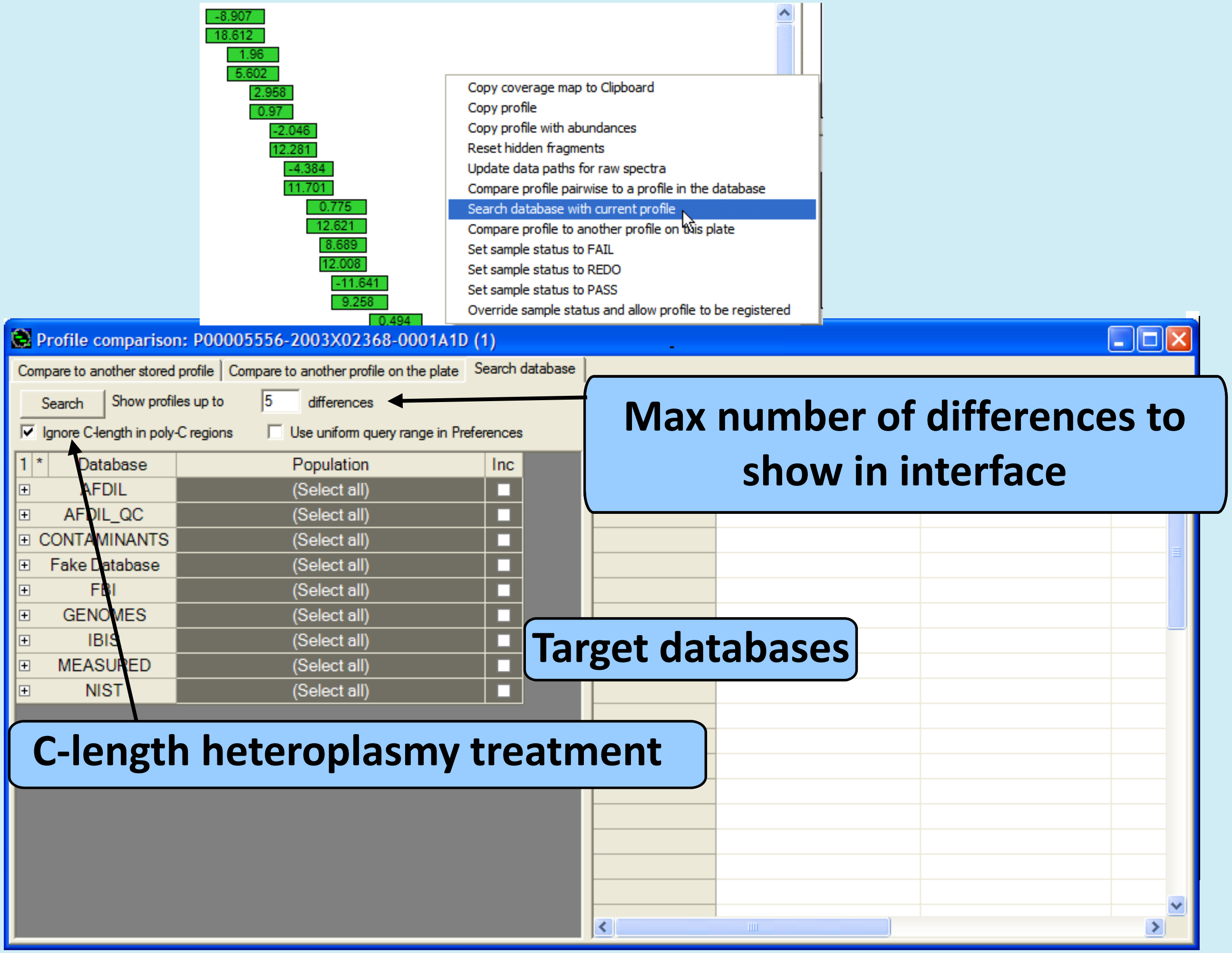
2901: 15893..16012: A47 G18 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C27 T25
2897: 16055..16155: A31 G13 C29 T28
2896: 16102..16224: A45 G13 C41 T24

num	error	exp. mass	obs. mass

Comments for analysis report

Tasks-Analyze mtDNA 2.3.0410 MTDNA MTDNA THALL

Search Database – mtDNA



Profile comparison: P00005556-2003X02368-0001A1D (1)

Show profiles up to 5 differences

☑ Ignore C-length in poly-C regions ☐ Use uniform query range in Preferences

Database	Population	Inc
AFDIL	(Select all)	☐
AFDIL_QC	(Select all)	☐
CONTAMINANTS	(Select all)	☐
Fake Database	(Select all)	☐
FBI	(Select all)	☐
GENOMES	(Select all)	☐
IBIS	(Select all)	☐
MEASURED	(Select all)	☐
NIST	(Select all)	☐

Max number of differences to show in interface

Target databases

C-length heteroplasmy treatment

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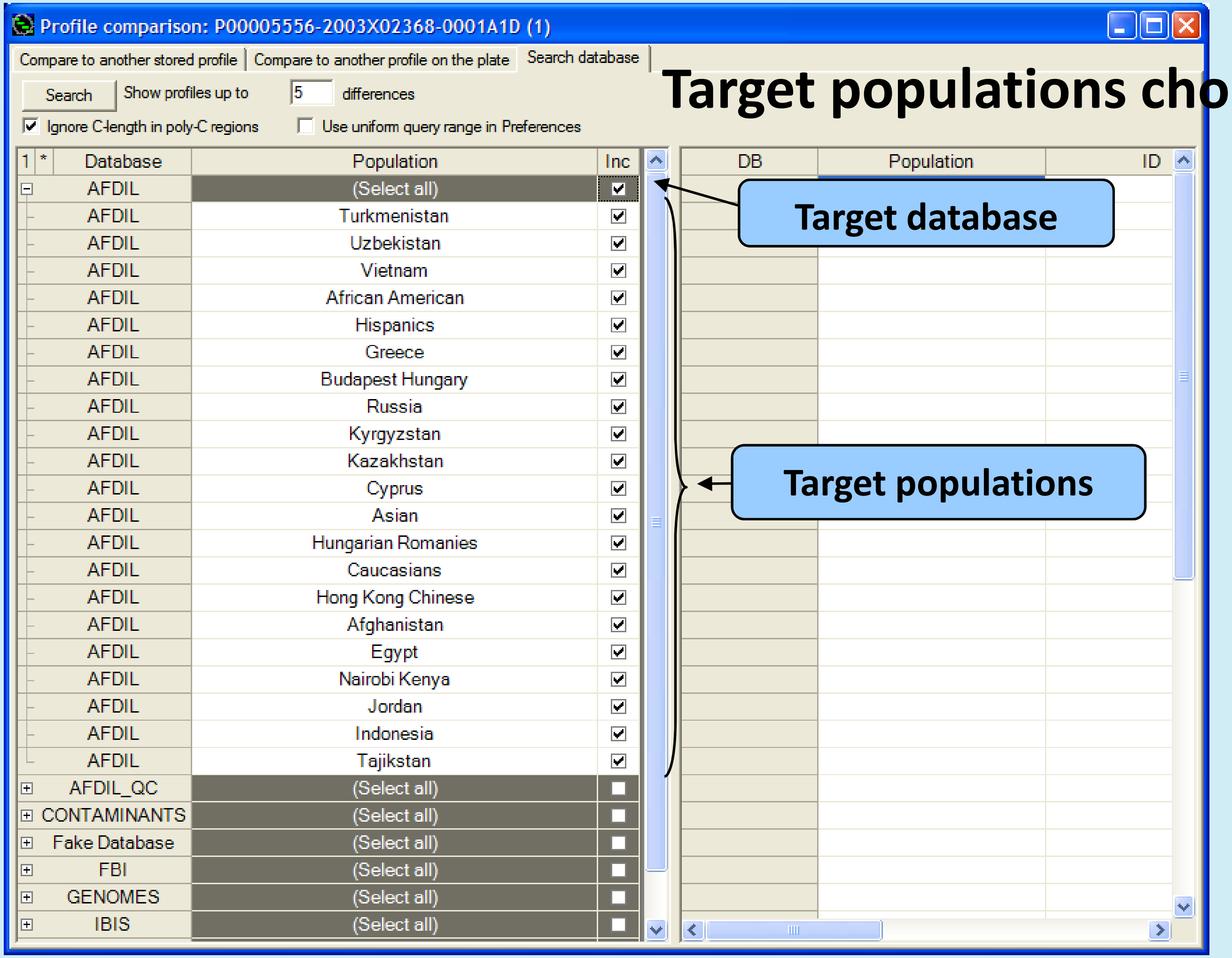
Search Database – mtDNA



Profile comparison: P00005556-2003X02368-0001A1D (1)

Show profiles up to 5 differences

☑ Ignore C-length in poly-C regions

☐ Use uniform query range in Preferences

1	*	Database	Population	Inc
⊟		AFDIL	(Select all)	☑
		AFDIL	Turkmenistan	☑
		AFDIL	Uzbekistan	☑
		AFDIL	Vietnam	☑
		AFDIL	African American	☑
		AFDIL	Hispanics	☑
		AFDIL	Greece	☑
		AFDIL	Budapest Hungary	☑
		AFDIL	Russia	☑
		AFDIL	Kyrgyzstan	☑
		AFDIL	Kazakhstan	☑
		AFDIL	Cyprus	☑
		AFDIL	Asian	☑
		AFDIL	Hungarian Romanies	☑
		AFDIL	Caucasians	☑
		AFDIL	Hong Kong Chinese	☑
		AFDIL	Afghanistan	☑
		AFDIL	Egypt	☑
		AFDIL	Nairobi Kenya	☑
		AFDIL	Jordan	☑
		AFDIL	Indonesia	☑
		AFDIL	Tajikstan	☑
⊞		AFDIL_QC	(Select all)	☐
⊞		CONTAMINANTS	(Select all)	☐
⊞		Fake Database	(Select all)	☐
⊞		FBI	(Select all)	☐
⊞		GENOMES	(Select all)	☐
⊞		IBIS	(Select all)	☐

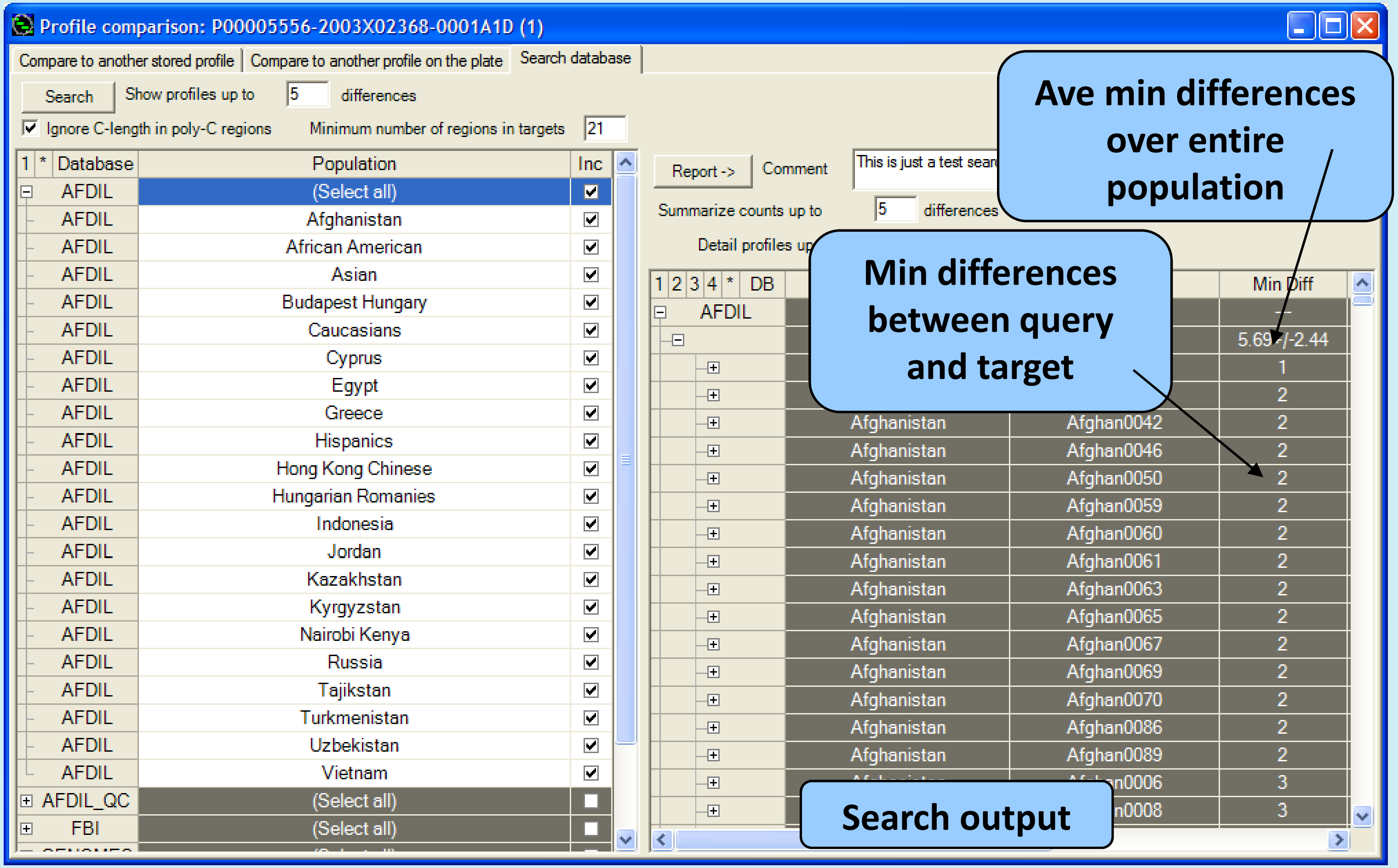
DB	Population	ID

Target populations choice

Target database

Target populations

Search Database - mtDNA



Profile comparison: P00005556-2003X02368-0001A1D (1)

Show profiles up to 5 differences

☑ Ignore C-length in poly-C regions

Minimum number of regions in targets 21

1	*	Database	Population	Inc
⊟		AFDIL	(Select all)	☑
		AFDIL	Afghanistan	☑
		AFDIL	African American	☑
		AFDIL	Asian	☑
		AFDIL	Budapest Hungary	☑
		AFDIL	Caucasians	☑
		AFDIL	Cyprus	☑
		AFDIL	Egypt	☑
		AFDIL	Greece	☑
		AFDIL	Hispanics	☑
		AFDIL	Hong Kong Chinese	☑
		AFDIL	Hungarian Romanies	☑
		AFDIL	Indonesia	☑
		AFDIL	Jordan	☑
		AFDIL	Kazakhstan	☑
		AFDIL	Kyrgyzstan	☑
		AFDIL	Nairobi Kenya	☑
		AFDIL	Russia	☑
		AFDIL	Tajikstan	☑
		AFDIL	Turkmenistan	☑
		AFDIL	Uzbekistan	☑
		AFDIL	Vietnam	☑
⊞		AFDIL_QC	(Select all)	☐
⊞		FBI	(Select all)	☐

Comment: This is just a test sear

Summarize counts up to 5 differences

Detail profiles up

1	2	3	4	*	DB			Min Diff
⊟					AFDIL			—
	⊟							5.69+/-2.44
		⊞						1
		⊞						2
		⊞				Afghanistan	Afghan0042	2
		⊞				Afghanistan	Afghan0046	2
		⊞				Afghanistan	Afghan0050	2
		⊞				Afghanistan	Afghan0059	2
		⊞				Afghanistan	Afghan0060	2
		⊞				Afghanistan	Afghan0061	2
		⊞				Afghanistan	Afghan0063	2
		⊞				Afghanistan	Afghan0065	2
		⊞				Afghanistan	Afghan0067	2
		⊞				Afghanistan	Afghan0069	2
		⊞				Afghanistan	Afghan0070	2
		⊞				Afghanistan	Afghan0086	2
		⊞				Afghanistan	Afghan0089	2
		⊞					n0006	3
		⊞					n0008	3

Ave min differences over entire population

Min differences between query and target

Search output

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Comparing mtDNA Profiles

AF-2		USA.FBI.000070	diff	Accounting for overlaps
2901: 15893..16012: no data		2901: 15893..16012: no data		
2925: 15937..16041: no data		2925: 15937..16041: no data		
2899: 15985..16073: no data		2899: 16015..16051: A7 G10 C5 T15		
2898: 16048..16098: A15 G7 C17 T12	X	2898: 16048..16098: A15 G7 C16 T13	1	1
2897: 16078..16129: A15 G8 C14 T15	➡	2897: 16078..16129: A15 G8 C14 T15		
2896: 16124..16201: A27 G7 C27 T17	X	2896: 16124..16201: A26 G7 C29 T16	2	2
2895: 16157..16201: A17 G1 C19 T8	X	2895: 16157..16201: A16 G1 C21 T7	2	
2893: 16182..16250: A24 G5 C28 T12	X	2893: 16182..16250: A23 G5 C29 T12	1	
2892: 16254..16305: A17 G4 C22 T8 N	➡	2892: 16254..16305: A18 G4 C22 T8		
2891: 16283..16344: A23 G5 C18 T15 N	➡	2891: 16283..16344: A24 G5 C19 T14		
2890: 16318..16402: no data		2890: 16342..16381: A8 G5 C17 T10		
2889: 16357..16451: no data		2889: 16377..16428: A12 G9 C20 T11		
2902: 5..97: no data		2902: 31..76: A5 G15 C12 T15		
2903: 20..139: no data		2903: 41..114: A12 G24 C21 T18		
2904: 103..162: A10 G10 C20 T20	X	2904: 103..162: A9 G11 C18 T22	3	3
2905: 138..217: A23 G9 C21 T27	X	2905: 138..217: A22 G10 C19 T29	3	
2906: 178..267: A32 G15 C15 T28	➡	2906: 178..267: A32 G15 C15 T28		
2908: 234..313: A30 G6 C30 T16	➡	2908: 234..313: A30 G6 C31 T16		
2907: 263..340: A25 G6 C35 T14	➡	2907: 263..340: A25 G6 C36 T14		
2923: 262..390: no data		2923: 289..367: A27 G6 C37 T12		
2910: 331..425: no data		2910: 355..401: A21 G3 C14 T9		
2916: 367..463: no data		2916: 389..437: A11 G7 C12 T19		
2912: 409..521: no data		2912: 431..501: A20 G1 C34 T16		
2913: 464..603: no data		2913: 493..576: A27 G6 C46 T5		

Total minimum differences 6

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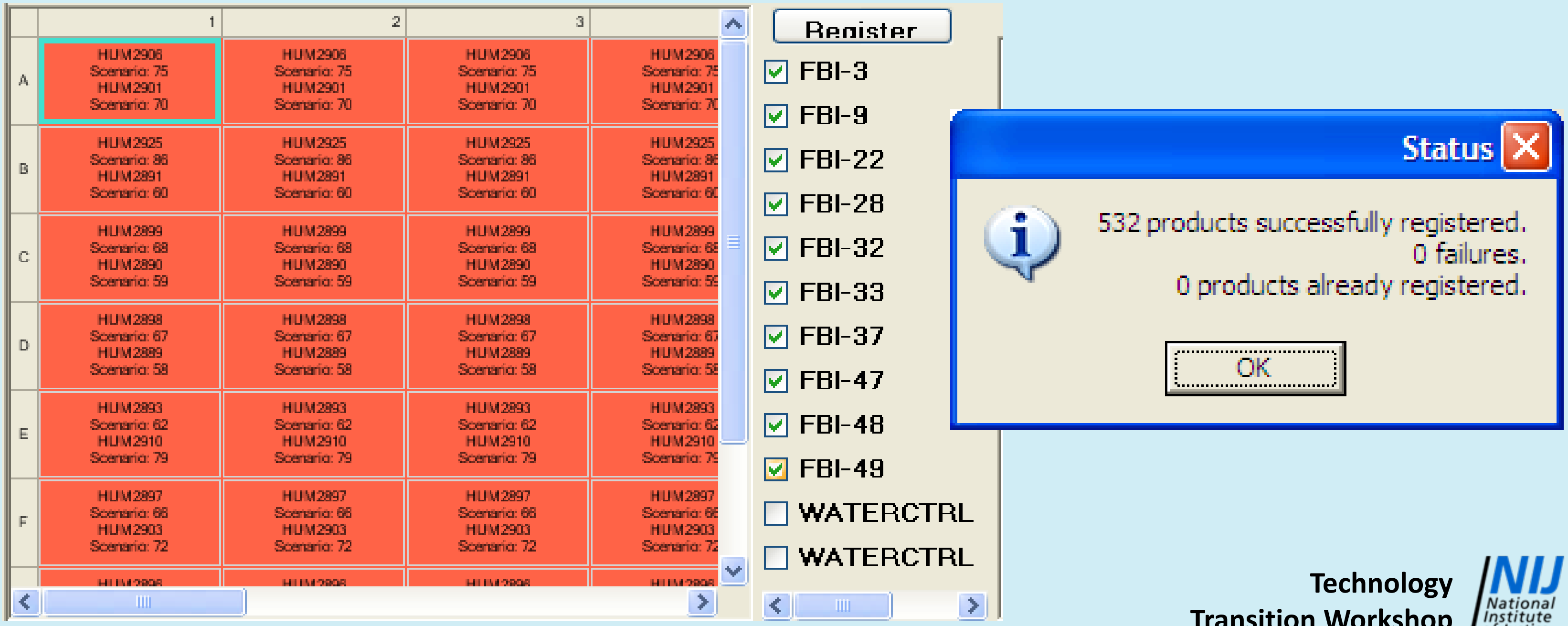
Profile Registration – mtDNA

- Analysis of a full plate exposes profile registration interface
  - Samples that pass auto-processing are checked by default
  - Controls are not checked by default
  - Samples that failed will be disabled
  - If positive or negative control fails, registration for entire plate will be disabled; disabled state can be overridden



	1	2	3	
A	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 75 HUM2901 Scenario: 70	HUM2906 Scenario: 7 HUM2901 Scenario: 7
B	HUM2925 Scenario: 86 HUM2891 Scenario: 60	HUM2925 Scenario: 86 HUM2891 Scenario: 60	HUM2925 Scenario: 86 HUM2891 Scenario: 60	HUM2925 Scenario: 8 HUM2891 Scenario: 6
C	HUM2899 Scenario: 68 HUM2890 Scenario: 59	HUM2899 Scenario: 68 HUM2890 Scenario: 59	HUM2899 Scenario: 68 HUM2890 Scenario: 59	HUM2899 Scenario: 6 HUM2890 Scenario: 5
D	HUM2898 Scenario: 67 HUM2889 Scenario: 58	HUM2898 Scenario: 67 HUM2889 Scenario: 58	HUM2898 Scenario: 67 HUM2889 Scenario: 58	HUM2898 Scenario: 6 HUM2889 Scenario: 5
E	HUM2893 Scenario: 62 HUM2910 Scenario: 79	HUM2893 Scenario: 62 HUM2910 Scenario: 79	HUM2893 Scenario: 62 HUM2910 Scenario: 79	HUM2893 Scenario: 6 HUM2910 Scenario: 7
F	HUM2897 Scenario: 66 HUM2903 Scenario: 72	HUM2897 Scenario: 66 HUM2903 Scenario: 72	HUM2897 Scenario: 66 HUM2903 Scenario: 72	HUM2897 Scenario: 6 HUM2903 Scenario: 7

- ☑ FBI-3
- ☑ FBI-9
- ☑ FBI-22
- ☑ FBI-28
- ☑ FBI-32
- ☑ FBI-33
- ☑ FBI-37
- ☑ FBI-47
- ☑ FBI-48
- ☑ FBI-49
- ☐ WATERCTRL
- ☐ WATERCTRL

532 products successfully registered.
0 failures.
0 products already registered.

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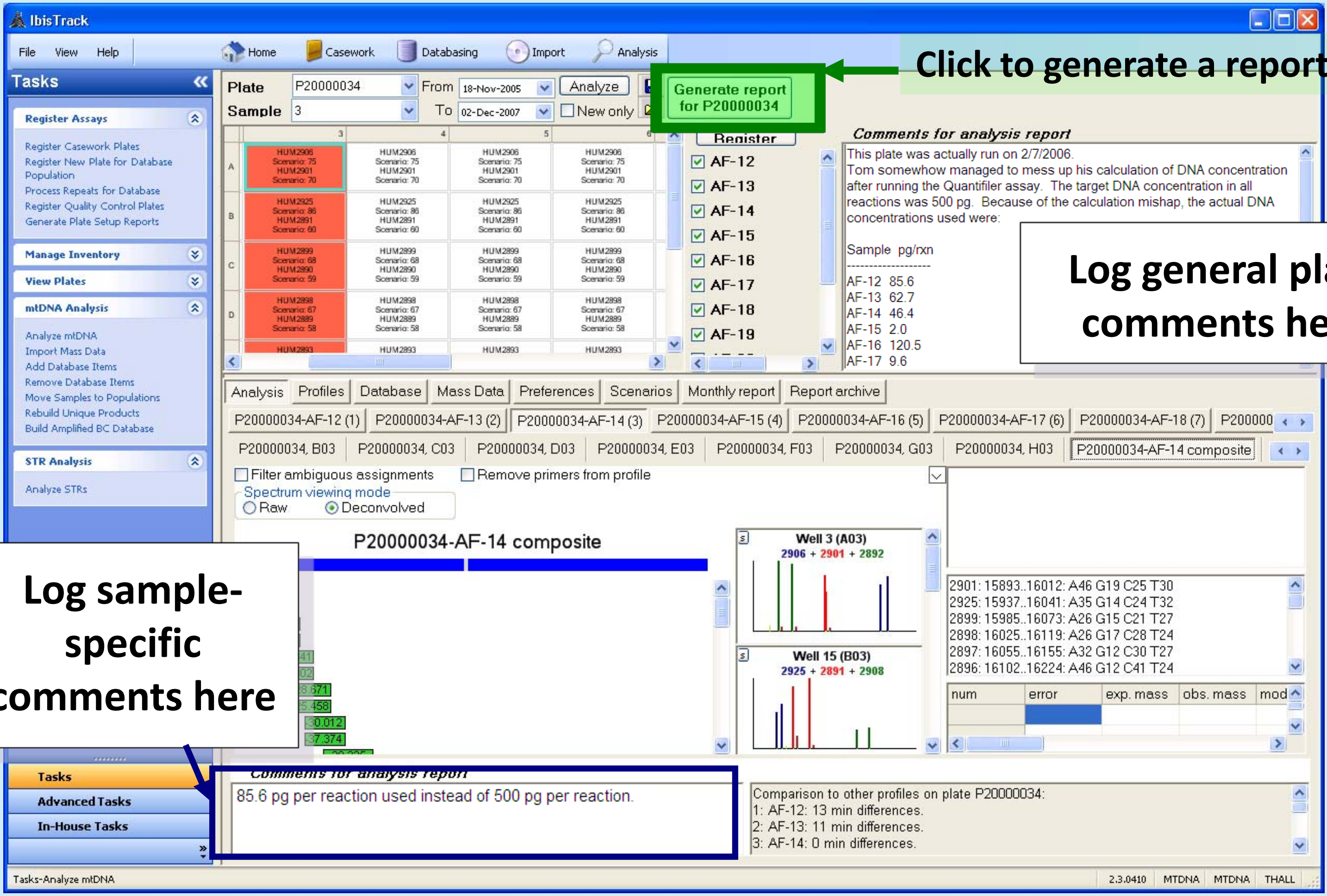
Report Generation – mtDNA



Comments for analysis report

This plate was actually run on 2/7/2006.
Tom somehow managed to mess up his calculation of DNA concentration after running the Quantifiler assay. The target DNA concentration in all reactions was 500 pg. Because of the calculation mishap, the actual DNA concentrations used were:

Sample pg/rxn

AF-12 85.6
AF-13 62.7
AF-14 46.4
AF-15 2.0
AF-16 120.5
AF-17 9.6

2901: 15893..16012: A46 G19 C25 T30
2925: 15937..16041: A35 G14 C24 T32
2899: 15985..16073: A26 G15 C21 T27
2898: 16025..16119: A26 G17 C28 T24
2897: 16055..16155: A32 G12 C30 T27
2896: 16102..16224: A46 G12 C41 T24

Comments for analysis report

85.6 pg per reaction used instead of 500 pg per reaction.

Comparison to other profiles on plate P20000034:
1: AF-12: 13 min differences.
2: AF-13: 11 min differences.
3: AF-14: 0 min differences.

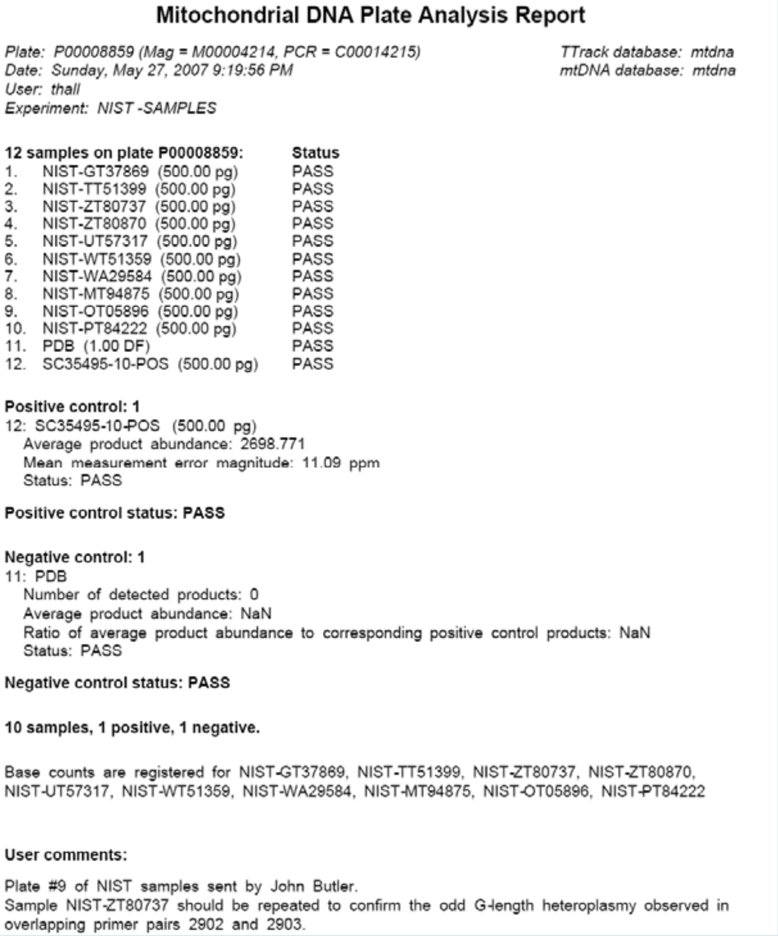
Click to generate a report

Log general plate comments here

Log sample-specific comments here

mtDNA Plate Analysis Report – Page 1

- **First page of report summarizes samples and controls on a plate**
  - **Quantity (or dilution) used**
  - **Status**
  - **Ave abundance (for controls)**
  - **Ave error (for positive)**
  - **Control status**
  - **Samples that have registered profiles**
  - **User comments**



Mitochondrial DNA Plate Analysis Report

Plate: P00008859 (Mag = M00004214, PCR = C00014215)
Date: Sunday, May 27, 2007 9:19:56 PM
User: thall
Experiment: NIST -SAMPLES

TTrack database: mtdna
mtDNA database: mtdna

12 samples on plate P00008859:		**Status**
1.	NIST-GT37869 (500.00 pg)	PASS
2.	NIST-TT51399 (500.00 pg)	PASS
3.	NIST-ZT80737 (500.00 pg)	PASS
4.	NIST-ZT80870 (500.00 pg)	PASS
5.	NIST-UT57317 (500.00 pg)	PASS
6.	NIST-WT51359 (500.00 pg)	PASS
7.	NIST-WA29584 (500.00 pg)	PASS
8.	NIST-MT94875 (500.00 pg)	PASS
9.	NIST-OT05896 (500.00 pg)	PASS
10.	NIST-PT84222 (500.00 pg)	PASS
11.	PDB (1.00 DF)	PASS
12.	SC35495-10-POS (500.00 pg)	PASS

Positive control: 1
12: SC35495-10-POS (500.00 pg)
Average product abundance: 2698.771
Mean measurement error magnitude: 11.09 ppm
Status: PASS

Positive control status: PASS

Negative control: 1
11: PDB
Number of detected products: 0
Average product abundance: NaN
Ratio of average product abundance to corresponding positive control products: NaN
Status: PASS

Negative control status: PASS

10 samples, 1 positive, 1 negative.

Base counts are registered for NIST-GT37869, NIST-TT51399, NIST-ZT80737, NIST-ZT80870, NIST-UT57317, NIST-WT51359, NIST-WA29584, NIST-MT94875, NIST-OT05896, NIST-PT84222

User comments:

Plate #9 of NIST samples sent by John Butler.
Sample NIST-ZT80737 should be repeated to confirm the odd G-length heteroplasmy observed in overlapping primer pairs 2902 and 2903.

mtDNA Plate Analysis Report – Page 2

Second page of report summarizes analysis parameters used

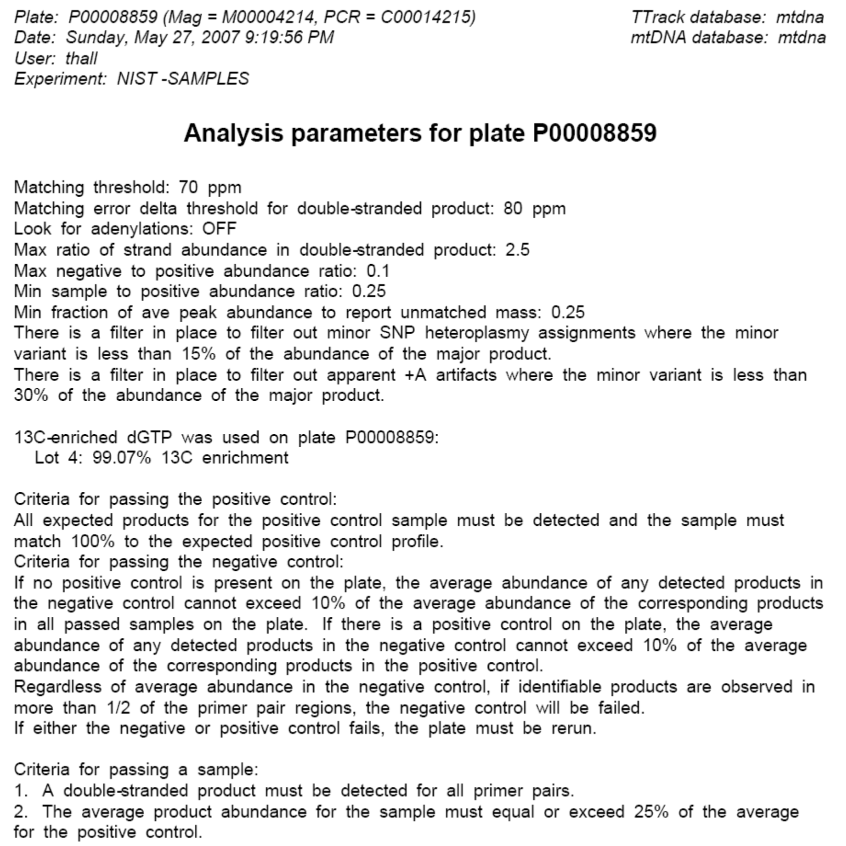


Plate: P00008859 (Mag = M00004214, PCR = C00014215)
Date: Sunday, May 27, 2007 9:19:56 PM
User: thall
Experiment: NIST -SAMPLES

TTrack database: mtdna
mtDNA database: mtdna

Analysis parameters for plate P00008859

Matching threshold: 70 ppm
Matching error delta threshold for double-stranded product: 80 ppm
Look for adenylations: OFF
Max ratio of strand abundance in double-stranded product: 2.5
Max negative to positive abundance ratio: 0.1
Min sample to positive abundance ratio: 0.25
Min fraction of ave peak abundance to report unmatched mass: 0.25
There is a filter in place to filter out minor SNP heteroplasmy assignments where the minor variant is less than 15% of the abundance of the major product.
There is a filter in place to filter out apparent +A artifacts where the minor variant is less than 30% of the abundance of the major product.

13C-enriched dGTP was used on plate P00008859:
Lot 4: 99.07% 13C enrichment

Criteria for passing the positive control:
All expected products for the positive control sample must be detected and the sample must match 100% to the expected positive control profile.
Criteria for passing the negative control:
If no positive control is present on the plate, the average abundance of any detected products in the negative control cannot exceed 10% of the average abundance of the corresponding products in all passed samples on the plate. If there is a positive control on the plate, the average abundance of any detected products in the negative control cannot exceed 10% of the average abundance of the corresponding products in the positive control.
Regardless of average abundance in the negative control, if identifiable products are observed in more than 1/2 of the primer pair regions, the negative control will be failed.
If either the negative or positive control fails, the plate must be rerun.

Criteria for passing a sample:
1. A double-stranded product must be detected for all primer pairs.
2. The average product abundance for the sample must equal or exceed 25% of the average for the positive control.

mtDNA Plate Analysis Report – Summary Page

- Each sample gets a summary page
  - Coverage map
  - Average abundance
  - Average error
  - Status
  - Comparison to other samples on the plate
  - Log of manual assignments and filtered assignments (if any)
  - User comments

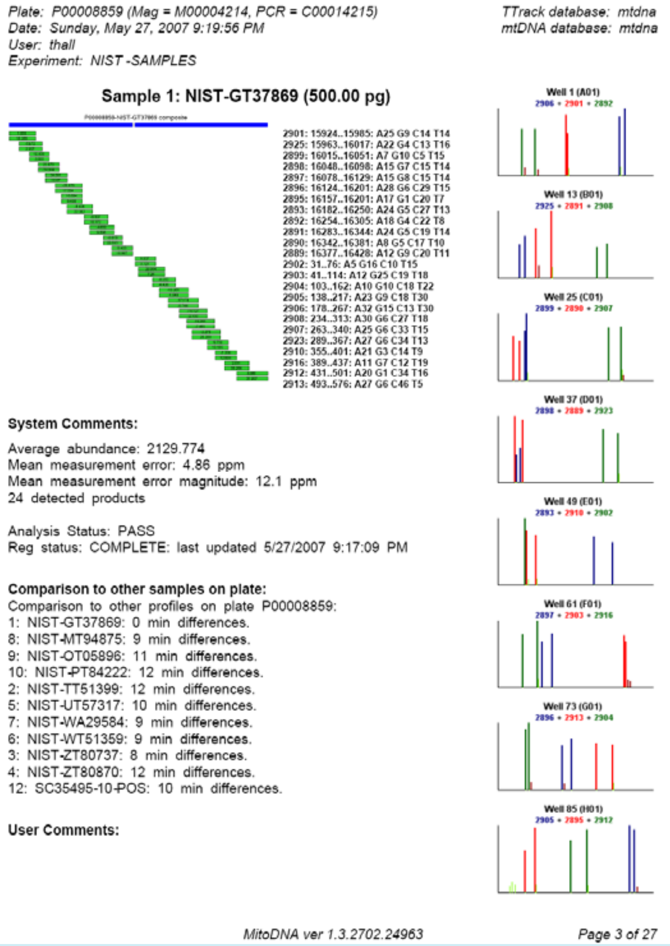


Plate: P00008859 (Mag = M00004214, PCR = C00014215)
Date: Sunday, May 27, 2007 9:19:56 PM
User: thall
Experiment: NIST -SAMPLES

TTrack database: mtdna
mtDNA database: mtdna

Sample 1: NIST-GT37869 (500.00 pg)

2901: 15924..15985: A25 G9 C14 T14
2925: 15963..16017: A22 G4 C13 T16
2899: 16015..16051: A7 G10 C5 T15
2898: 16048..16098: A15 G7 C15 T14
2897: 16078..16129: A15 G8 C15 T14
2896: 16124..16201: A28 G6 C29 T15
2895: 16157..16201: A17 G1 C20 T7
2893: 16182..16250: A24 G5 C27 T13
2892: 16254..16305: A18 G4 C22 T8
2891: 16283..16344: A24 G5 C19 T14
2890: 16342..16381: A8 G5 C17 T10
2889: 16377..16428: A12 G9 C20 T11
2902: 31..76: A5 G16 C10 T15
2903: 41..114: A12 G25 C19 T18
2904: 103..162: A10 G10 C18 T22
2905: 138..217: A23 G9 C18 T30
2906: 178..267: A32 G15 C13 T30
2908: 234..313: A30 G6 C27 T18
2907: 263..340: A25 G6 C33 T15
2923: 289..367: A27 G6 C34 T13
2910: 355..401: A21 G3 C14 T9
2916: 389..437: A11 G7 C12 T19
2912: 431..501: A20 G1 C34 T16
2913: 493..576: A27 G6 C46 T5

System Comments:

Average abundance: 2129.774
Mean measurement error: 4.86 ppm
Mean measurement error magnitude: 12.1 ppm
24 detected products

Analysis Status: PASS
Reg status: COMPLETE: last updated 5/27/2007 9:17:09 PM

Comparison to other samples on plate:
Comparison to other profiles on plate P00008859:
1: NIST-GT37869: 0 min differences.
8: NIST-MT94875: 9 min differences.
9: NIST-OT05896: 11 min differences.
10: NIST-PT84222: 12 min differences.
2: NIST-TT51399: 12 min differences.
5: NIST-UT57317: 10 min differences.
7: NIST-WA29584: 9 min differences.
6: NIST-WT51359: 9 min differences.
3: NIST-ZT80737: 8 min differences.
4: NIST-ZT80870: 12 min differences.
12: SC35495-10-POS: 10 min differences.

User Comments:

MitoDNA ver 1.3.2702.24963

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mtDNA Plate Analysis Report – Assignment Report

- At the end of the report is a comprehensive raw mass-to-product assignment report in table format for the entire plate that serves as a raw data log
- Every report generated is automatically archived into the database

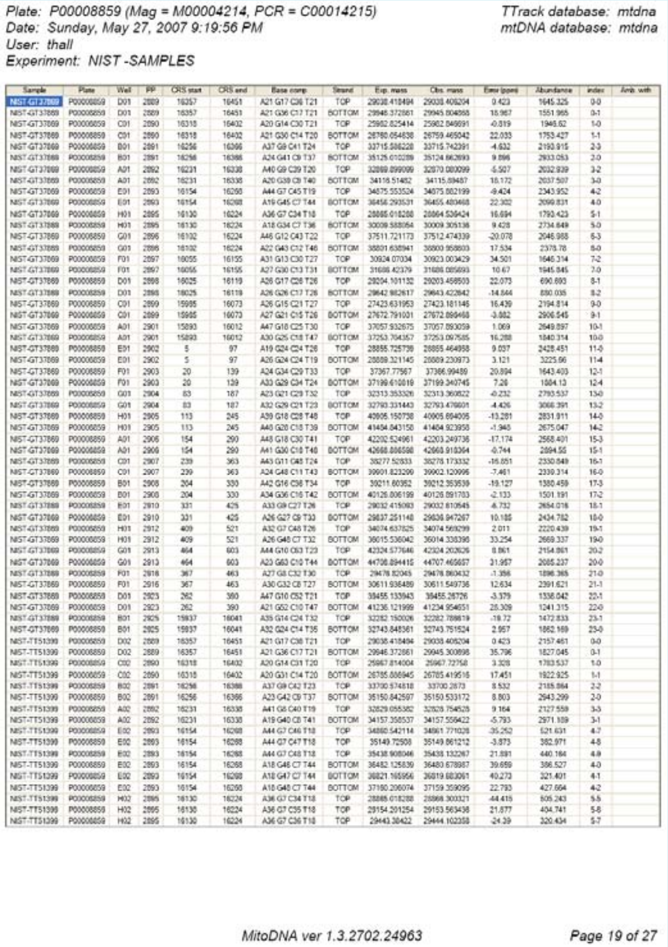

Plate: P00008859 (Mag = M00004214, PCR = C00014215)
Date: Sunday, May 27, 2007 9:19:56 PM
User: thall
Experiment: NIST -SAMPLES

TTrack database: mtdna
mtDNA database: mtdna

MitoDNA ver 1.3.2702.24963

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SNP Sample Analysis

- **Data processing is automated and utilizes the same code as mtDNA data processing**
- **SNP analysis interface is similar to mtDNA interface**
- **Allele names displayed (as SNP identities) instead of base compositions**

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SNP Sample Analysis

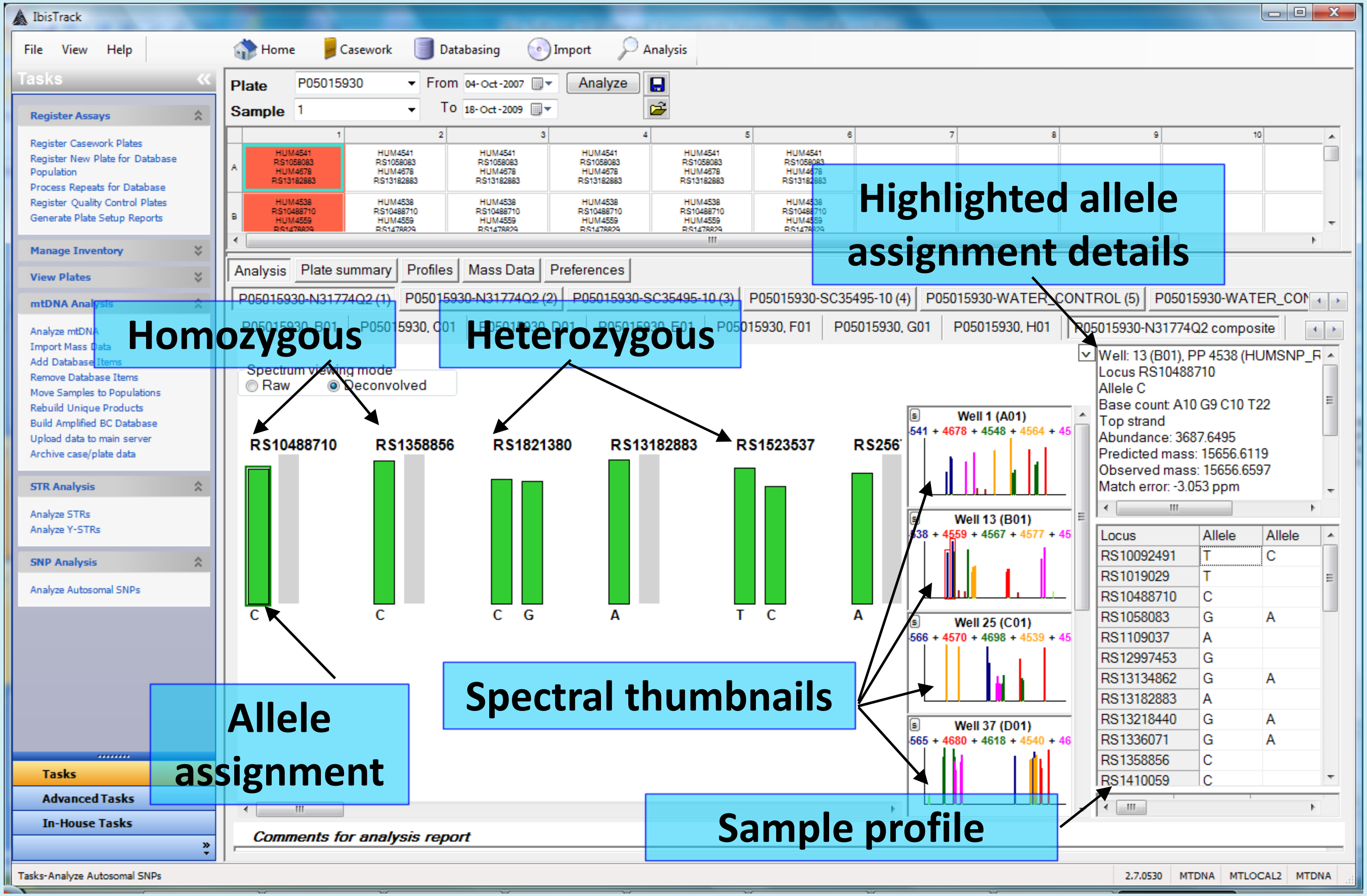

Plate P05015930 From 04-Oct-2007 To 18-Oct-2009

Sample 1

Spectrum viewing mode
○ Raw ● Deconvolved

Well: 13 (B01), PP 4538 (HUMSNP_R
Locus RS10488710
Allele C
Base count: A10 G9 C10 T22
Top strand
Abundance: 3687.6495
Predicted mass: 15656.6119
Observed mass: 15656.6597
Match error: -3.053 ppm

Locus	Allele	Allele
RS10092491	T	C
RS1019029	T	
RS10488710	C	
RS1058083	G	A
RS1109037	A	
RS12997453	G	
RS13134862	G	A
RS13182883	A	
RS13218440	G	A
RS1336071	G	A
RS1358856	C	
RS1410059	C	

Comments for analysis report

Highlighted allele assignment details

Homozygous

Heterozygous

Allele assignment

Spectral thumbnails

Sample profile

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SNP Sample Analysis – Plate Profile Summary

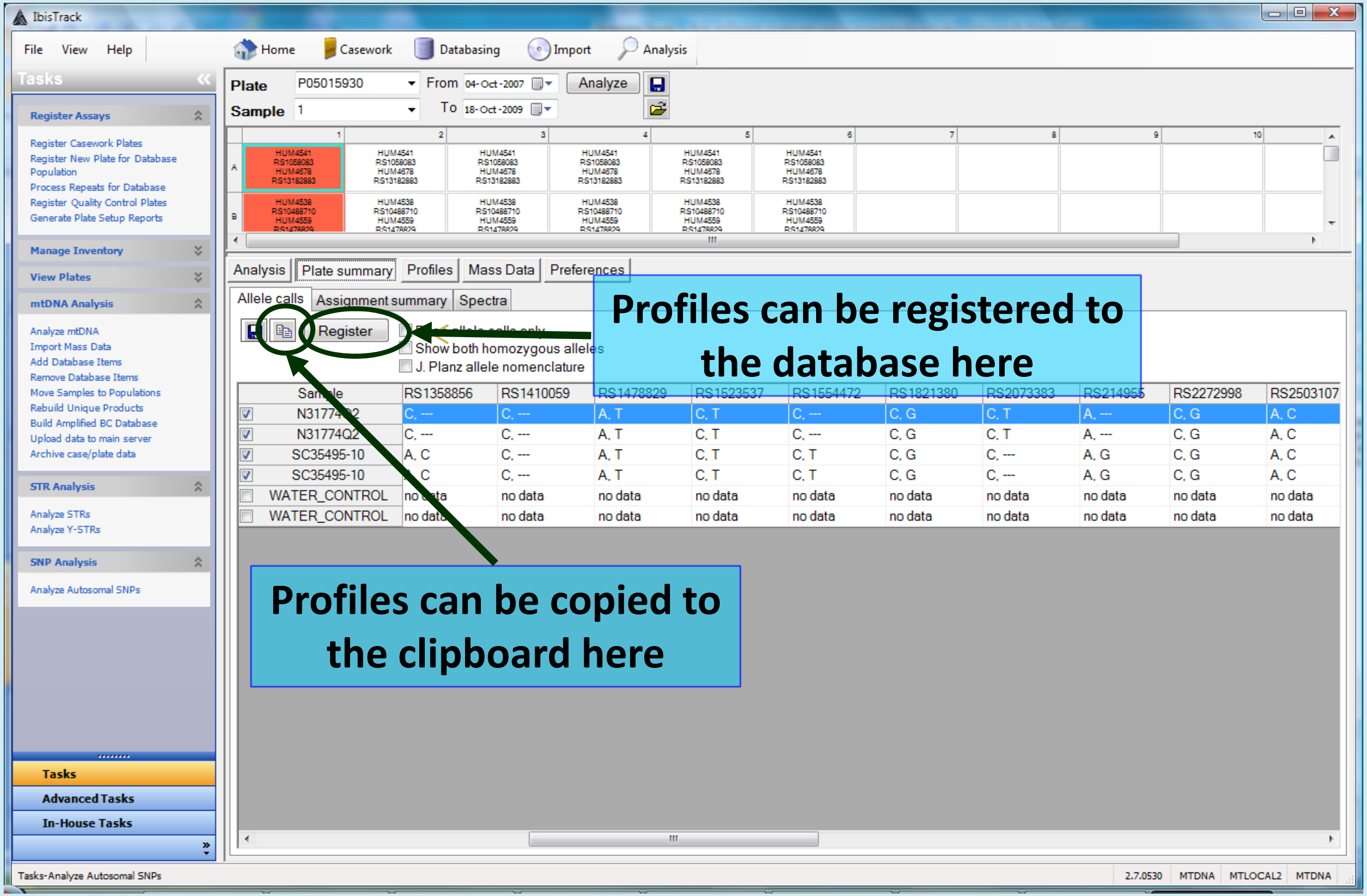

Plate P05015930 From 04-Oct-2007
Sample 1 To 18-Oct-2009

	Sample	RS1358856	RS1410059	RS1478829	RS1523537	RS1554472	RS1821380	RS2073383	RS214955	RS2272998	RS2503107
☑	N31774Q2	C, ---	C, ---	A, T	C, T	C, ---	C, G	C, T	A, ---	C, G	A, C
☑	N31774Q2	C, ---	C, ---	A, T	C, T	C, ---	C, G	C, T	A, ---	C, G	A, C
☑	SC35495-10	A, C	C, ---	A, T	C, T	C, T	C, G	C, ---	A, G	C, G	A, C
☑	SC35495-10	A, C	C, ---	A, T	C, T	C, T	C, G	C, ---	A, G	C, G	A, C
☐	WATER_CONTROL	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
☐	WATER_CONTROL	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data

Profiles can be registered to the database here

Profiles can be copied to the clipboard here

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SNP Sample Analysis – Profile Query

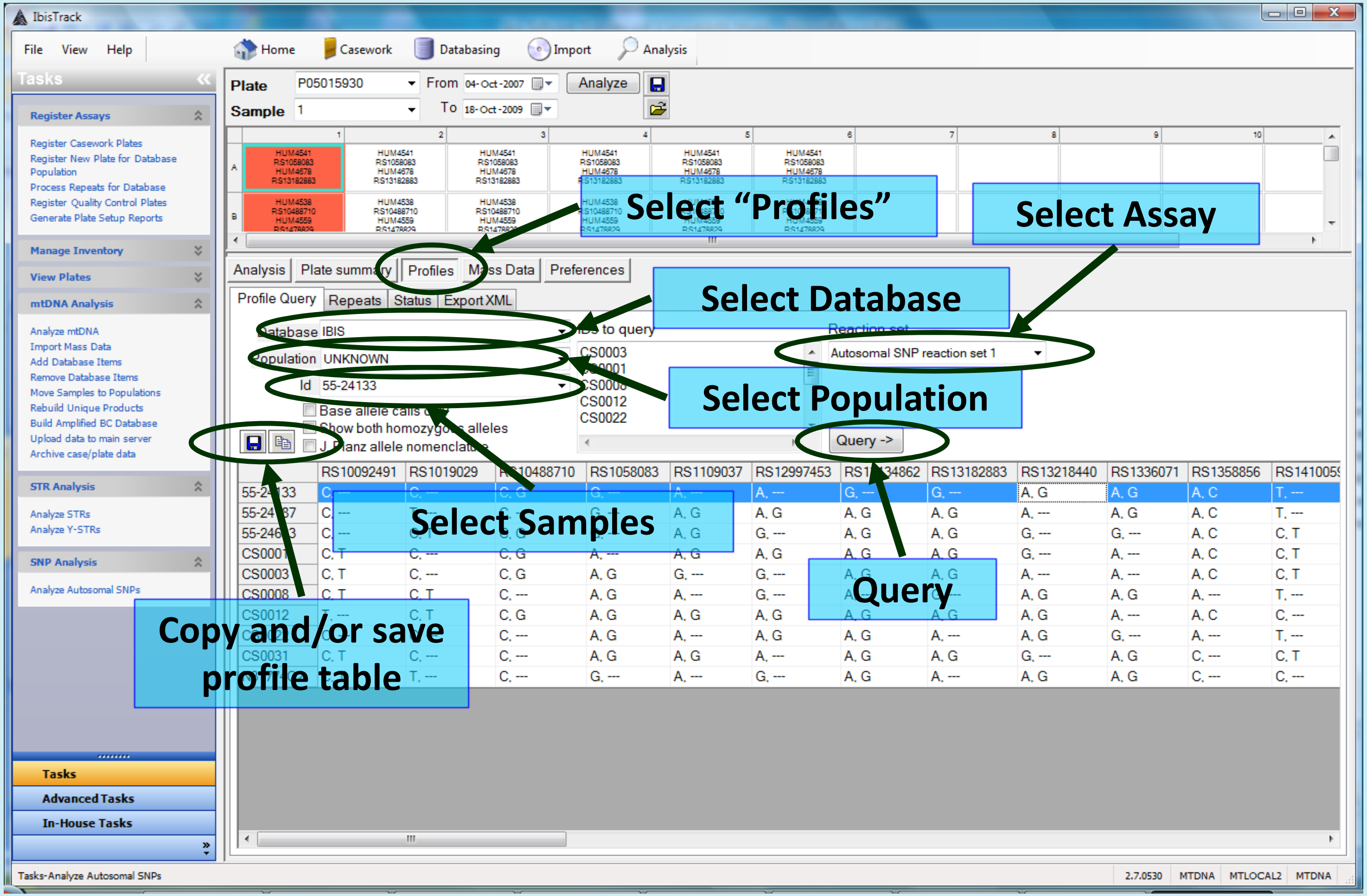

Plate P05015930 From 04-Oct-2007
Sample 1 To 18-Oct-2009

Database IBIS
Population UNKNOWN
Id 55-24133

IDs to query
CS0003
CS0001
CS0008
CS0012
CS0022

Reaction set
Autosomal SNP reaction set 1

☐ Base allele calls only
☐ Show both homozygous alleles
☐ J. Planz allele nomenclature

	RS10092491	RS1019029	RS10488710	RS1058083	RS1109037	RS12997453	RS1[illegible]34862	RS13182883	RS13218440	RS1336071	RS1358856	RS141005[illegible]
55-24133	C, ---	C, ---	C, G	G, ---	A, ---	A, ---	G, ---	G, ---	A, G	A, G	A, C	T, ---
[illegible]	C, ---	[illegible]	[illegible]	[illegible]	A, G	A, G	A, G	A, G	A, ---	A, G	A, C	T, ---
[illegible]	C, ---	C, T	C, G	[illegible]	A, G	G, ---	A, G	A, G	G, ---	G, ---	A, C	C, T
CS0001	C, T	C, ---	C, G	A, ---	A, G	A, G	A, G	A, G	G, ---	A, ---	A, C	C, T
CS0003	C, T	C, ---	C, G	A, G	G, ---	G, ---	A, G	A, G	A, ---	A, ---	A, C	C, T
CS0008	C, T	C, T	C, ---	A, G	A, ---	G, ---	[illegible]	[illegible]	A, G	A, G	A, ---	T, ---
CS0012	T, ---	C, T	C, G	A, G	A, G	A, G	A, G	A, G	A, G	A, ---	A, C	C, ---
[illegible]	[illegible]	[illegible]	C, ---	A, G	A, ---	A, G	A, G	A, ---	A, G	G, ---	A, ---	T, ---
CS0031	C, T	C, ---	C, ---	A, G	A, G	A, ---	A, G	A, G	G, ---	A, G	C, ---	C, T
[illegible]	[illegible]	T, ---	C, ---	G, ---	A, ---	G, ---	A, G	A, ---	A, G	A, G	C, ---	C, ---

Tasks-Analyze Autosomal SNPs 2.7.0530 MTDNA MTLOCAL2 MTDNA

Select "Profiles"

Select Assay

Select Database

Select Population

Select Samples

Query

Copy and/or save profile table

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Summary

- Data processing after MS is automated
- Processed data persist in database with registered sample and plate information
- Sample analysis is automated (triggered manually)
- Interface provides dynamic graphical functions to evaluate, QC, and adjust (if necessary) profile assignments
- Profiles can be registered a plate at a time
- Query profiles from database
- Automated comparison of profiles to database entries (mtDNA)
- Search database (mtDNA)
- Add sequences and convert to base compositions in the database (mtDNA)

Questions?

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***Note**: All images and charts courtesy of Tom Hall, Ph.D.*