Coffalyser Data analysis software

- What is it / what can it do
- Supported file formats
- Coffalyser data structure
- Installation & requirements (key content)
- Importing sample data
- Basic analysis settings / Normalization methods
- Troubleshooting with the log file
- Evaluation of quality check marks
- Troubleshooting on peak detection and size calling
- MLPA probe panelling & data filtering
- Result exploration & interpretation
- Effect of normalization methods
- Trouble shooting on slope correction data

COFFALYSER

MRC-Holland b.v.
MLPA

Coffalyser software updates

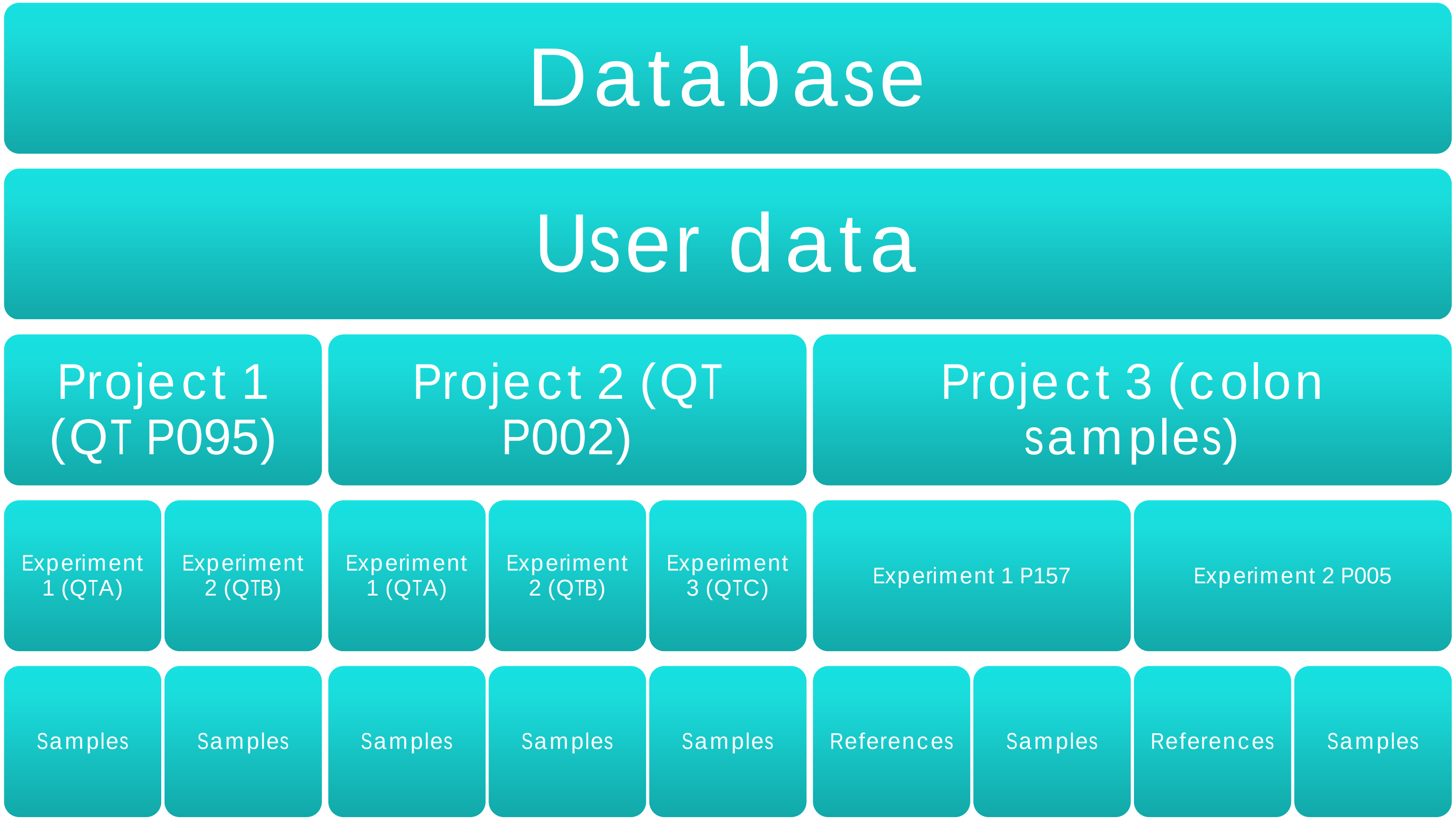
- Alpha test version
- Direct import of raw data from fragment analyzer
- Automatic software upgrades
- Automatic MLPA probe mix sheet updates
- Automatic raw data troubleshooting
- Wide array of analysis strategies
- Recommended analysis methods from MRC-holland
- Extensive result reporting options
- Current version has a file based database but future versions will have a SQL server database with secured data storage and sample oriented

Supported systems and file formats

- Raw data files
  - ABI all series (ABIF files, *.*fsa)
  - CEQ all series (raw data *.*SCF)
  - Megabace (RSD files)
  - Agilent (XML files)
- Fragment lists
  - Exported from the programs Genescan, Genemapper, Peak scanner, CEQ fragment analyzer
  - Exported from slabgels (LICOR, Spectrophotometrix)
  - From gel-scans (using Genetools)

- Raw data files allow for much more extensive troubleshooting methods and are therefore recommended

Coffalyser Data structure (files)

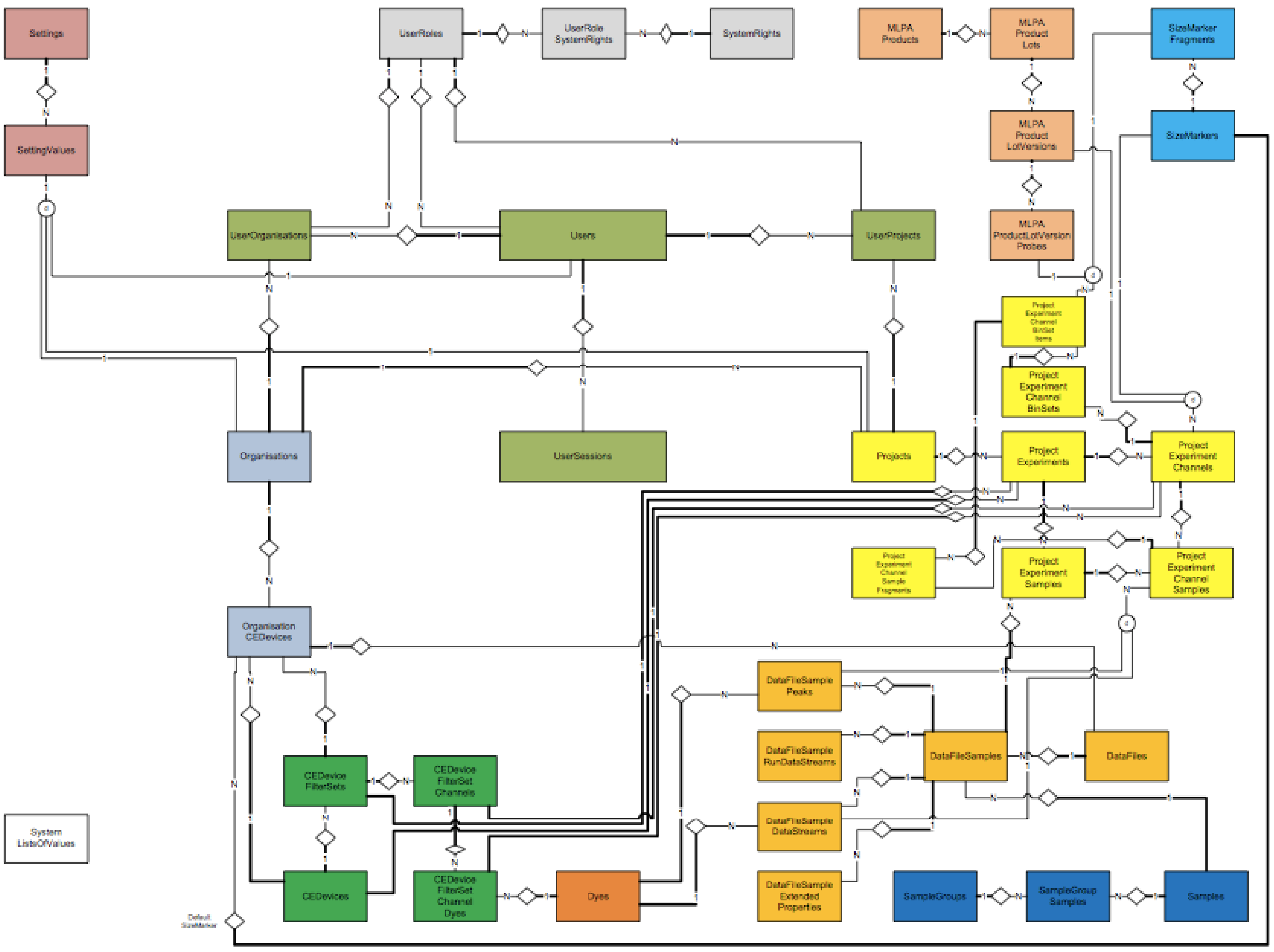


Database

User data

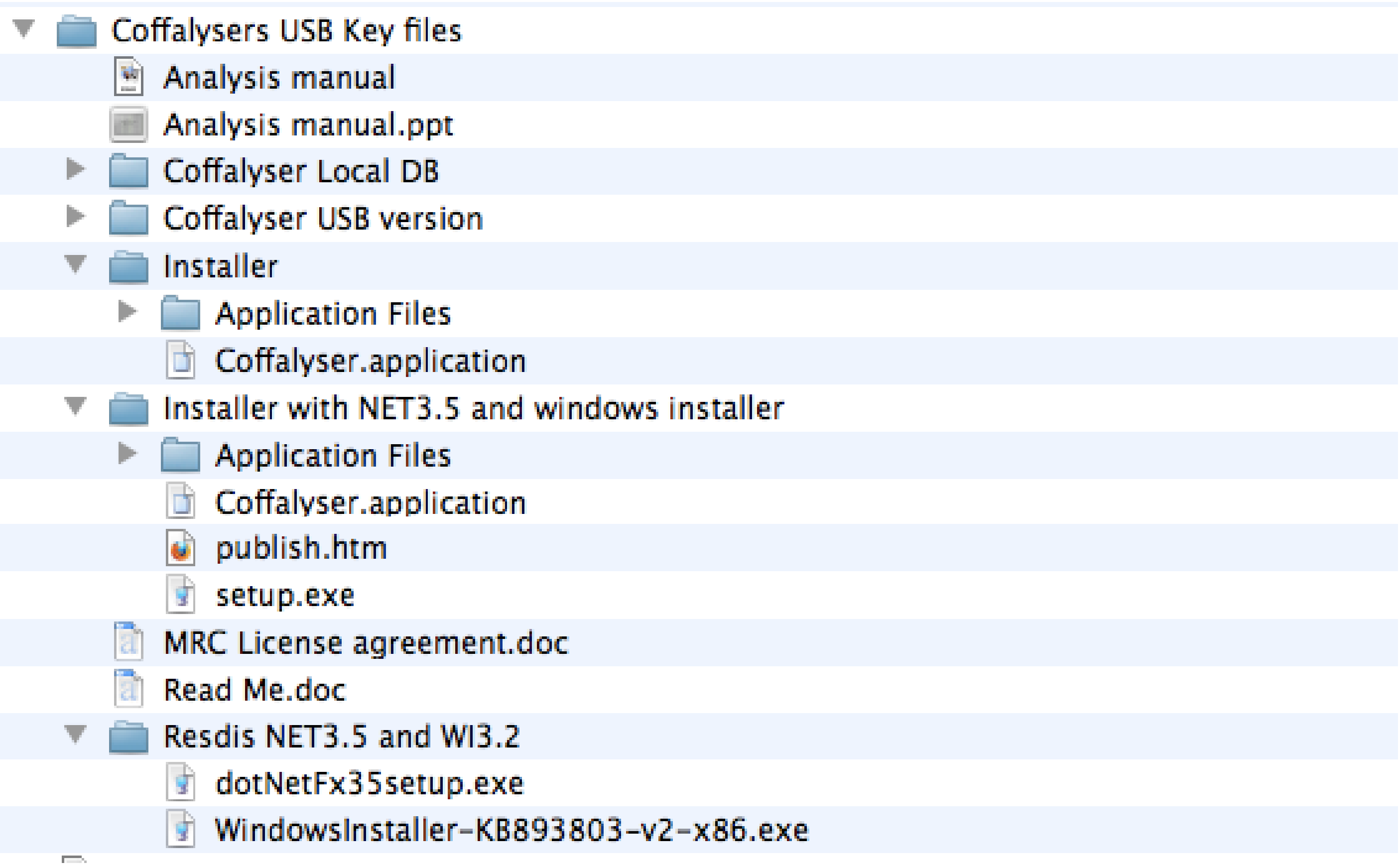
- Project 1 (QT P095)
  - Experiment 1 (QTA)
    - Samples
  - Experiment 2 (QTB)
    - Samples
- Project 2 (QT P002)
  - Experiment 1 (QTA)
    - Samples
  - Experiment 2 (QTB)
    - Samples
  - Experiment 3 (QTC)
    - Samples
- Project 3 (colon samples)
  - Experiment 1 P157
    - References
    - Samples
  - Experiment 2 P005
    - References
    - Samples

Coffalyser data structure (sql structure)



MRC-Holl

Key content



- Coffalysers USB Key files
  - Analysis manual
  - Analysis manual.ppt
  - Coffalyser Local DB
  - Coffalyser USB version
  - Installer
    - Application Files
    - Coffalyser.application
  - Installer with NET3.5 and windows installer
    - Application Files
    - Coffalyser.application
    - publish.htm
    - setup.exe
  - MRC License agreement.doc
  - Read Me.doc
  - Resdis NET3.5 and WI3.2
    - dotNetFx35setup.exe
    - WindowsInstaller-KB893803-v2-x86.exe

Run the installer from the webpage online or run the coffalyser.application file

MRC-Holland
Coffalyser

Name: Coffalyser

Version: 1.0.0.27

Publisher: MRC-Holland

System requirements

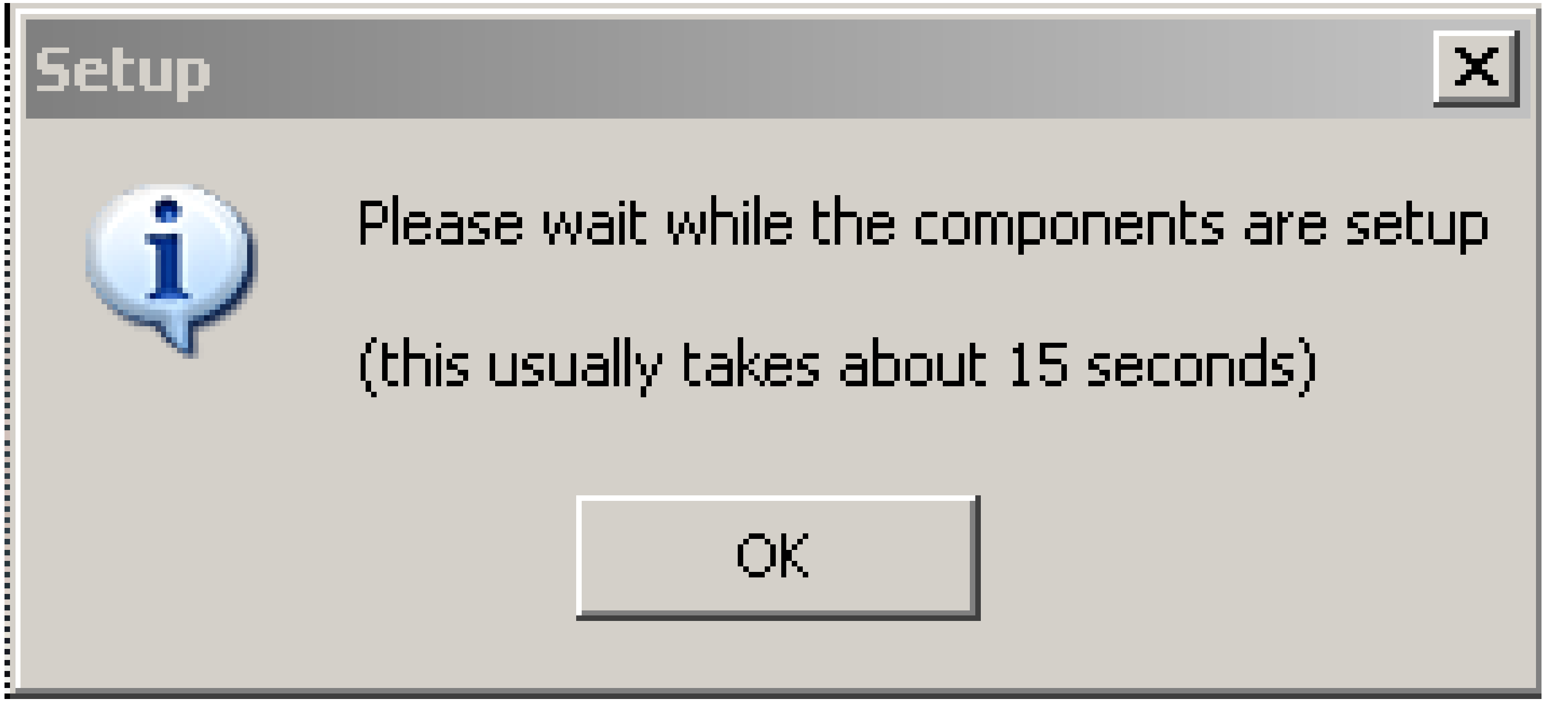
- The Coffalyser requires a computer with windows Xp or higher installed and updated with:

- (.NET 2.0), .NET 3.5 or higher
- Windows installer 3.1 (or higher)

If your computer is not updated with .Net 2.0 or windows installer

- Windows will not recognize the coffalyser.application file
- Update windows with .Net 2.0 and windows installer 3.1 and run the coffalyser.application file
- Or run the setup.exe file on the usb disk and following the installation instructions

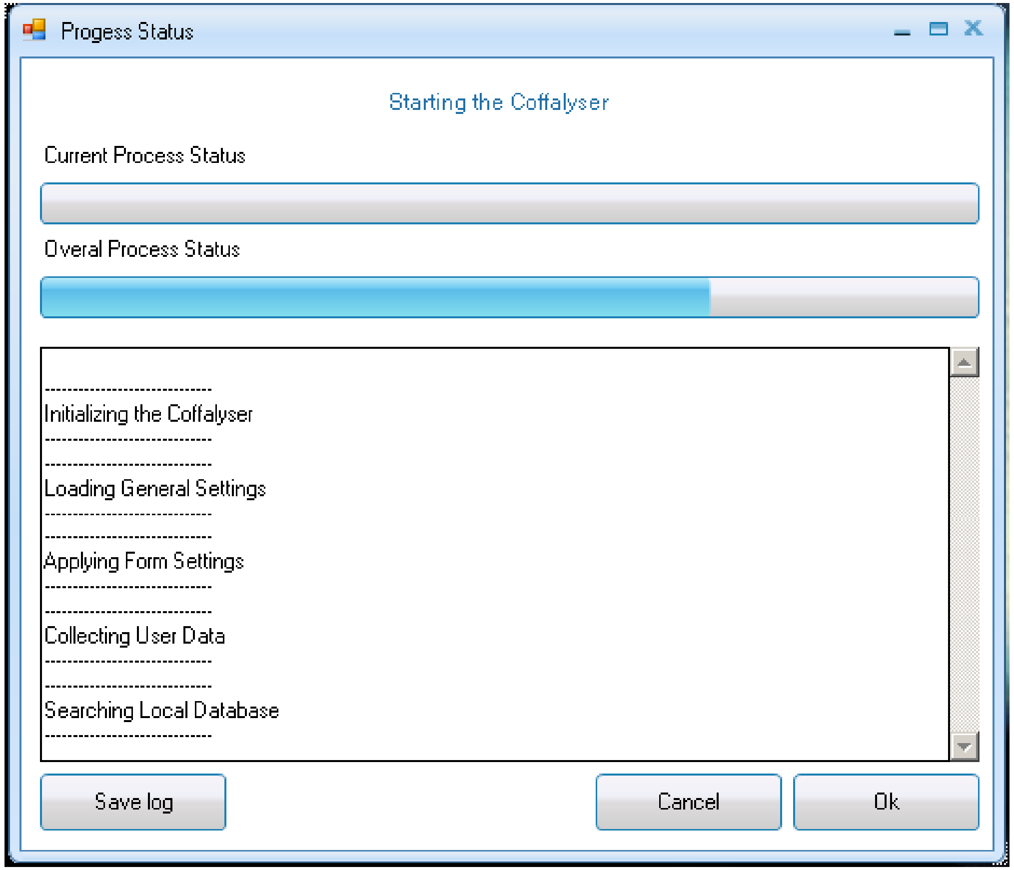
Next setup will install the needed components



Please wait while the components are setup

(this usually takes about 15 seconds)

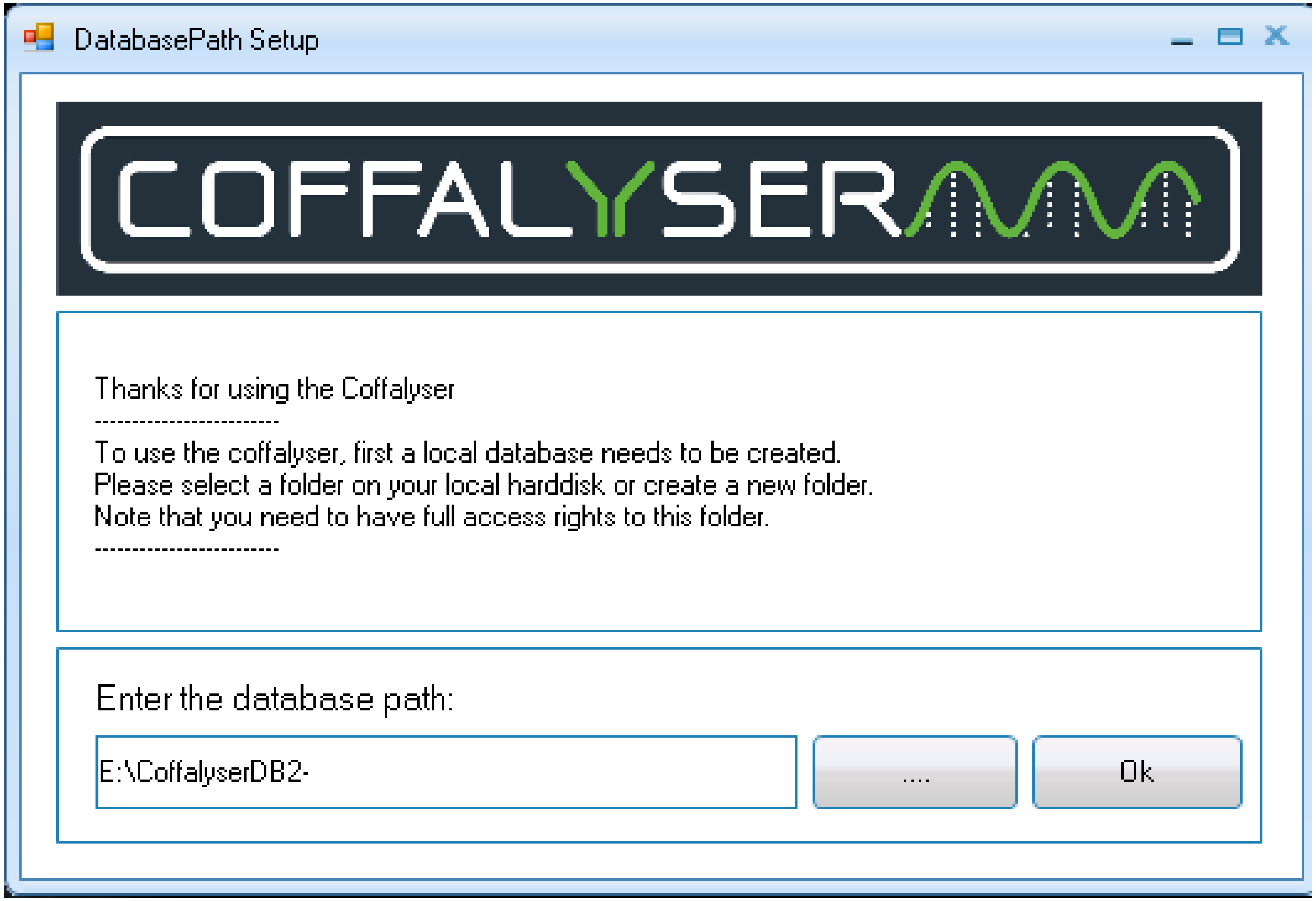
If the progress form appears the installation succeeded



Starting the Coffalyser

```
------------------------------
Initializing the Coffalyser
------------------------------
------------------------------
Loading General Settings
------------------------------
------------------------------
Applying Form Settings
------------------------------
------------------------------
Collecting User Data
------------------------------
------------------------------
Searching Local Database
------------------------------
```

The current Coffalyser database is file based. It is therefore recommended to install the database on your local hard drive. Do not keep the selected folder open windows explorer when the program is running.

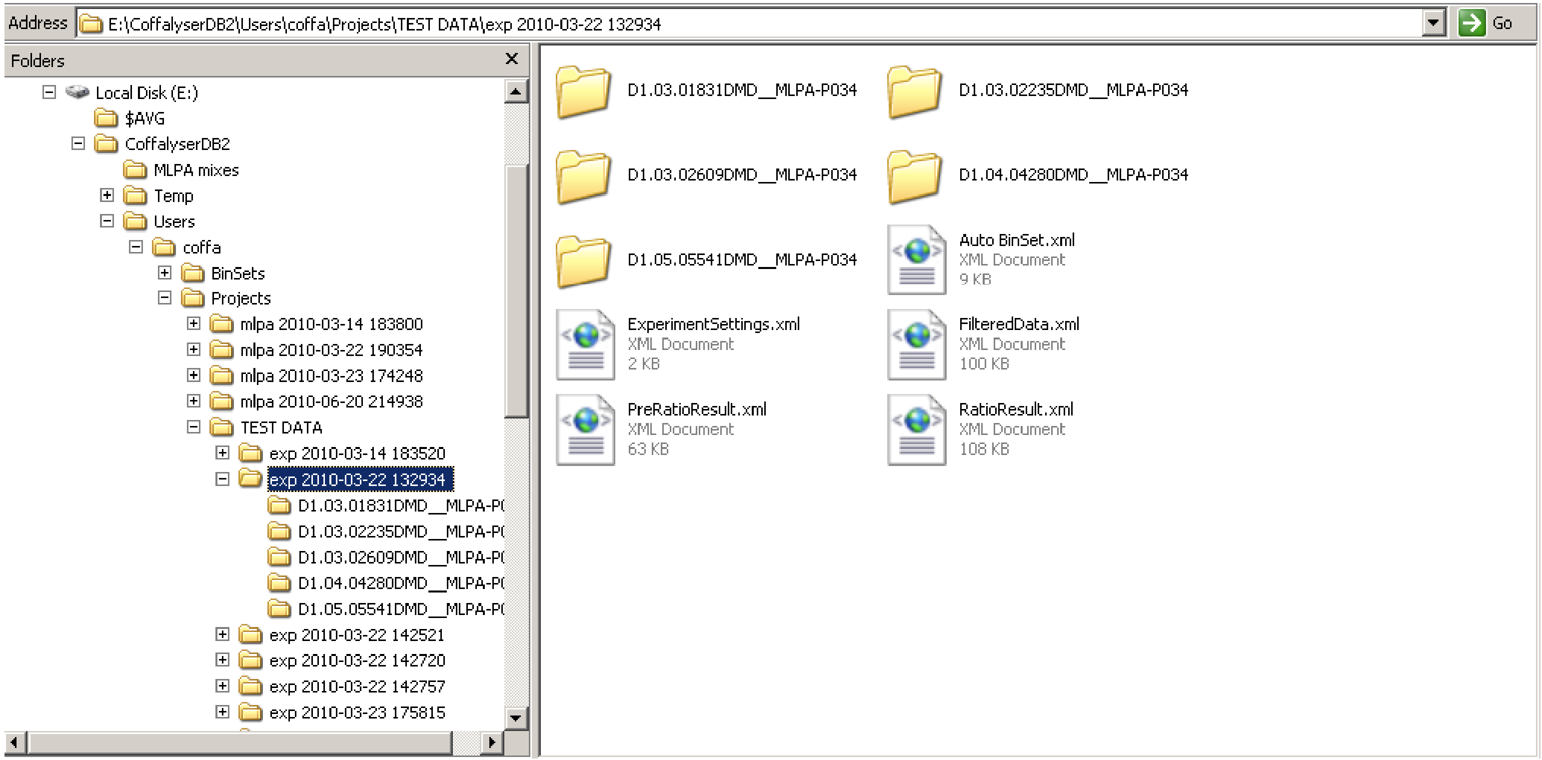


Thanks for using the Coffalyser

To use the coffalyser, first a local database needs to be created.
Please select a folder on your local harddisk or create a new folder.
Note that you need to have full access rights to this folder.

Enter the database path: E:\CoffalyserDB2-

Database folder structure



Address: E:\CoffalyserDB2\Users\coffa\Projects\TEST DATA\exp 2010-03-22 132934

Folders

- Local Disk (E:)
  - $AVG
  - CoffalyserDB2
    - MLPA mixes
    - Temp
    - Users
      - coffa
        - BinSets
        - Projects
          - mlpa 2010-03-14 183800
          - mlpa 2010-03-22 190354
          - mlpa 2010-03-23 174248
          - mlpa 2010-06-20 214938
          - TEST DATA
            - exp 2010-03-14 183520
            - exp 2010-03-22 132934
              - D1.03.01831DMD__MLPA-P
              - D1.03.02235DMD__MLPA-P
              - D1.03.02609DMD__MLPA-P
              - D1.04.04280DMD__MLPA-P
              - D1.05.05541DMD__MLPA-P
            - exp 2010-03-22 142521
            - exp 2010-03-22 142720
            - exp 2010-03-22 142757
            - exp 2010-03-23 175815

D1.03.01831DMD__MLPA-P034

D1.03.02235DMD__MLPA-P034

D1.03.02609DMD__MLPA-P034

D1.04.04280DMD__MLPA-P034

D1.05.05541DMD__MLPA-P034

Auto BinSet.xml
XML Document
9 KB

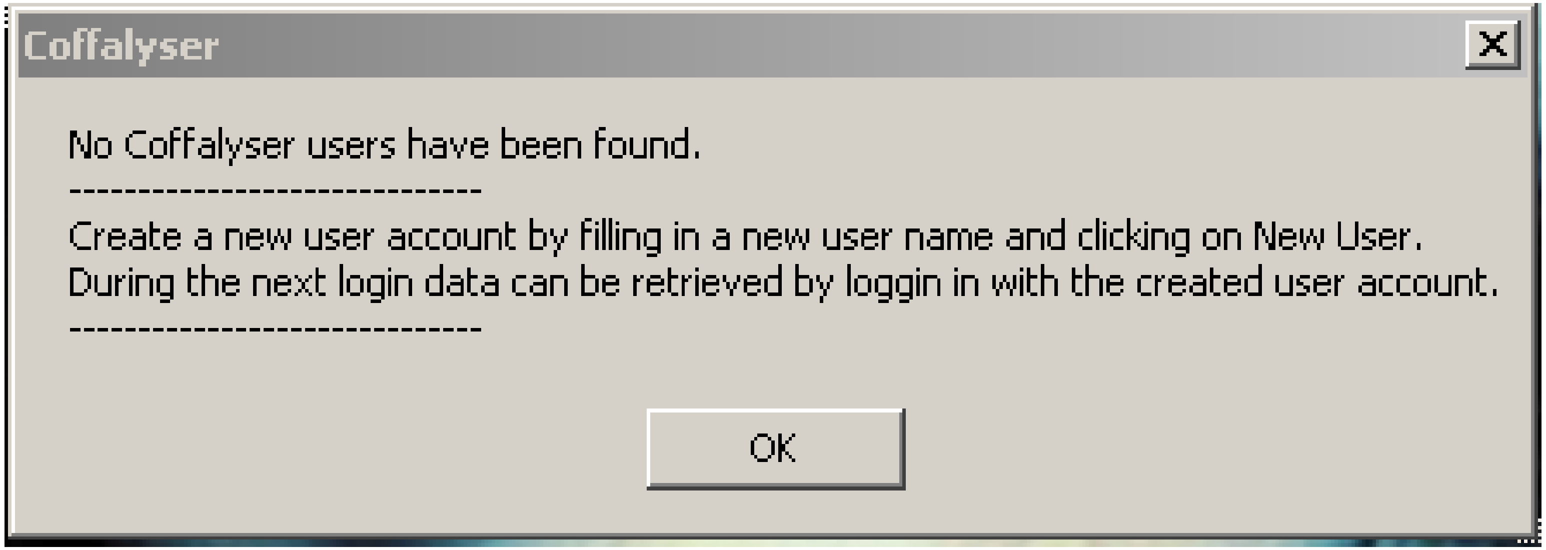
ExperimentSettings.xml
XML Document
2 KB

FilteredData.xml
XML Document
100 KB

PreRatioResult.xml
XML Document
63 KB

RatioResult.xml
XML Document
108 KB

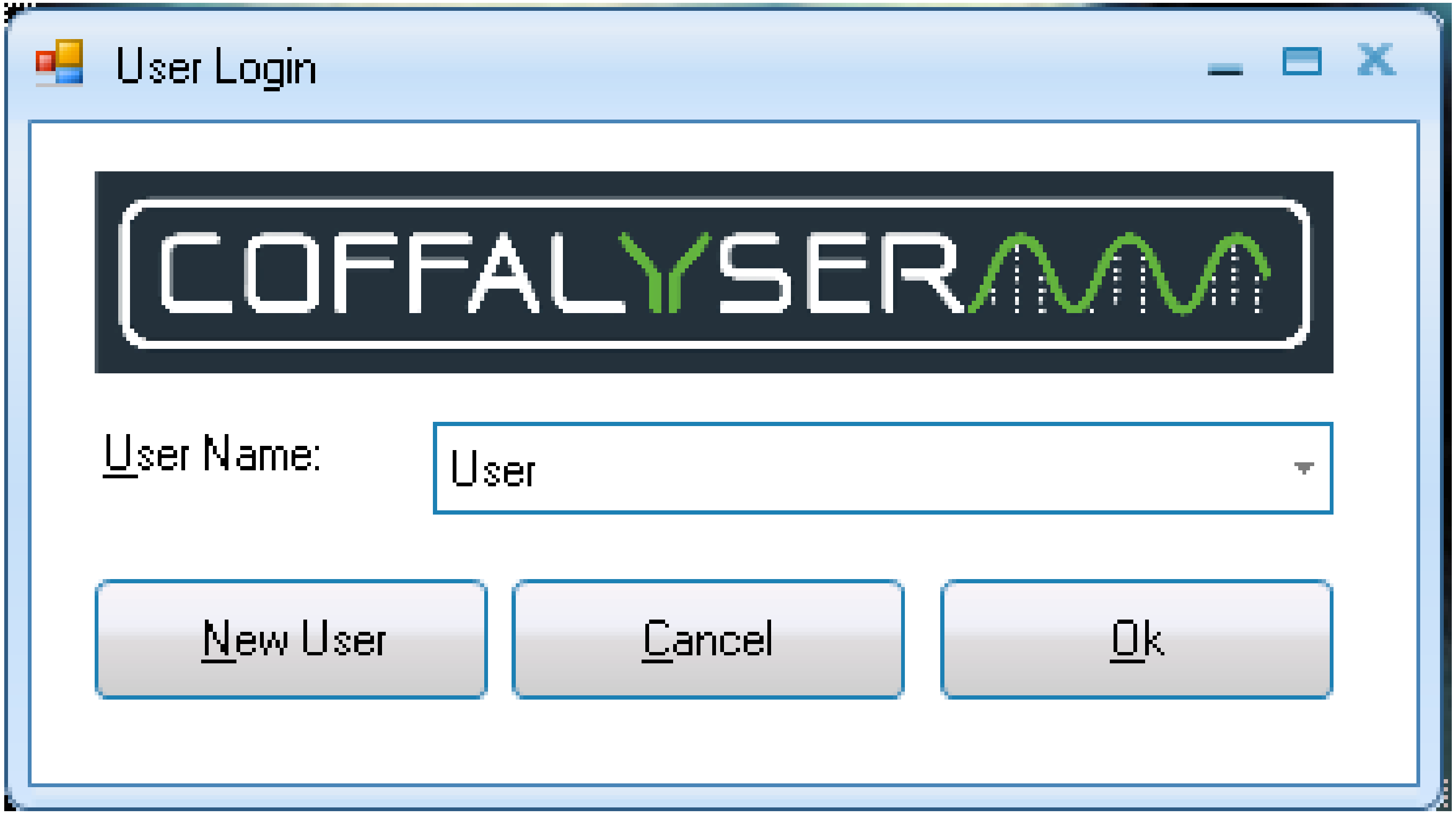
Select ok to create a new user



No Coffalyser users have been found.

Create a new user account by filling in a new user name and clicking on New User.
During the next login data can be retrieved by loggin in with the created user account.

Select New user

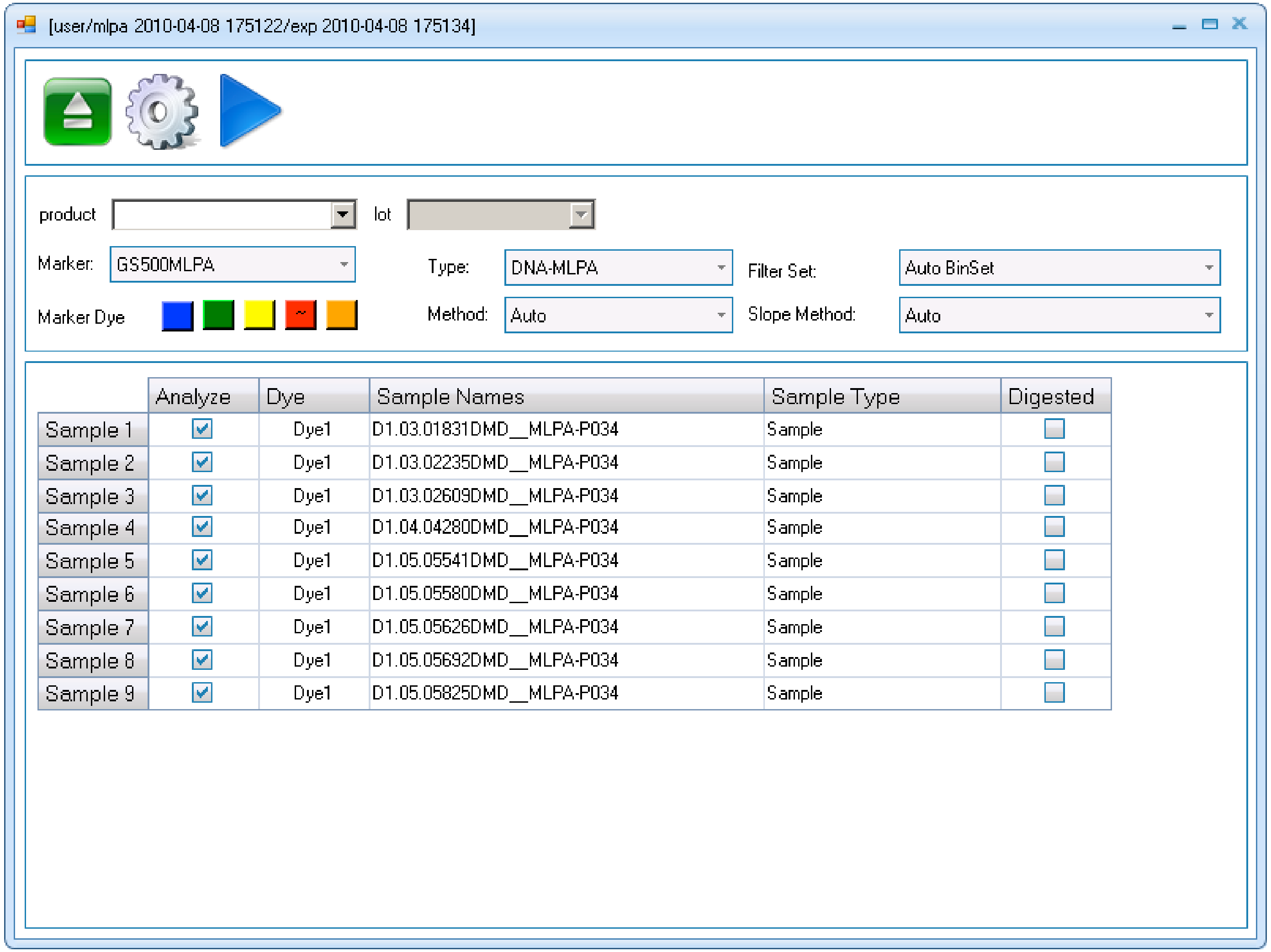


User Name: User

Importing sample data

- Test 1 P034 DMD 0707 (ABI-310)
    - With reference data
    - Using block method
    - Using population method (6014)
- Test 2 P034 DMD 0508 (ABI-3130)
    - Using population wo reference data
- P335 tumor analysis
    - Block method
    - Slope correction block & population

Basic analysis settings



[user/mlpa 2010-04-08 175122/exp 2010-04-08 175134]

product:

lot:

Marker: GS500MLPA

Marker Dye: 🟦 🟩 🟨 🟥 🟧

Type: DNA-MLPA

Method: Auto

Filter Set: Auto BinSet

Slope Method: Auto

	Analyze	Dye	Sample Names	Sample Type	Digested
Sample 1	☑	Dye1	D1.03.01831DMD__MLPA-P034	Sample	☐
Sample 2	☑	Dye1	D1.03.02235DMD__MLPA-P034	Sample	☐
Sample 3	☑	Dye1	D1.03.02609DMD__MLPA-P034	Sample	☐
Sample 4	☑	Dye1	D1.04.04280DMD__MLPA-P034	Sample	☐
Sample 5	☑	Dye1	D1.05.05541DMD__MLPA-P034	Sample	☐
Sample 6	☑	Dye1	D1.05.05580DMD__MLPA-P034	Sample	☐
Sample 7	☑	Dye1	D1.05.05626DMD__MLPA-P034	Sample	☐
Sample 8	☑	Dye1	D1.05.05692DMD__MLPA-P034	Sample	☐
Sample 9	☑	Dye1	D1.05.05825DMD__MLPA-P034	Sample	☐

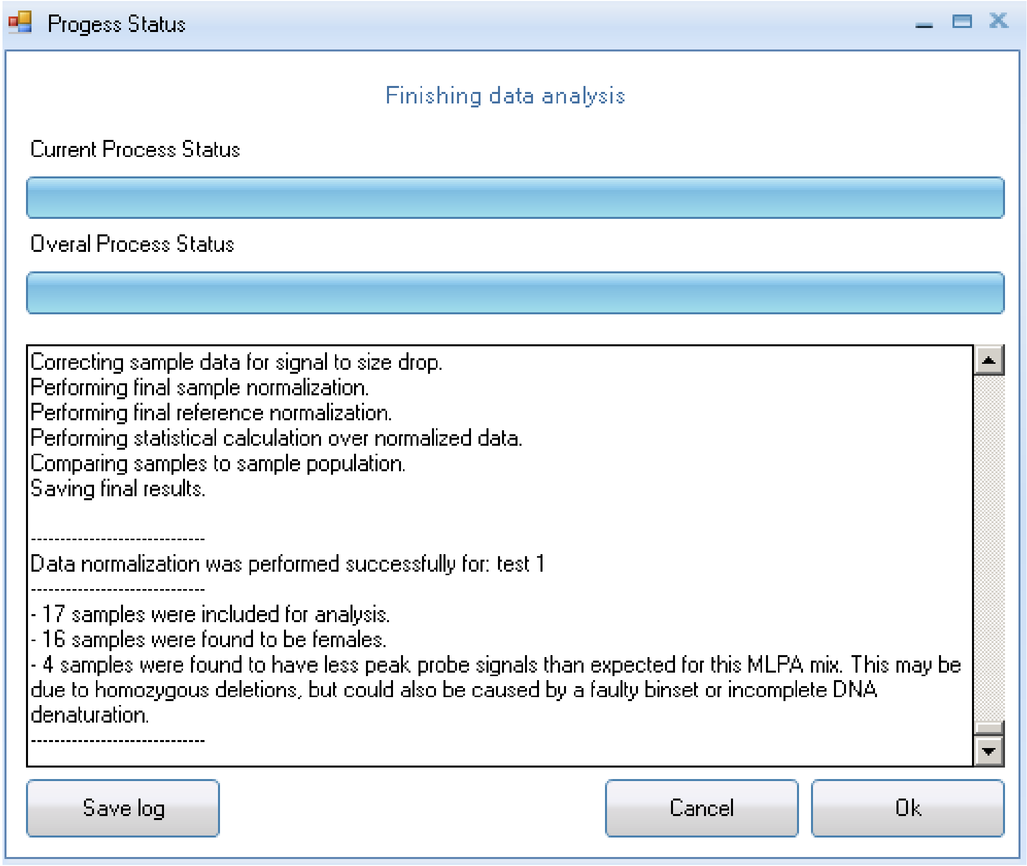
Analysis pre sets

- Size marker and dye color
- Normalization methods
  - auto
  - Block normalization
  - Population normalization
- Binset
  - Auto
  - Manual binsets
- Slope correction
  - auto
  - Block normalization
  - Population normalization
- Setting references samples type (key/click)

Data analysis steps

- Spectral signal correction
- Peak detection
- Size marker matcher
- Peak size calling
- Peak to probe matching
- Data filtering
- Data pre-normalization
- Size to signal drop correction
- Final normalization
- Calculation of population distribution and signal comparison

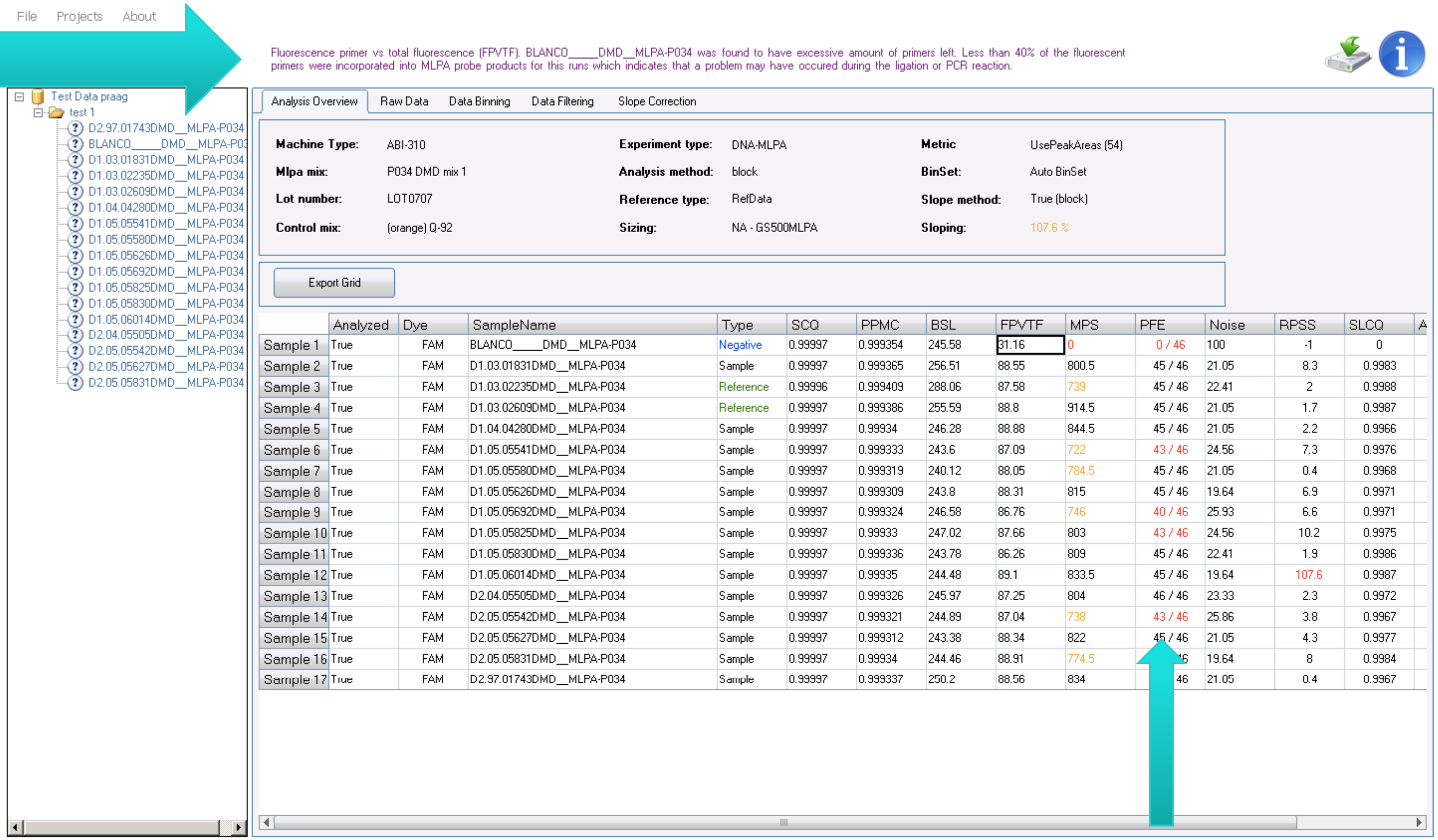
Troubleshooting with the log file & support



```
Correcting sample data for signal to size drop.
Performing final sample normalization.
Performing final reference normalization.
Performing statistical calculation over normalized data.
Comparing samples to sample population.
Saving final results.

------------------------------
Data normalization was performed successfully for: test 1
------------------------------
- 17 samples were included for analysis.
- 16 samples were found to be females.
- 4 samples were found to have less peak probe signals than expected for this MLPA mix. This may be
due to homozygous deletions, but could also be caused by a faulty binset or incomplete DNA
denaturation.
------------------------------
```

Evaluation of quality check marks



Fluorescence primer vs total fluorescence (FPVTF). BLANCO_____DMD__MLPA-P034 was found to have excessive amount of primers left. Less than 40% of the fluorescent primers were incorporated into MLPA probe products for this runs which indicates that a problem may have occured during the ligation or PCR reaction.

Machine Type: ABI-310
Mlpa mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92

Experiment type: DNA-MLPA
Analysis method: block
Reference type: RefData
Sizing: NA - GS500MLPA

Metric UsePeakAreas (54)
BinSet: Auto BinSet
Slope method: True (block)
Sloping: 107.6 %

	Analyzed	Dye	SampleName	Type	SCQ	PPMC	BSL	FPVTF	MPS	PFE	Noise	RPSS	SLCQ	A
Sample 1	True	FAM	BLANCO_____DMD__MLPA-P034	Negative	0.99997	0.999354	245.58	31.16	0	0 / 46	100	-1	0	
Sample 2	True	FAM	D1.03.01831DMD__MLPA-P034	Sample	0.99997	0.999365	256.51	88.55	800.5	45 / 46	21.05	8.3	0.9983	
Sample 3	True	FAM	D1.03.02235DMD__MLPA-P034	Reference	0.99996	0.999409	288.06	87.58	739	45 / 46	22.41	2	0.9988	
Sample 4	True	FAM	D1.03.02609DMD__MLPA-P034	Reference	0.99997	0.999386	255.59	88.8	914.5	45 / 46	21.05	1.7	0.9987	
Sample 5	True	FAM	D1.04.04280DMD__MLPA-P034	Sample	0.99997	0.99934	246.28	88.88	844.5	45 / 46	21.05	2.2	0.9966	
Sample 6	True	FAM	D1.05.05541DMD__MLPA-P034	Sample	0.99997	0.999333	243.6	87.09	722	43 / 46	24.56	7.3	0.9976	
Sample 7	True	FAM	D1.05.05580DMD__MLPA-P034	Sample	0.99997	0.999319	240.12	88.05	784.5	45 / 46	21.05	0.4	0.9968	
Sample 8	True	FAM	D1.05.05626DMD__MLPA-P034	Sample	0.99997	0.999309	243.8	88.31	815	45 / 46	19.64	6.9	0.9971	
Sample 9	True	FAM	D1.05.05692DMD__MLPA-P034	Sample	0.99997	0.999324	246.58	86.76	746	40 / 46	25.93	6.6	0.9971	
Sample 10	True	FAM	D1.05.05825DMD__MLPA-P034	Sample	0.99997	0.99933	247.02	87.66	803	43 / 46	24.56	10.2	0.9975	
Sample 11	True	FAM	D1.05.05830DMD__MLPA-P034	Sample	0.99997	0.999336	243.78	86.26	809	45 / 46	22.41	1.9	0.9986	
Sample 12	True	FAM	D1.05.06014DMD__MLPA-P034	Sample	0.99997	0.99935	244.48	89.1	833.5	45 / 46	19.64	107.6	0.9987	
Sample 13	True	FAM	D2.04.05505DMD__MLPA-P034	Sample	0.99997	0.999326	245.97	87.25	804	46 / 46	23.33	2.3	0.9972	
Sample 14	True	FAM	D2.05.05542DMD__MLPA-P034	Sample	0.99997	0.999321	244.89	87.04	738	43 / 46	25.86	3.8	0.9967	
Sample 15	True	FAM	D2.05.05627DMD__MLPA-P034	Sample	0.99997	0.999312	243.38	88.34	822	45 / 46	21.05	4.3	0.9977	
Sample 16	True	FAM	D2.05.05831DMD__MLPA-P034	Sample	0.99997	0.99934	244.46	88.91	774.5	6	19.64	8	0.9984	
Sample 17	True	FAM	D2.97.01743DMD__MLPA-P034	Sample	0.99997	0.999337	250.2	88.56	834	46	21.05	0.4	0.9967	

Missing signals

Fragment separation quality

- Size call quality
  - R^2 correlation (SCQ)
  - Pearson product moment correlation (PPMC)
- Baseline height (BSL)
- Noise
- Maximal run signal check (MAS)
- Maximal Probe signal check (MAPS)
- Median probe signal check (MPS)

- Troubleshooting
  - Adjust fragment separation conditions
  - Adjust Injection mixture composition

MLPA DNA quality

- DNA concentration check (DNA)
- DNA denaturation check (DD)
- Presence of X and Y fragment (X/Y –Frag)
- Number of expected / found probe fragments

MLPA PCR quality

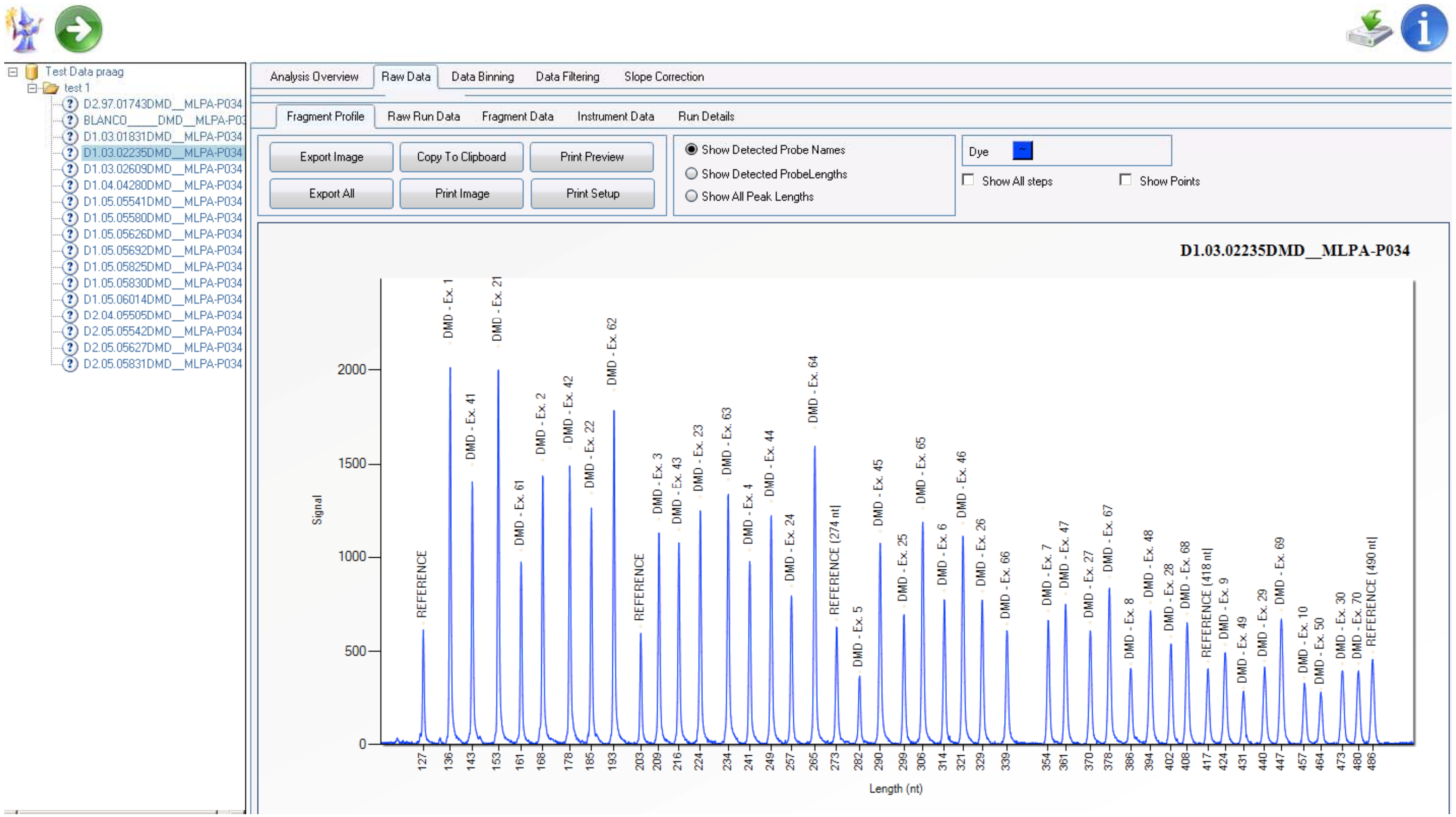
- Percentage of primer incorporated in MLPA probes (FPVSTF)
- Relative signals sloping difference between average/median reference and test samples (RPSS)
- Percentage signal drop on probe heights (machine)
- Percentage signal drop on probe areas (PCR)

Quality of normalization

- Slope correction quality (SLCQ)
- Normalization factor quality (AMAD)

- Probe distribution calculations

Checking electropherograms



● Show Detected Probe Names
○ Show Detected ProbeLengths
○ Show All Peak Lengths

Dye

☐ Show All steps
☐ Show Points

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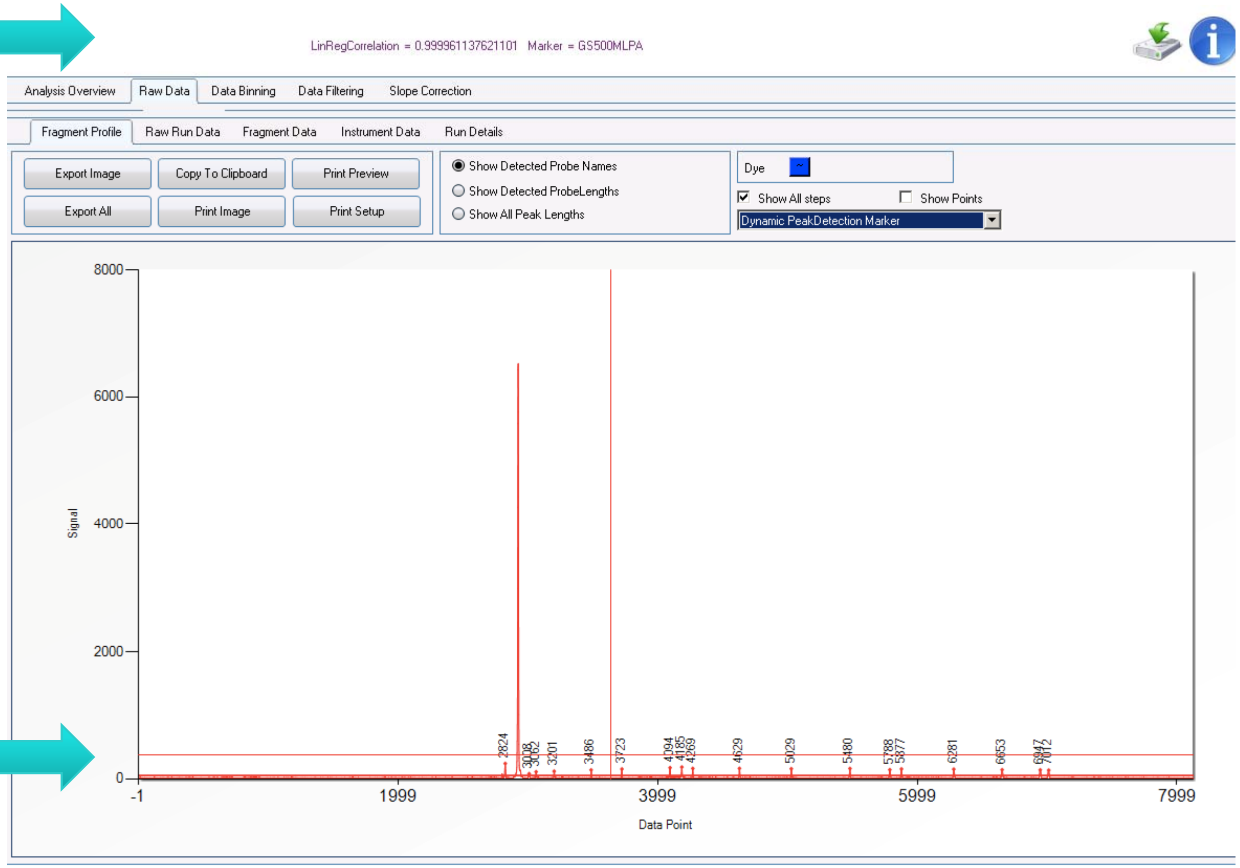
Electropherogram views

- Filtered view with probe ID
- Filtered view with probe lengths
- Unfiltered detected peaks line chart view with start end point
- Unfiltered detected peaks data-point view with start end point
- Step by steps peak detection and size calling

Troubleshooting with peak detection

Step info

Peak height line



LinRegCorrelation = 0.999961137621101 Marker = GS500MLPA

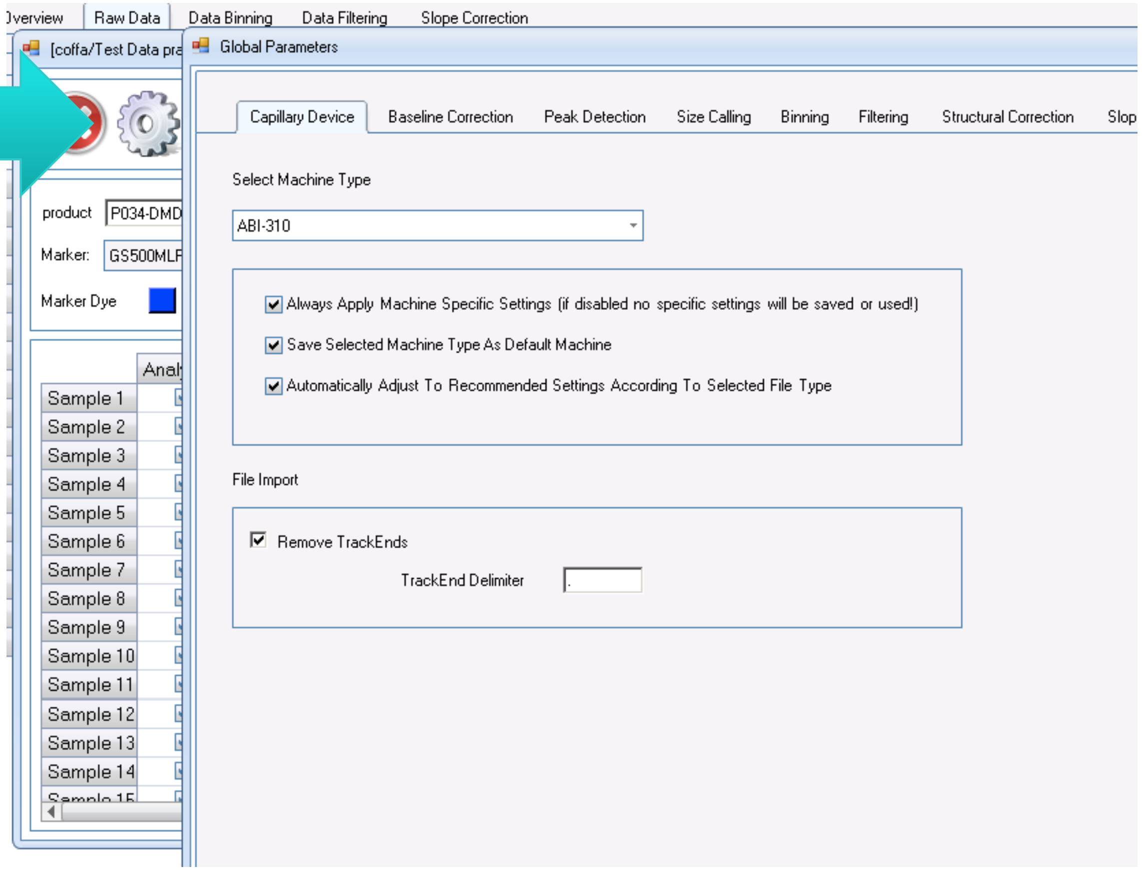
● Show Detected Probe Names
○ Show Detected ProbeLengths
○ Show All Peak Lengths

Dye 🟦

☑ Show All steps ☐ Show Points

Dynamic PeakDetection Marker

About machine profiles and peak detection settings



product P034-DMD

Marker: GS500MLF

Marker Dye 🟦

Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
Sample 11
Sample 12
Sample 13
Sample 14
Sample 15

Select Machine Type

ABI-310

☑ Always Apply Machine Specific Settings (if disabled no specific settings will be saved or used!)

☑ Save Selected Machine Type As Default Machine

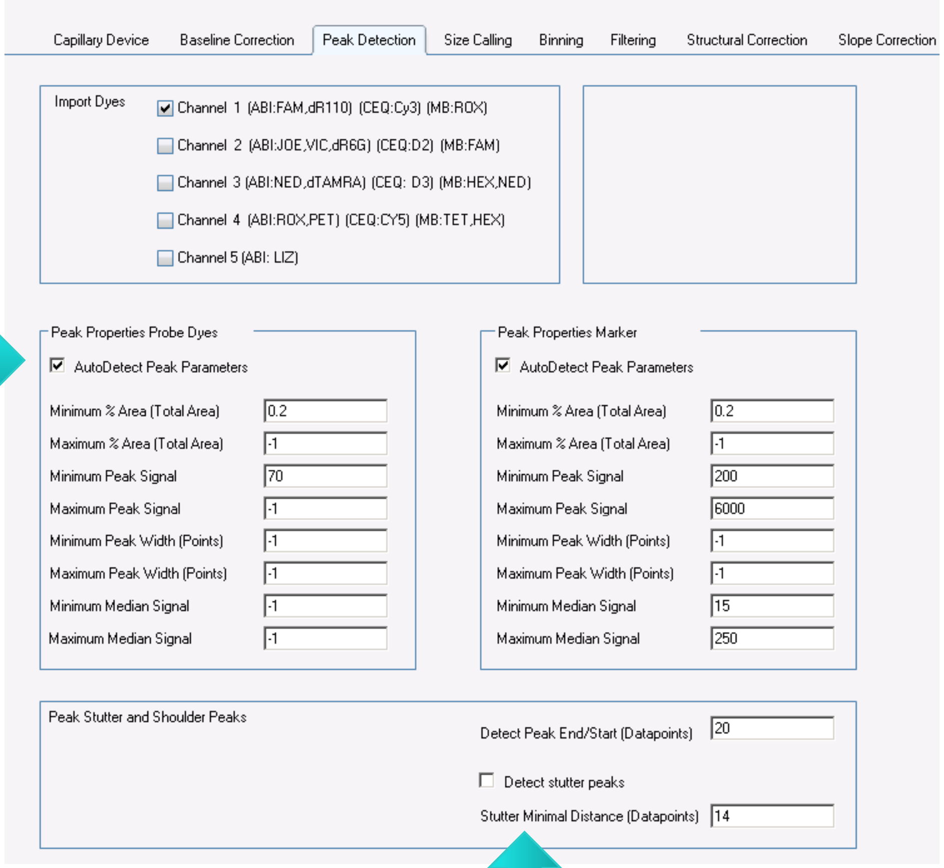
☑ Automatically Adjust To Recommended Settings According To Selected File Type

File Import

☑ Remove TrackEnds

TrackEnd Delimiter .

About peak detection settings



Import Dyes
- ☑ Channel 1 (ABI:FAM,dR110) (CEQ:Cy3) (MB:ROX)
- ☐ Channel 2 (ABI:JOE,VIC,dR6G) (CEQ:D2) (MB:FAM)
- ☐ Channel 3 (ABI:NED,dTAMRA) (CEQ: D3) (MB:HEX,NED)
- ☐ Channel 4 (ABI:ROX,PET) (CEQ:CY5) (MB:TET,HEX)
- ☐ Channel 5 (ABI: LIZ)

Peak Properties Probe Dyes

☑ AutoDetect Peak Parameters

Minimum % Area (Total Area)	0.2
Maximum % Area (Total Area)	-1
Minimum Peak Signal	70
Maximum Peak Signal	-1
Minimum Peak Width (Points)	-1
Maximum Peak Width (Points)	-1
Minimum Median Signal	-1
Maximum Median Signal	-1

Peak Properties Marker

☑ AutoDetect Peak Parameters

Minimum % Area (Total Area)	0.2
Maximum % Area (Total Area)	-1
Minimum Peak Signal	200
Maximum Peak Signal	6000
Minimum Peak Width (Points)	-1
Maximum Peak Width (Points)	-1
Minimum Median Signal	15
Maximum Median Signal	250

Peak Stutter and Shoulder Peaks

Detect Peak End/Start (Datapoints) 20

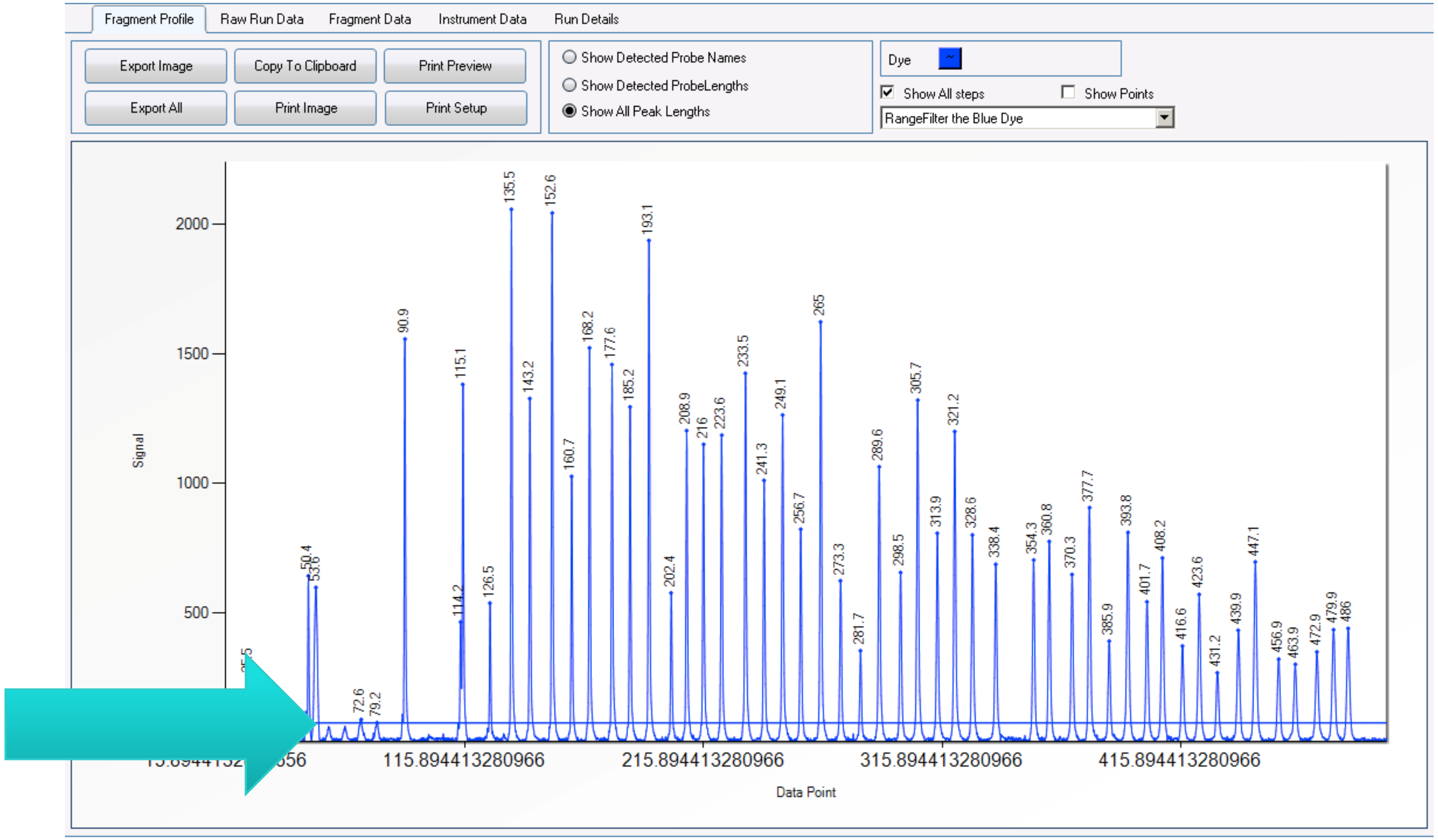
☐ Detect stutter peaks

Stutter Minimal Distance (Datapoints) 14

Peak detection settings

- Peak signal height (min/max)
- Percentage peak area vs total fluorescence
- Peak width
- Peak median signal (min/max)
- Stutter peak

Minimal probe peak signal



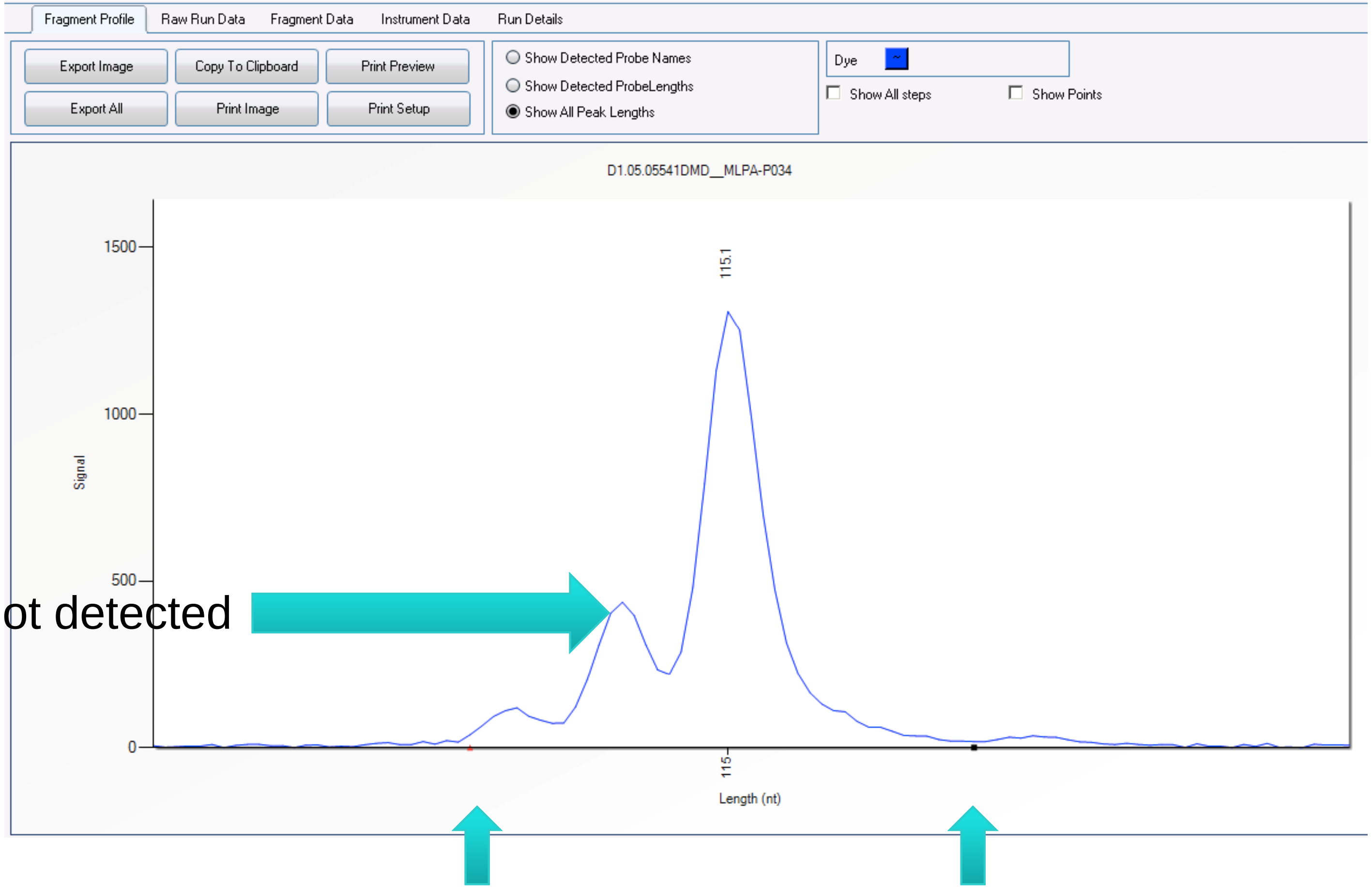
○ Show Detected Probe Names
○ Show Detected ProbeLengths
● Show All Peak Lengths

Dye

☑ Show All steps
☐ Show Points

RangeFilter the Blue Dye

Stutter peak / stutter peak detection off



○ Show Detected Probe Names
○ Show Detected ProbeLengths
● Show All Peak Lengths
☐ Show All steps
☐ Show Points

Stutter peak not detected

Peak area signal

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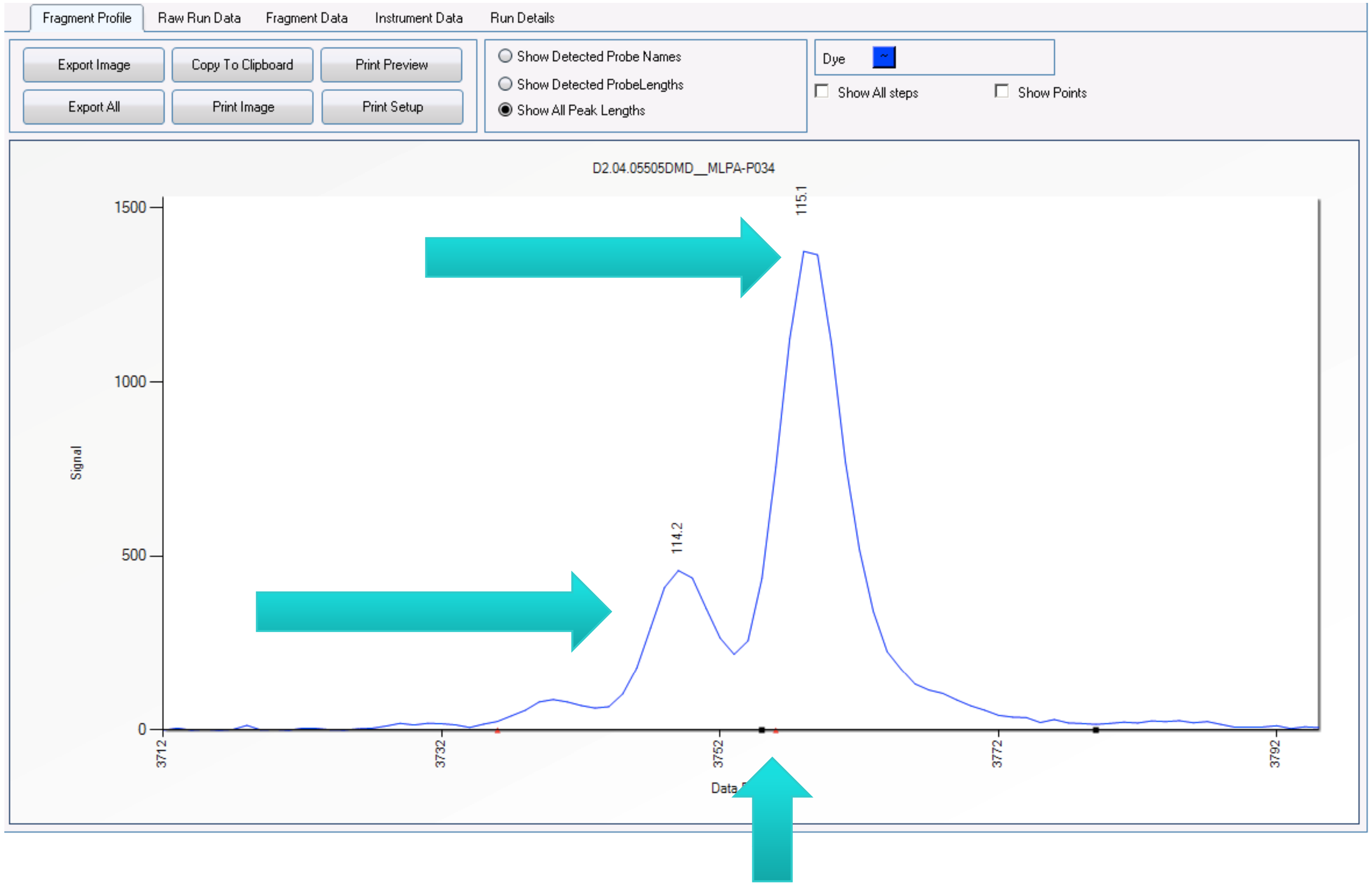
Stutter peak / split peak detection on

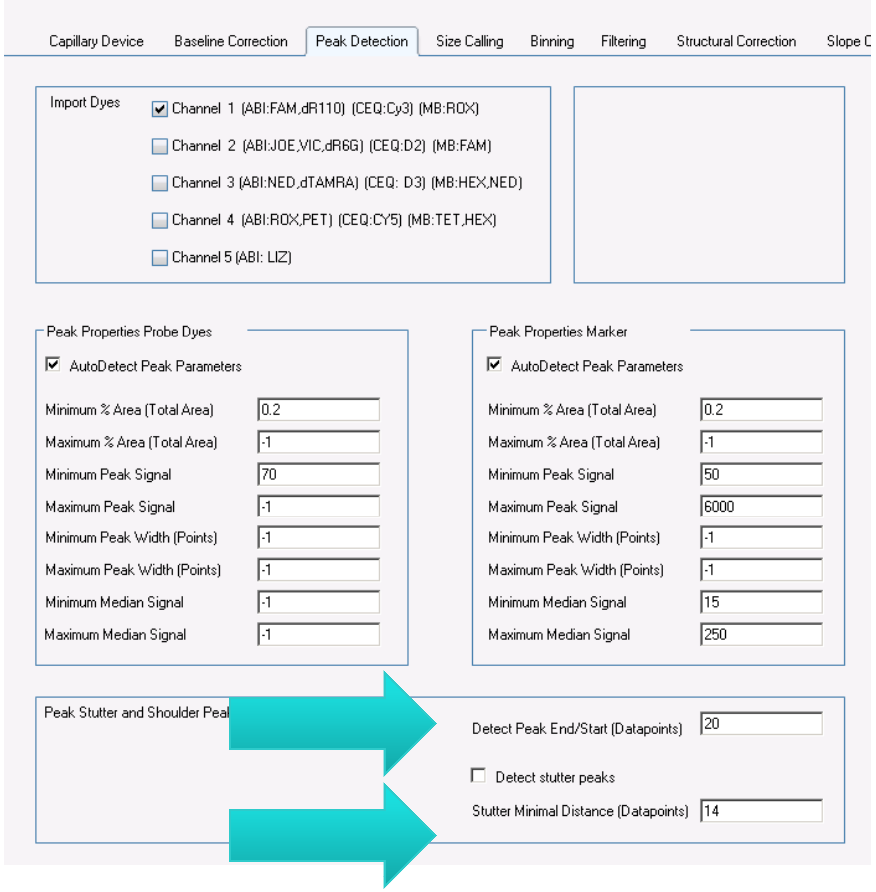


○ Show Detected Probe Names
○ Show Detected ProbeLengths
● Show All Peak Lengths

Dye

☐ Show All steps
☐ Show Points

Stutter peak detection settings



Import Dyes
☑ Channel 1 (ABI:FAM,dR110) (CEQ:Cy3) (MB:ROX)
☐ Channel 2 (ABI:JOE,VIC,dR6G) (CEQ:D2) (MB:FAM)
☐ Channel 3 (ABI:NED,dTAMRA) (CEQ: D3) (MB:HEX,NED)
☐ Channel 4 (ABI:ROX,PET) (CEQ:CY5) (MB:TET,HEX)
☐ Channel 5 (ABI: LIZ)

	Peak Properties Probe Dyes	Peak Properties Marker
AutoDetect Peak Parameters	☑	☑
Minimum % Area (Total Area)	0.2	0.2
Maximum % Area (Total Area)	-1	-1
Minimum Peak Signal	70	50
Maximum Peak Signal	-1	6000
Minimum Peak Width (Points)	-1	-1
Maximum Peak Width (Points)	-1	-1
Minimum Median Signal	-1	15
Maximum Median Signal	-1	250

Peak Stutter and Shoulder Pea

Detect Peak End/Start (Datapoints): 20
☐ Detect stutter peaks
Stutter Minimal Distance (Datapoints): 14

Minimal data-point width
Of peaks

Minimal data-point width
of stutter peak if on

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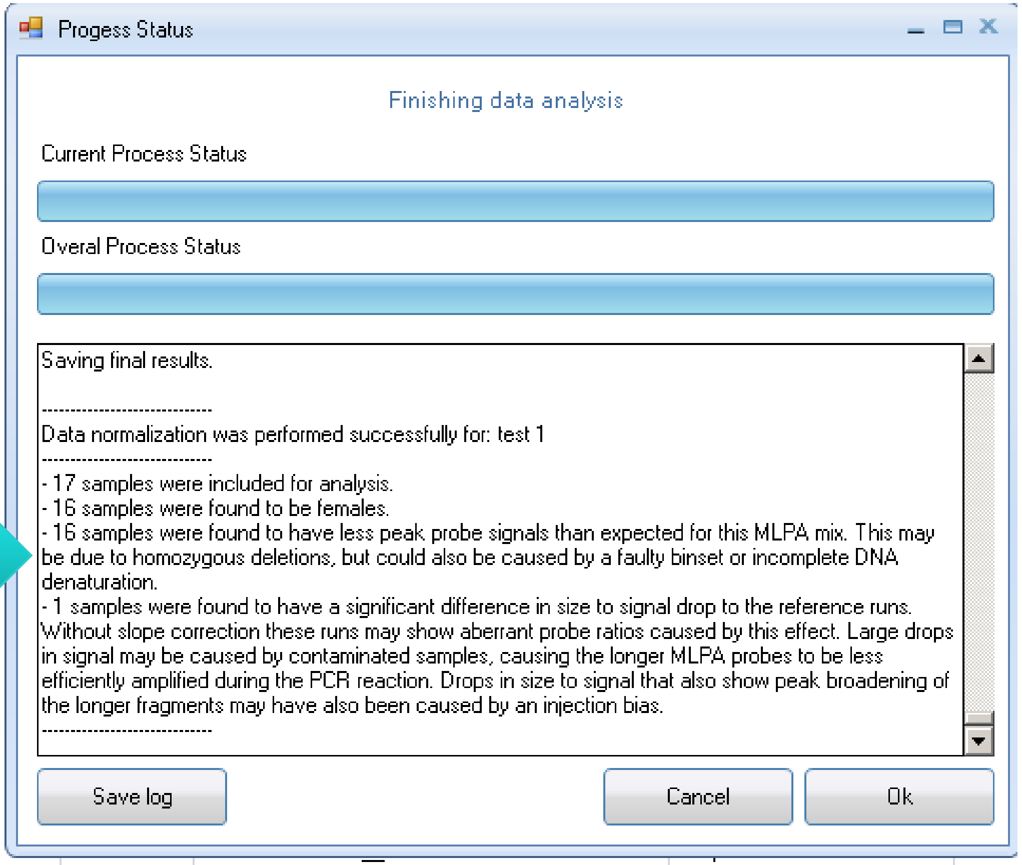
Troubleshooting on peak panelling



```
Saving final results.

-----------------------------
Data normalization was performed successfully for: test 1
-----------------------------
- 17 samples were included for analysis.
- 16 samples were found to be females.
- 16 samples were found to have less peak probe signals than expected for this MLPA mix. This may
be due to homozygous deletions, but could also be caused by a faulty binset or incomplete DNA
denaturation.
- 1 samples were found to have a significant difference in size to signal drop to the reference runs.
Without slope correction these runs may show aberrant probe ratios caused by this effect. Large drops
in signal may be caused by contaminated samples, causing the longer MLPA probes to be less
efficiently amplified during the PCR reaction. Drops in size to signal that also show peak broadening of
the longer fragments may have also been caused by an injection bias.
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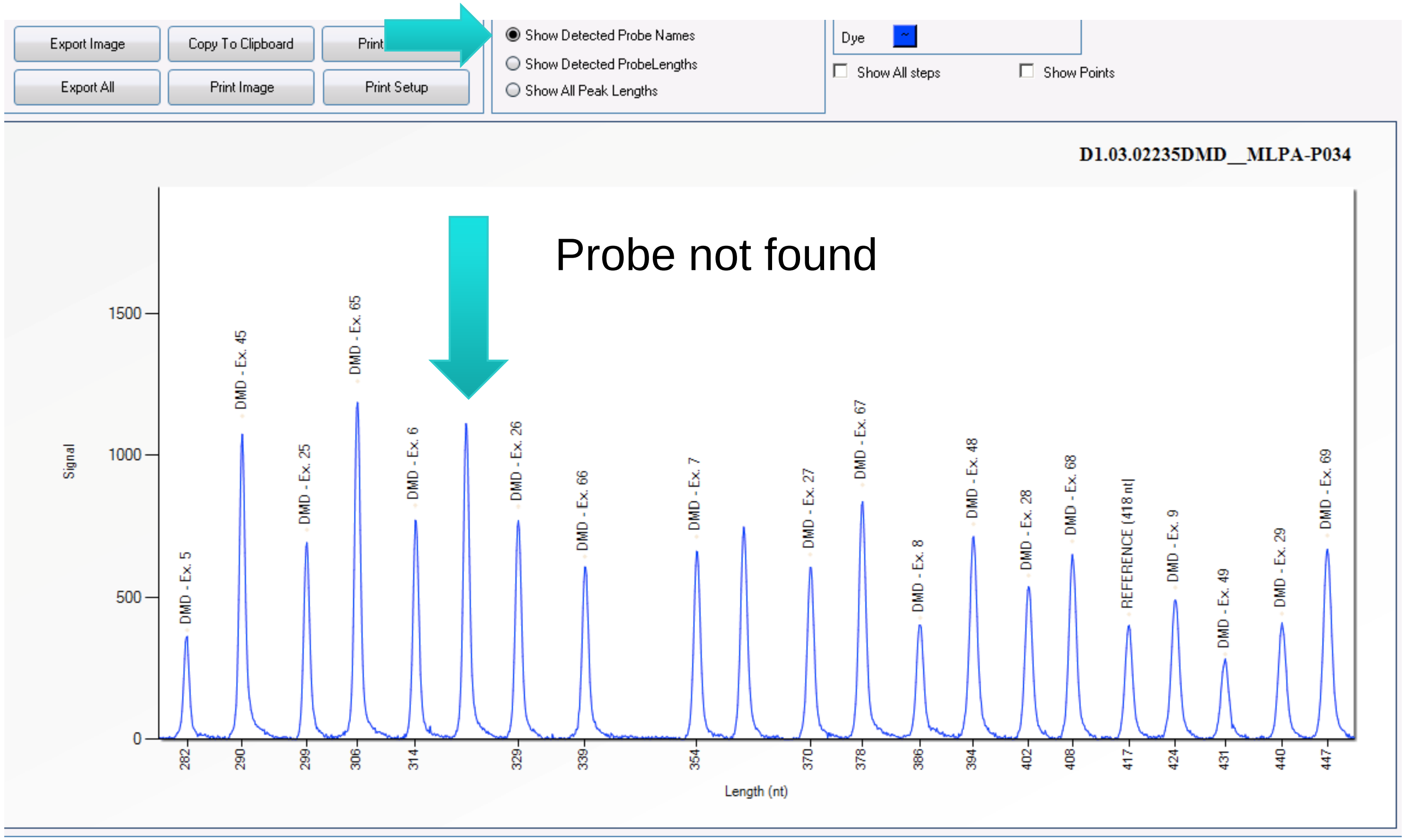
MLPA

MLPA probe panelling & data filtering



● Show Detected Probe Names
○ Show Detected ProbeLengths
○ Show All Peak Lengths

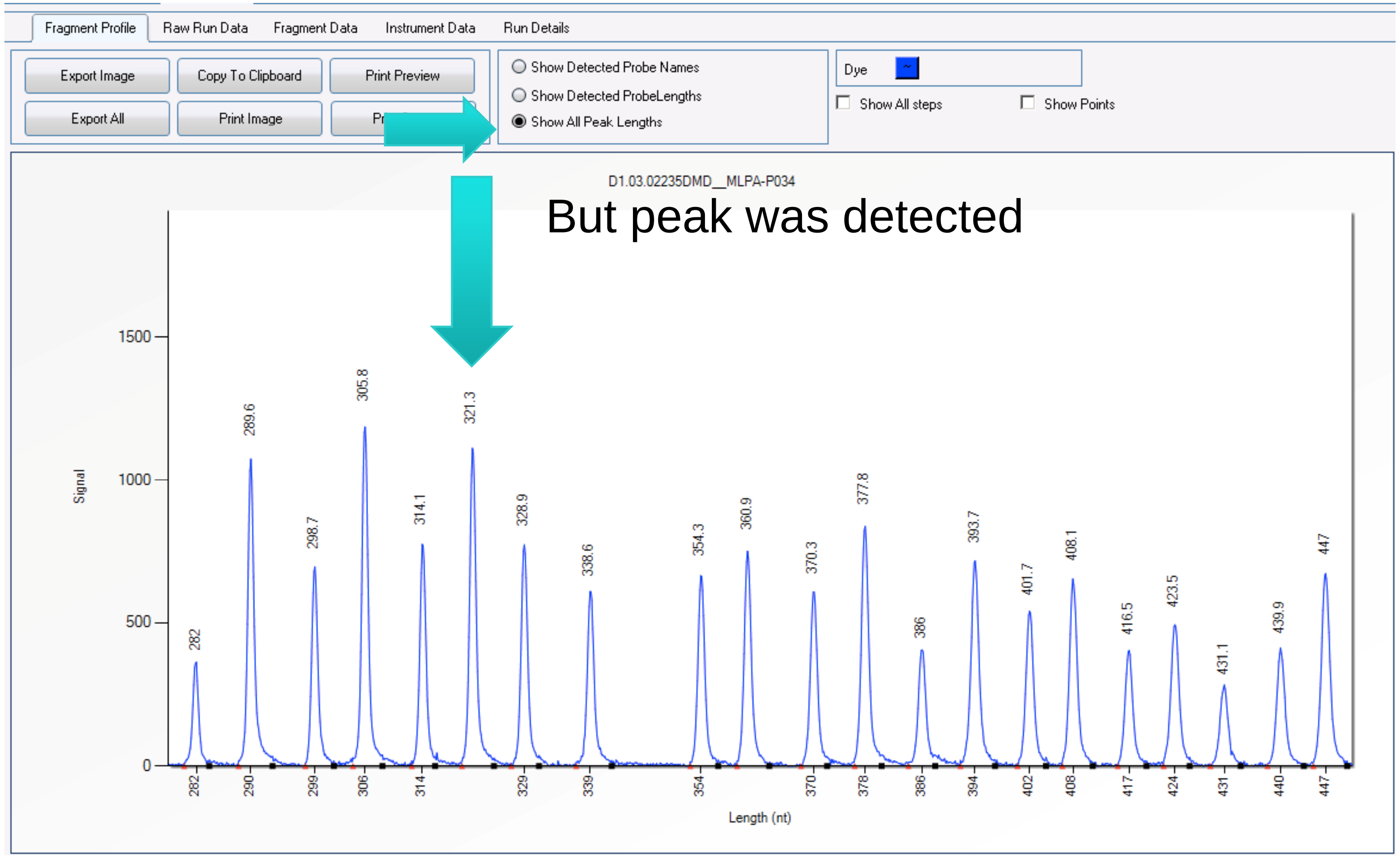
Dye

☐ Show All steps ☐ Show Points

D1.03.02235DMD__MLPA-P034

Probe not found

MLPA probe panelling & data filtering

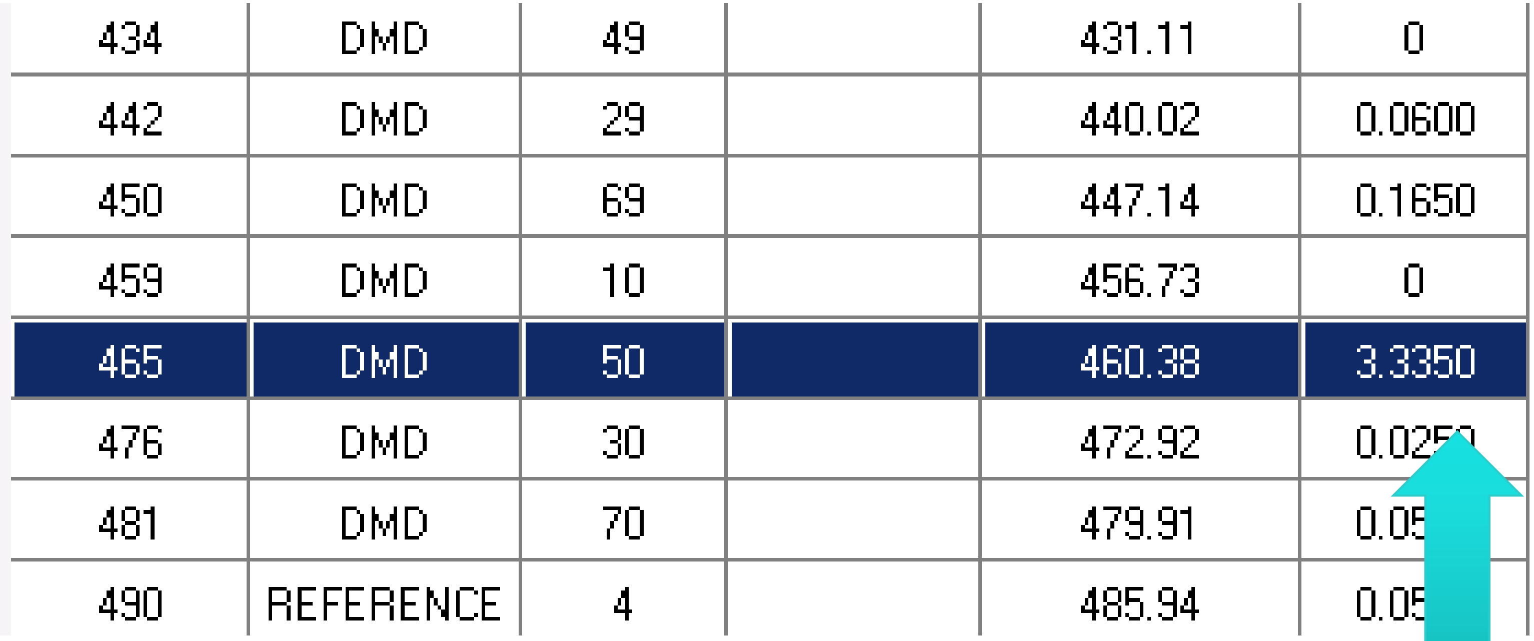


○ Show Detected Probe Names
○ Show Detected ProbeLengths
● Show All Peak Lengths

☐ Show All steps
☐ Show Points

But peak was detected

Checking automatic generated binset

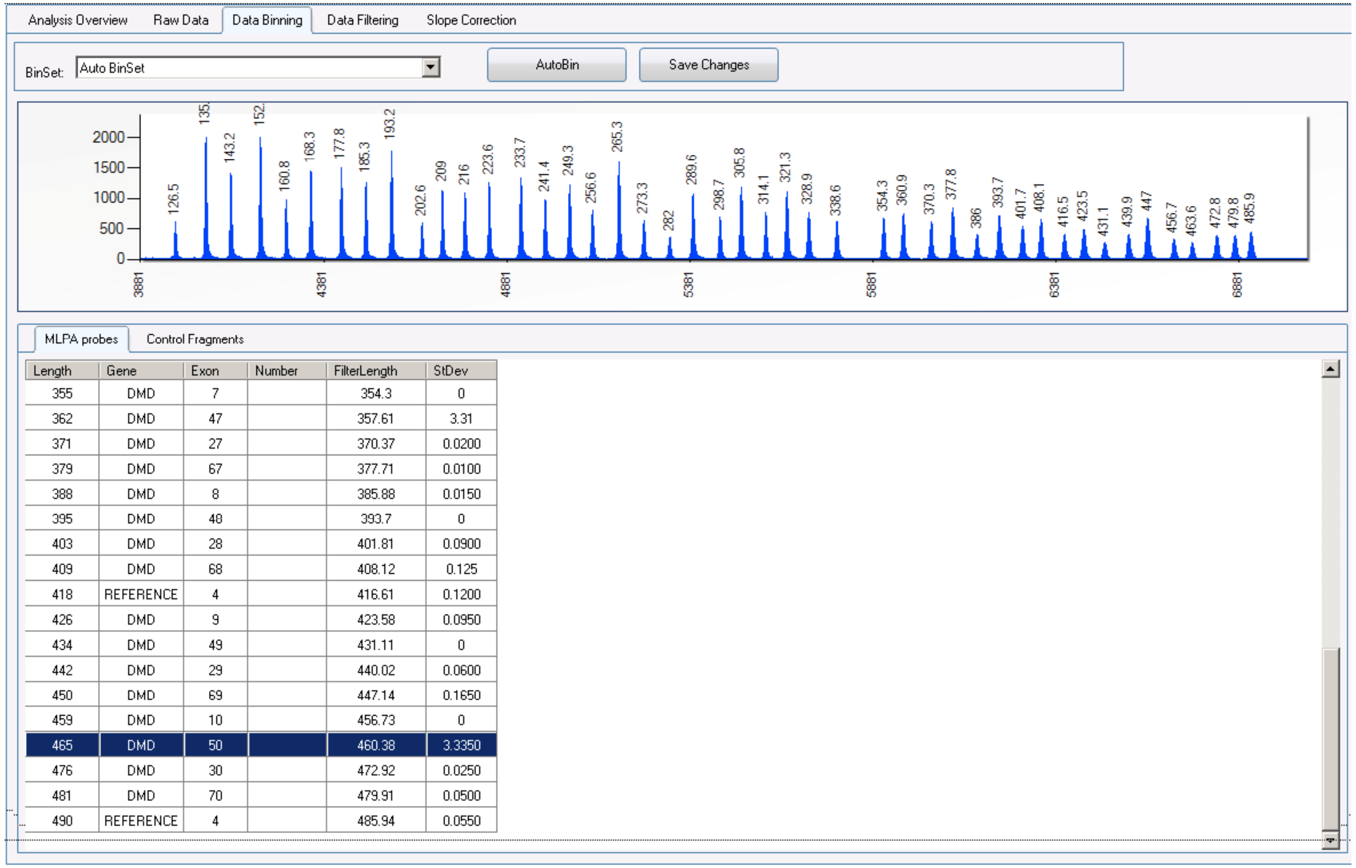


434	DMD	49		431.11	0
442	DMD	29		440.02	0.0600
450	DMD	69		447.14	0.1650
459	DMD	10		456.73	0
465	DMD	50		460.38	3.3350
476	DMD	30		472.92	0.0250
481	DMD	70		479.91	0.05
490	REFERENCE	4		485.94	0.05

A high stdev indicated that there was an uncertainty about the estimated length

A stdev of zero means that a probe length was only found a single time or not at all

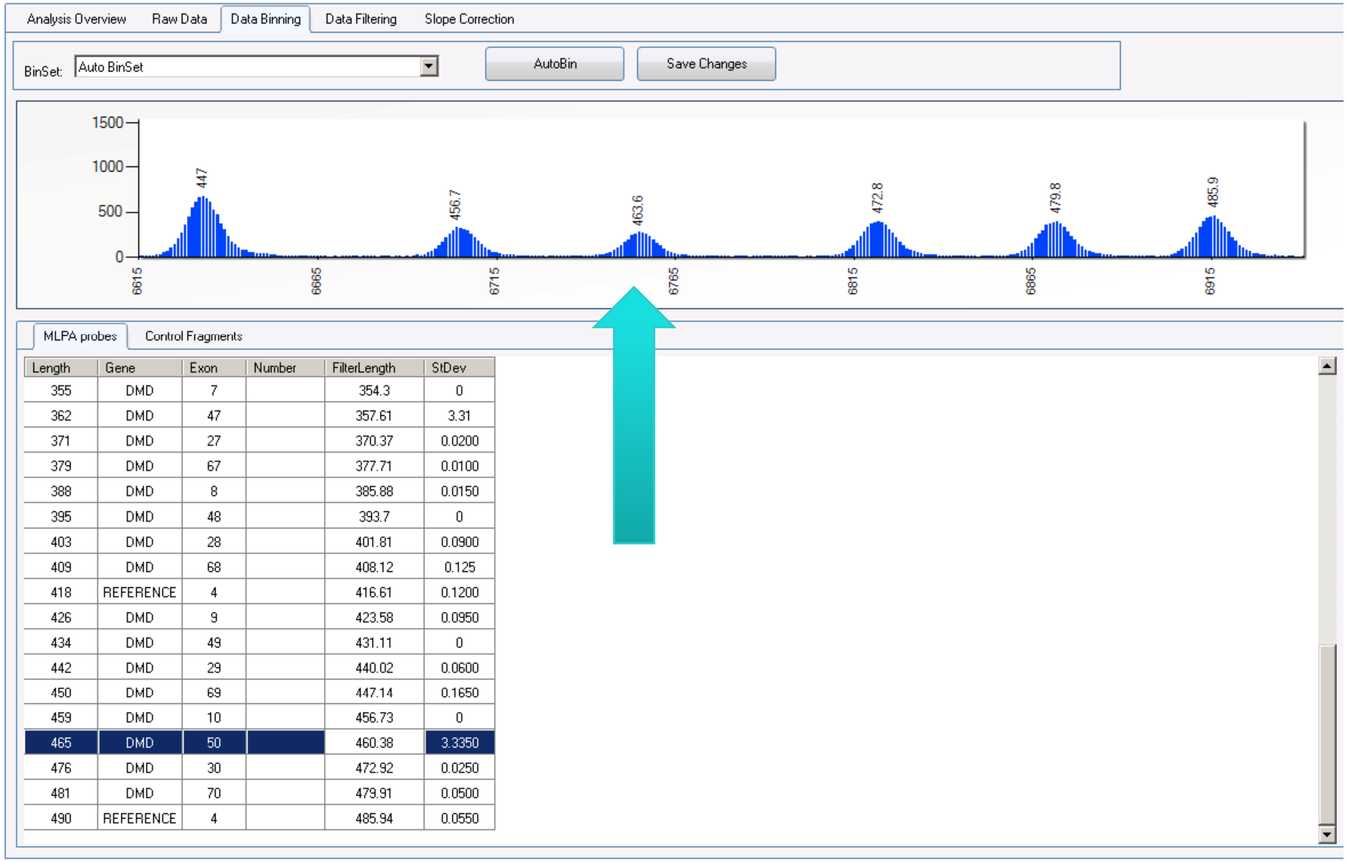
Adjusting bin set manually



BinSet: Auto BinSet

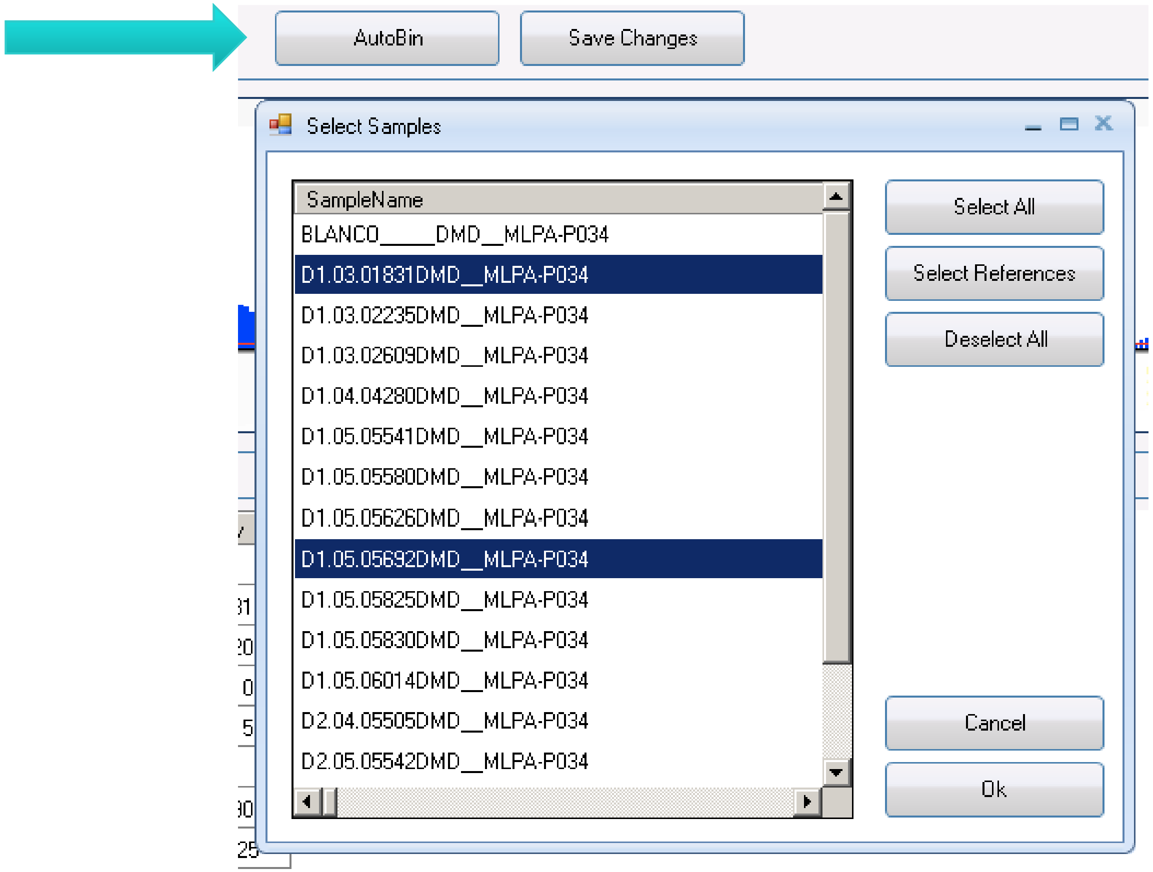
Length	Gene	Exon	Number	FilterLength	StDev
355	DMD	7		354.3	0
362	DMD	47		357.61	3.31
371	DMD	27		370.37	0.0200
379	DMD	67		377.71	0.0100
388	DMD	8		385.88	0.0150
395	DMD	48		393.7	0
403	DMD	28		401.81	0.0900
409	DMD	68		408.12	0.125
418	REFERENCE	4		416.61	0.1200
426	DMD	9		423.58	0.0950
434	DMD	49		431.11	0
442	DMD	29		440.02	0.0600
450	DMD	69		447.14	0.1650
459	DMD	10		456.73	0
465	DMD	50		460.38	3.3350
476	DMD	30		472.92	0.0250
481	DMD	70		479.91	0.0500
490	REFERENCE	4		485.94	0.0550

Obtaining the correct detected probe length



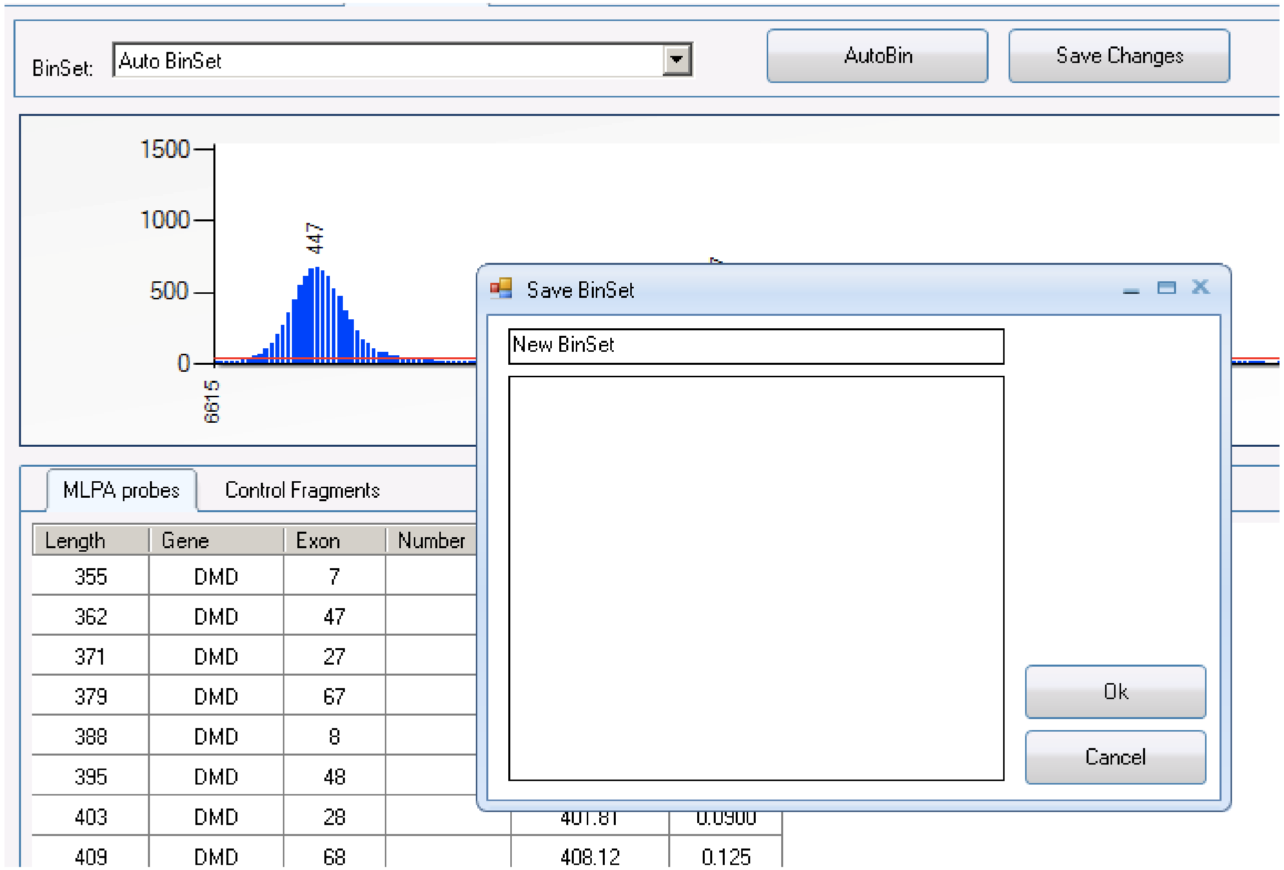
Length	Gene	Exon	Number	FilterLength	StDev
355	DMD	7		354.3	0
362	DMD	47		357.61	3.31
371	DMD	27		370.37	0.0200
379	DMD	67		377.71	0.0100
388	DMD	8		385.88	0.0150
395	DMD	48		393.7	0
403	DMD	28		401.81	0.0900
409	DMD	68		408.12	0.125
418	REFERENCE	4		416.61	0.1200
426	DMD	9		423.58	0.0950
434	DMD	49		431.11	0
442	DMD	29		440.02	0.0600
450	DMD	69		447.14	0.1650
459	DMD	10		456.73	0
465	DMD	50		460.38	3.3350
476	DMD	30		472.92	0.0250
481	DMD	70		479.91	0.0500
490	REFERENCE	4		485.94	0.0550

Redoing automatic binning using a selection



SampleName
BLANCO_____DMD__MLPA-P034
D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034

Saving manually adjusted bin set



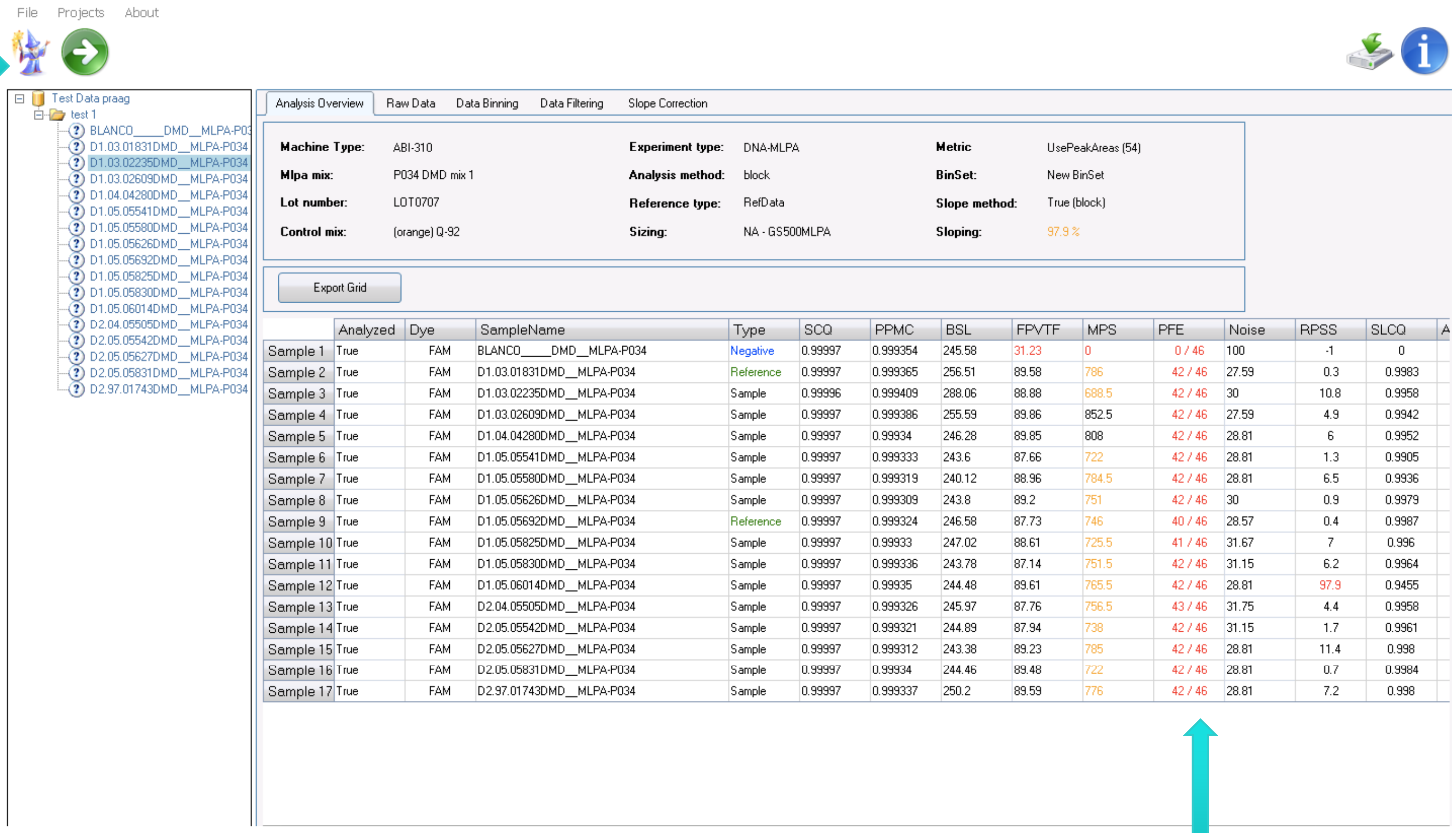
BinSet: Auto BinSet

New BinSet

Length	Gene	Exon	Number		
355	DMD	7			
362	DMD	47			
371	DMD	27			
379	DMD	67			
388	DMD	8			
395	DMD	48			
403	DMD	28		401.81	0.0900
409	DMD	68		408.12	0.125

Re-analyzing experiment data

Re -open wizard



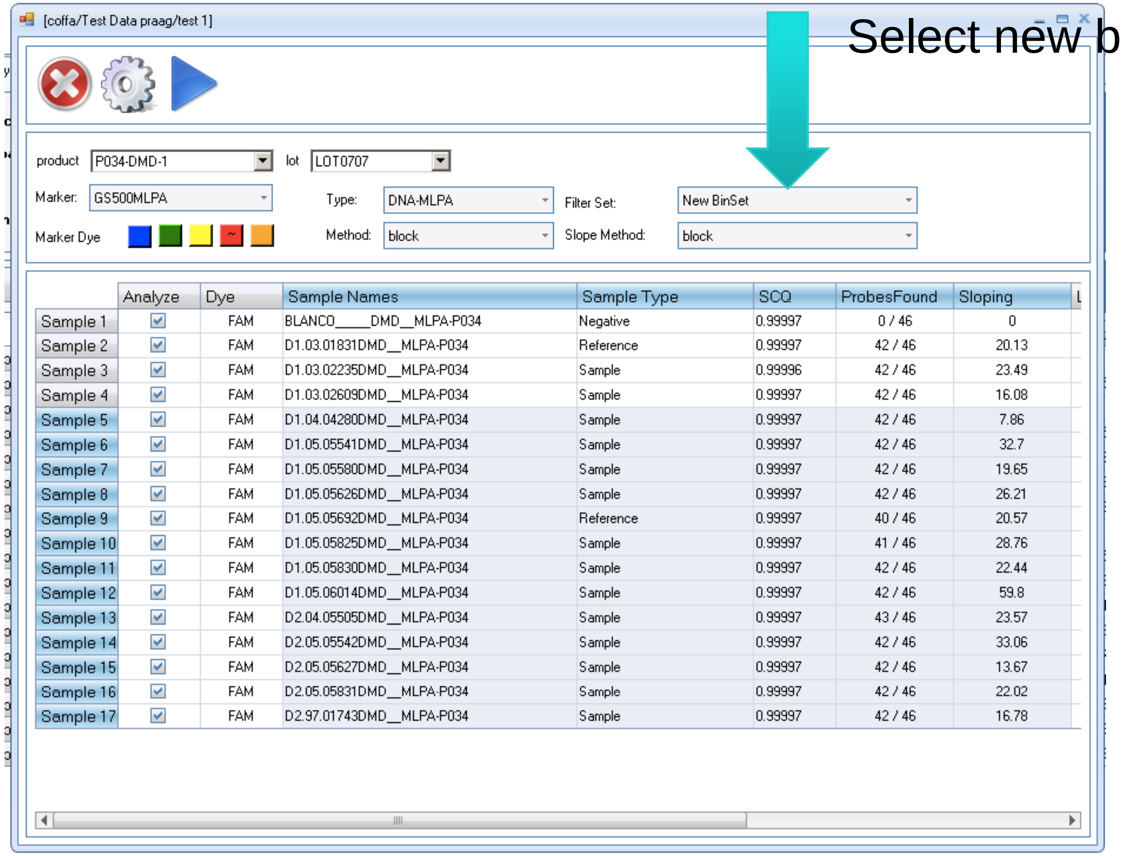
Test Data praag
test 1
BLANCO_____DMD__MLPA-P03
D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034
D2.05.05627DMD__MLPA-P034
D2.05.05831DMD__MLPA-P034
D2.97.01743DMD__MLPA-P034

Machine Type:	ABI-310	**Experiment type:**	DNA-MLPA	**Metric**	UsePeakAreas (54)
Mlpa mix:	P034 DMD mix 1	**Analysis method:**	block	**BinSet:**	New BinSet
Lot number:	LOT0707	**Reference type:**	RefData	**Slope method:**	True (block)
Control mix:	(orange) Q-92	**Sizing:**	NA - GS500MLPA	**Sloping:**	97.9 %

	Analyzed	Dye	SampleName	Type	SCQ	PPMC	BSL	FPVTF	MPS	PFE	Noise	RPSS	SLCQ
Sample 1	True	FAM	BLANCO_____DMD__MLPA-P034	Negative	0.99997	0.999354	245.58	31.23	0	0 / 46	100	-1	0
Sample 2	True	FAM	D1.03.01831DMD__MLPA-P034	Reference	0.99997	0.999365	256.51	89.58	786	42 / 46	27.59	0.3	0.9983
Sample 3	True	FAM	D1.03.02235DMD__MLPA-P034	Sample	0.99996	0.999409	288.06	88.88	688.5	42 / 46	30	10.8	0.9958
Sample 4	True	FAM	D1.03.02609DMD__MLPA-P034	Sample	0.99997	0.999386	255.59	89.86	852.5	42 / 46	27.59	4.9	0.9942
Sample 5	True	FAM	D1.04.04280DMD__MLPA-P034	Sample	0.99997	0.99934	246.28	89.85	808	42 / 46	28.81	6	0.9952
Sample 6	True	FAM	D1.05.05541DMD__MLPA-P034	Sample	0.99997	0.999333	243.6	87.66	722	42 / 46	28.81	1.3	0.9905
Sample 7	True	FAM	D1.05.05580DMD__MLPA-P034	Sample	0.99997	0.999319	240.12	88.96	784.5	42 / 46	28.81	6.5	0.9936
Sample 8	True	FAM	D1.05.05626DMD__MLPA-P034	Sample	0.99997	0.999309	243.8	89.2	751	42 / 46	30	0.9	0.9979
Sample 9	True	FAM	D1.05.05692DMD__MLPA-P034	Reference	0.99997	0.999324	246.58	87.73	746	40 / 46	28.57	0.4	0.9987
Sample 10	True	FAM	D1.05.05825DMD__MLPA-P034	Sample	0.99997	0.99933	247.02	88.61	725.5	41 / 46	31.67	7	0.996
Sample 11	True	FAM	D1.05.05830DMD__MLPA-P034	Sample	0.99997	0.999336	243.78	87.14	751.5	42 / 46	31.15	6.2	0.9964
Sample 12	True	FAM	D1.05.06014DMD__MLPA-P034	Sample	0.99997	0.99935	244.48	89.61	765.5	42 / 46	28.81	97.9	0.9455
Sample 13	True	FAM	D2.04.05505DMD__MLPA-P034	Sample	0.99997	0.999326	245.97	87.76	756.5	43 / 46	31.75	4.4	0.9958
Sample 14	True	FAM	D2.05.05542DMD__MLPA-P034	Sample	0.99997	0.999321	244.89	87.94	738	42 / 46	31.15	1.7	0.9961
Sample 15	True	FAM	D2.05.05627DMD__MLPA-P034	Sample	0.99997	0.999312	243.38	89.23	785	42 / 46	28.81	11.4	0.998
Sample 16	True	FAM	D2.05.05831DMD__MLPA-P034	Sample	0.99997	0.99934	244.46	89.48	722	42 / 46	28.81	0.7	0.9984
Sample 17	True	FAM	D2.97.01743DMD__MLPA-P034	Sample	0.99997	0.999337	250.2	89.59	776	42 / 46	28.81	7.2	0.998

Missing signal in all runs

Reanalyzing using an adjusted binset

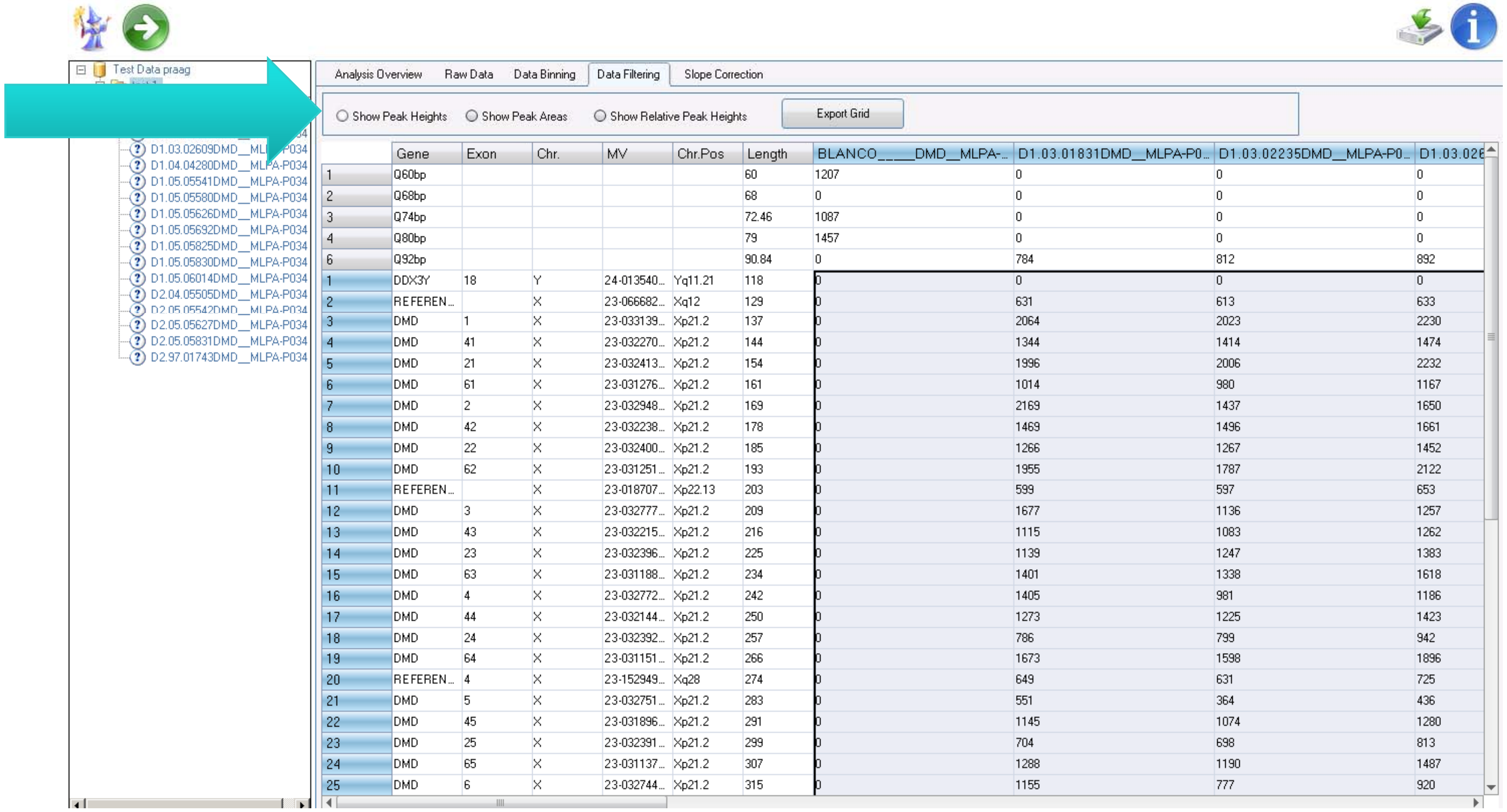


Select new bin set

product: P034-DMD-1
lot: LOT0707
Marker: GS500MLPA
Type: DNA-MLPA
Filter Set: New BinSet
Marker Dye: 🟦 🟩 🟨 🟥 🟧
Method: block
Slope Method: block

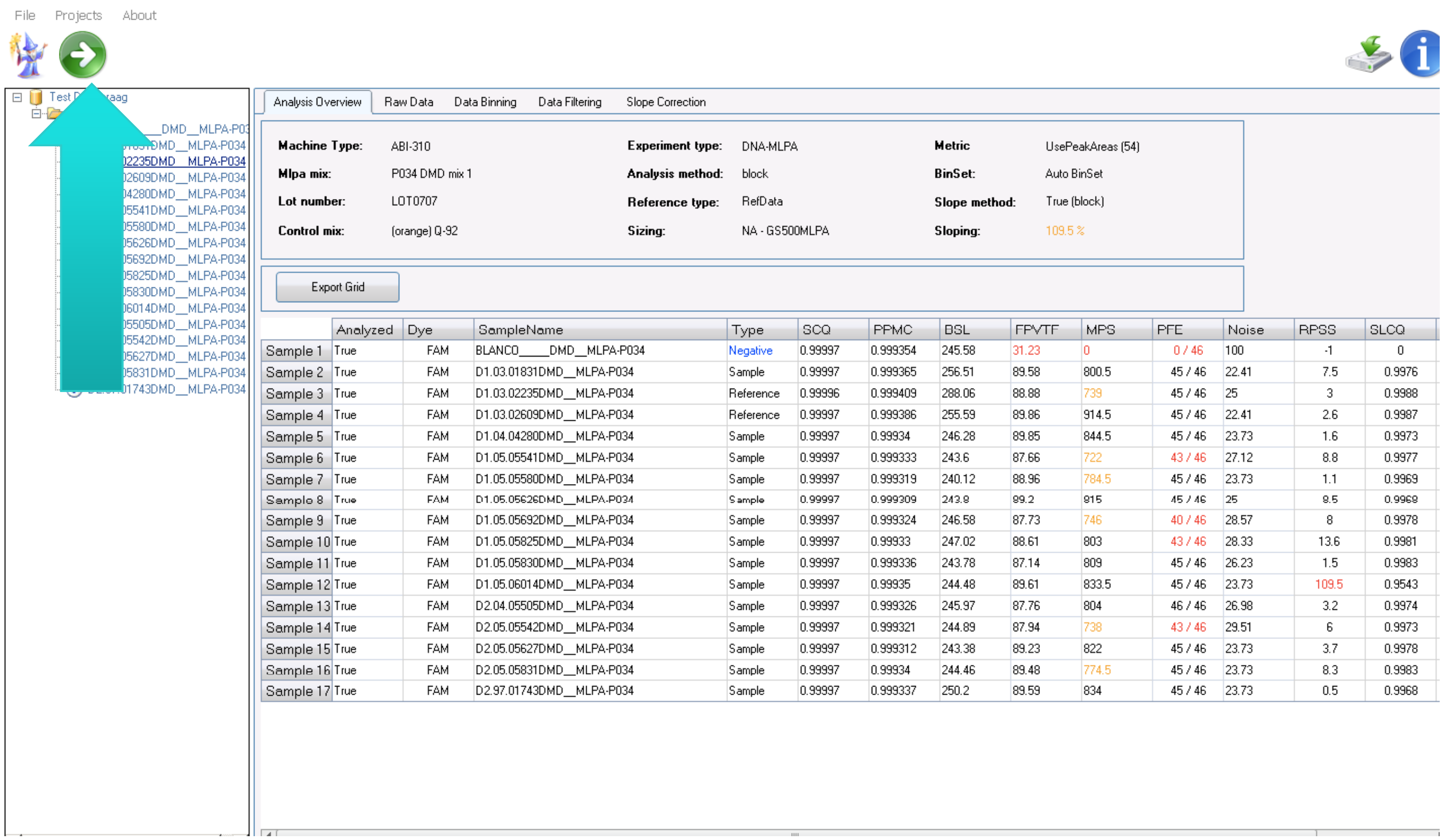
	Analyze	Dye	Sample Names	Sample Type	SCQ	ProbesFound	Sloping
Sample 1	☑	FAM	BLANCO_____DMD__MLPA-P034	Negative	0.99997	0 / 46	0
Sample 2	☑	FAM	D1.03.01831DMD__MLPA-P034	Reference	0.99997	42 / 46	20.13
Sample 3	☑	FAM	D1.03.02235DMD__MLPA-P034	Sample	0.99996	42 / 46	23.49
Sample 4	☑	FAM	D1.03.02609DMD__MLPA-P034	Sample	0.99997	42 / 46	16.08
Sample 5	☑	FAM	D1.04.04280DMD__MLPA-P034	Sample	0.99997	42 / 46	7.86
Sample 6	☑	FAM	D1.05.05541DMD__MLPA-P034	Sample	0.99997	42 / 46	32.7
Sample 7	☑	FAM	D1.05.05580DMD__MLPA-P034	Sample	0.99997	42 / 46	19.65
Sample 8	☑	FAM	D1.05.05626DMD__MLPA-P034	Sample	0.99997	42 / 46	26.21
Sample 9	☑	FAM	D1.05.05692DMD__MLPA-P034	Reference	0.99997	40 / 46	20.57
Sample 10	☑	FAM	D1.05.05825DMD__MLPA-P034	Sample	0.99997	41 / 46	28.76
Sample 11	☑	FAM	D1.05.05830DMD__MLPA-P034	Sample	0.99997	42 / 46	22.44
Sample 12	☑	FAM	D1.05.06014DMD__MLPA-P034	Sample	0.99997	42 / 46	59.8
Sample 13	☑	FAM	D2.04.05505DMD__MLPA-P034	Sample	0.99997	43 / 46	23.57
Sample 14	☑	FAM	D2.05.05542DMD__MLPA-P034	Sample	0.99997	42 / 46	33.06
Sample 15	☑	FAM	D2.05.05627DMD__MLPA-P034	Sample	0.99997	42 / 46	13.67
Sample 16	☑	FAM	D2.05.05831DMD__MLPA-P034	Sample	0.99997	42 / 46	22.02
Sample 17	☑	FAM	D2.97.01743DMD__MLPA-P034	Sample	0.99997	42 / 46	16.78

Exploring & exporting filtered data



	Gene	Exon	Chr.	MV	Chr.Pos	Length	BLANCO____DMD__MLPA-...	D1.03.01831DMD__MLPA-P0...	D1.03.02235DMD__MLPA-P0...	D1.03.026
1	Q60bp					60	1207	0	0	0
2	Q68bp					68	0	0	0	0
3	Q74bp					72.46	1087	0	0	0
4	Q80bp					79	1457	0	0	0
6	Q92bp					90.84	0	784	812	892
1	DDX3Y	18	Y	24-013540...	Yq11.21	118	0	0	0	0
2	REFEREN...		X	23-066682...	Xq12	129	0	631	613	633
3	DMD	1	X	23-033139...	Xp21.2	137	0	2064	2023	2230
4	DMD	41	X	23-032270...	Xp21.2	144	0	1344	1414	1474
5	DMD	21	X	23-032413...	Xp21.2	154	0	1996	2006	2232
6	DMD	61	X	23-031276...	Xp21.2	161	0	1014	980	1167
7	DMD	2	X	23-032948...	Xp21.2	169	0	2169	1437	1650
8	DMD	42	X	23-032238...	Xp21.2	178	0	1469	1496	1661
9	DMD	22	X	23-032400...	Xp21.2	185	0	1266	1267	1452
10	DMD	62	X	23-031251...	Xp21.2	193	0	1955	1787	2122
11	REFEREN...		X	23-018707...	Xp22.13	203	0	599	597	653
12	DMD	3	X	23-032777...	Xp21.2	209	0	1677	1136	1257
13	DMD	43	X	23-032215...	Xp21.2	216	0	1115	1083	1262
14	DMD	23	X	23-032396...	Xp21.2	225	0	1139	1247	1383
15	DMD	63	X	23-031188...	Xp21.2	234	0	1401	1338	1618
16	DMD	4	X	23-032772...	Xp21.2	242	0	1405	981	1186
17	DMD	44	X	23-032144...	Xp21.2	250	0	1273	1225	1423
18	DMD	24	X	23-032392...	Xp21.2	257	0	786	799	942
19	DMD	64	X	23-031151...	Xp21.2	266	0	1673	1598	1896
20	REFEREN...	4	X	23-152949...	Xq28	274	0	649	631	725
21	DMD	5	X	23-032751...	Xp21.2	283	0	551	364	436
22	DMD	45	X	23-031896...	Xp21.2	291	0	1145	1074	1280
23	DMD	25	X	23-032391...	Xp21.2	299	0	704	698	813
24	DMD	65	X	23-031137...	Xp21.2	307	0	1288	1190	1487
25	DMD	6	X	23-032744...	Xp21.2	315	0	1155	777	920

Open Results form



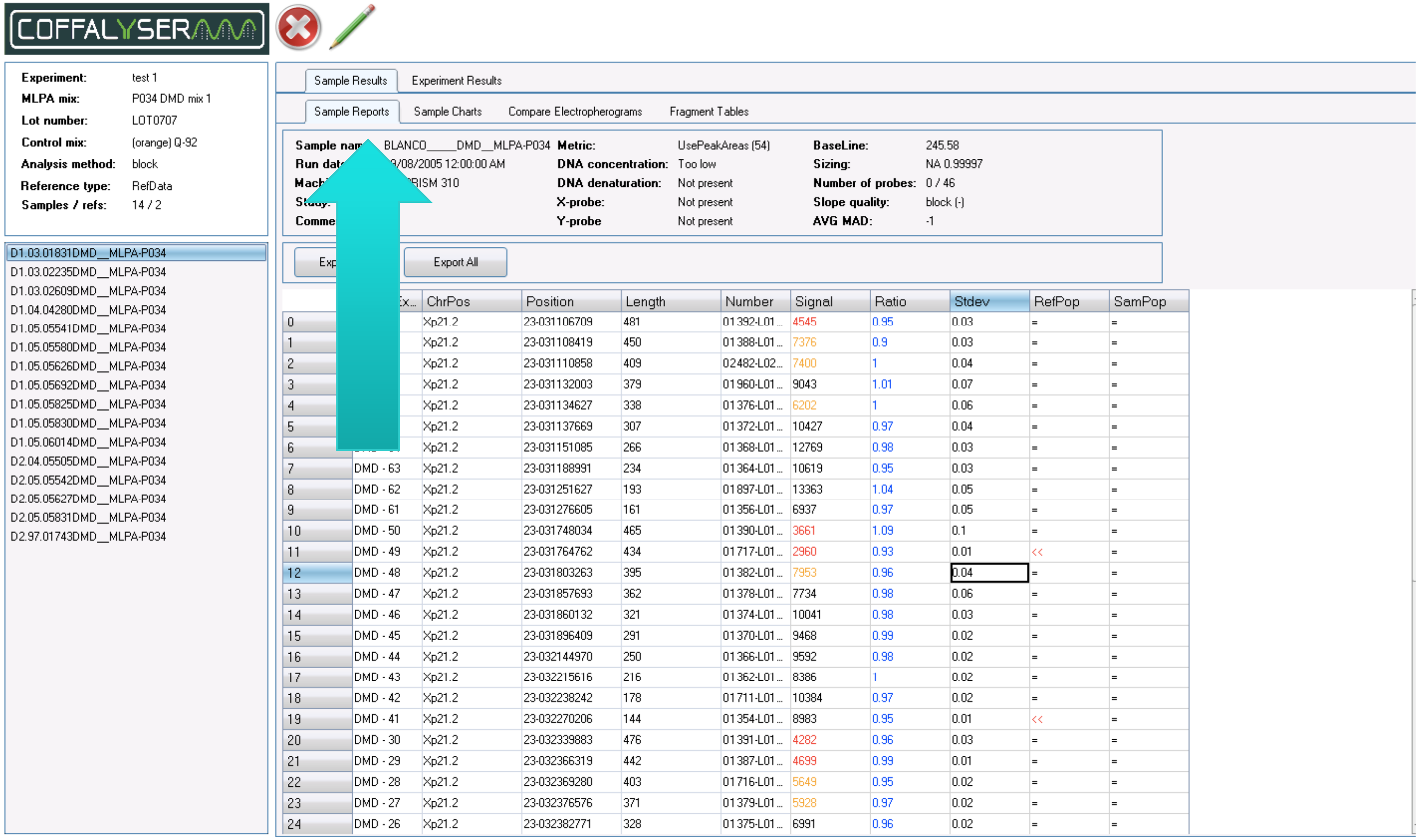
Machine Type: ABI-310
Mlpa mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92

Experiment type: DNA-MLPA
Analysis method: block
Reference type: RefData
Sizing: NA - GS500MLPA

Metric UsePeakAreas (54)
BinSet: Auto BinSet
Slope method: True (block)
Sloping: 109.5 %

	Analyzed	Dye	SampleName	Type	SCQ	PPMC	BSL	FPVTF	MPS	PFE	Noise	RPSS	SLCQ
Sample 1	True	FAM	BLANCO____DMD__MLPA-P034	Negative	0.99997	0.999354	245.58	31.23	0	0 / 46	100	-1	0
Sample 2	True	FAM	D1.03.01831DMD__MLPA-P034	Sample	0.99997	0.999365	256.51	89.58	800.5	45 / 46	22.41	7.5	0.9976
Sample 3	True	FAM	D1.03.02235DMD__MLPA-P034	Reference	0.99996	0.999409	288.06	88.88	739	45 / 46	25	3	0.9988
Sample 4	True	FAM	D1.03.02609DMD__MLPA-P034	Reference	0.99997	0.999386	255.59	89.86	914.5	45 / 46	22.41	2.6	0.9987
Sample 5	True	FAM	D1.04.04280DMD__MLPA-P034	Sample	0.99997	0.99934	246.28	89.85	844.5	45 / 46	23.73	1.6	0.9973
Sample 6	True	FAM	D1.05.05541DMD__MLPA-P034	Sample	0.99997	0.999333	243.6	87.66	722	43 / 46	27.12	8.8	0.9977
Sample 7	True	FAM	D1.05.05580DMD__MLPA-P034	Sample	0.99997	0.999319	240.12	88.96	784.5	45 / 46	23.73	1.1	0.9969
Sample 8	True	FAM	D1.05.05626DMD__MLPA-P034	Sample	0.99997	0.999309	243.8	99.2	815	45 / 46	25	8.5	0.9969
Sample 9	True	FAM	D1.05.05692DMD__MLPA-P034	Sample	0.99997	0.999324	246.58	87.73	746	40 / 46	28.57	8	0.9978
Sample 10	True	FAM	D1.05.05825DMD__MLPA-P034	Sample	0.99997	0.99933	247.02	88.61	803	43 / 46	28.33	13.6	0.9981
Sample 11	True	FAM	D1.05.05830DMD__MLPA-P034	Sample	0.99997	0.999336	243.78	87.14	809	45 / 46	26.23	1.5	0.9983
Sample 12	True	FAM	D1.05.06014DMD__MLPA-P034	Sample	0.99997	0.99935	244.48	89.61	833.5	45 / 46	23.73	109.5	0.9543
Sample 13	True	FAM	D2.04.05505DMD__MLPA-P034	Sample	0.99997	0.999326	245.97	87.76	804	46 / 46	26.98	3.2	0.9974
Sample 14	True	FAM	D2.05.05542DMD__MLPA-P034	Sample	0.99997	0.999321	244.89	87.94	738	43 / 46	29.51	6	0.9973
Sample 15	True	FAM	D2.05.05627DMD__MLPA-P034	Sample	0.99997	0.999312	243.38	89.23	822	45 / 46	23.73	3.7	0.9978
Sample 16	True	FAM	D2.05.05831DMD__MLPA-P034	Sample	0.99997	0.99934	244.46	89.48	774.5	45 / 46	23.73	8.3	0.9983
Sample 17	True	FAM	D2.97.01743DMD__MLPA-P034	Sample	0.99997	0.999337	250.2	89.59	834	45 / 46	23.73	0.5	0.9968

Results exploration form



Experiment: test 1
MLPA mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92
Analysis method: block
Reference type: RefData
Samples / refs: 14 / 2

D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034
D2.05.05627DMD__MLPA-P034
D2.05.05831DMD__MLPA-P034
D2.97.01743DMD__MLPA-P034

Sample nam BLANCO_____DMD__MLPA-P034
Run dat 9/08/2005 12:00:00 AM
Mach RISM 310
Study:
Comme

Metric: UsePeakAreas (54)
DNA concentration: Too low
DNA denaturation: Not present
X-probe: Not present
Y-probe Not present

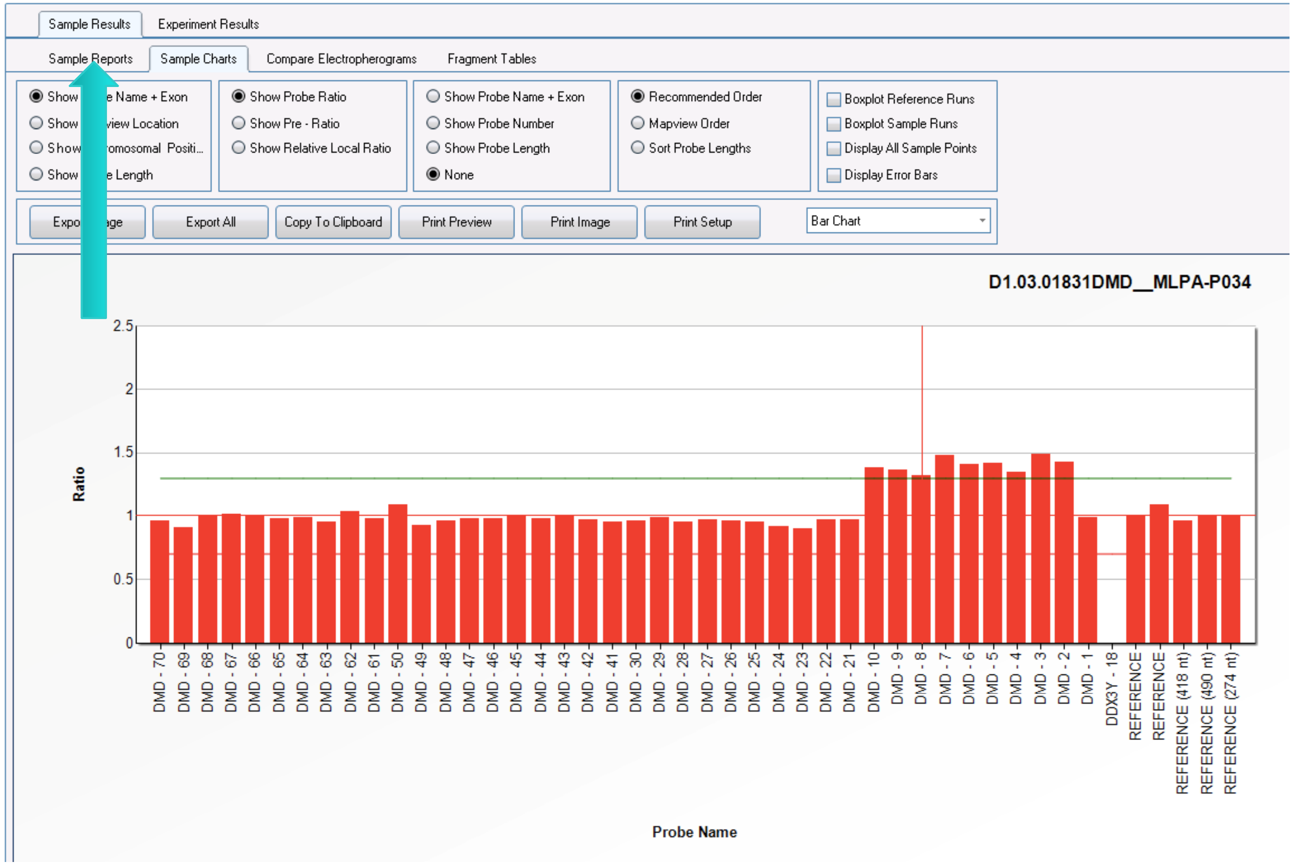
BaseLine: 245.58
Sizing: NA 0.99997
Number of probes: 0 / 46
Slope quality: block (-)
AVG MAD: -1

	Ex...	ChrPos	Position	Length	Number	Signal	Ratio	Stdev	RefPop	SamPop
0		Xp21.2	23-031106709	481	01392-L01...	4545	0.95	0.03	=	=
1		Xp21.2	23-031108419	450	01388-L01...	7376	0.9	0.03	=	=
2		Xp21.2	23-031110858	409	02482-L02...	7400	1	0.04	=	=
3		Xp21.2	23-031132003	379	01960-L01...	9043	1.01	0.07	=	=
4		Xp21.2	23-031134627	338	01376-L01...	6202	1	0.06	=	=
5		Xp21.2	23-031137669	307	01372-L01...	10427	0.97	0.04	=	=
6		Xp21.2	23-031151085	266	01368-L01...	12769	0.98	0.03	=	=
7	DMD - 63	Xp21.2	23-031188991	234	01364-L01...	10619	0.95	0.03	=	=
8	DMD - 62	Xp21.2	23-031251627	193	01897-L01...	13363	1.04	0.05	=	=
9	DMD - 61	Xp21.2	23-031276605	161	01356-L01...	6937	0.97	0.05	=	=
10	DMD - 50	Xp21.2	23-031748034	465	01390-L01...	3661	1.09	0.1	=	=
11	DMD - 49	Xp21.2	23-031764762	434	01717-L01...	2960	0.93	0.01	<<	=
12	DMD - 48	Xp21.2	23-031803263	395	01382-L01...	7953	0.96	0.04	=	=
13	DMD - 47	Xp21.2	23-031857693	362	01378-L01...	7734	0.98	0.06	=	=
14	DMD - 46	Xp21.2	23-031860132	321	01374-L01...	10041	0.98	0.03	=	=
15	DMD - 45	Xp21.2	23-031896409	291	01370-L01...	9468	0.99	0.02	=	=
16	DMD - 44	Xp21.2	23-032144970	250	01366-L01...	9592	0.98	0.02	=	=
17	DMD - 43	Xp21.2	23-032215616	216	01362-L01...	8386	1	0.02	=	=
18	DMD - 42	Xp21.2	23-032238242	178	01711-L01...	10384	0.97	0.02	=	=
19	DMD - 41	Xp21.2	23-032270206	144	01354-L01...	8983	0.95	0.01	<<	=
20	DMD - 30	Xp21.2	23-032339883	476	01391-L01...	4282	0.96	0.03	=	=
21	DMD - 29	Xp21.2	23-032366319	442	01387-L01...	4699	0.99	0.01	=	=
22	DMD - 28	Xp21.2	23-032369280	403	01716-L01...	5649	0.95	0.02	=	=
23	DMD - 27	Xp21.2	23-032376576	371	01379-L01...	5928	0.97	0.02	=	=
24	DMD - 26	Xp21.2	23-032382771	328	01375-L01...	6991	0.96	0.02	=	=

Sample results view

- Single sample exploration
    - Reports (signal, ratio, stdev, comparison to average reference population and median complete sample population)
    - Ratio chart visualization and distribution comparison
    - Electropherogram overlay and stacked charts
    - Export of result reports to pdf
    - Export of charts to jpg, gif, tiff, png, bmp
    - Export of grids to Excel, HTML, XML & csv

Ratio charts



Sample Results | Experiment Results

Sample Reports | Sample Charts | Compare Electropherograms | Fragment Tables

● Show e Name + Exon
○ Show view Location
○ Show omosomal Positi...
○ Show e Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

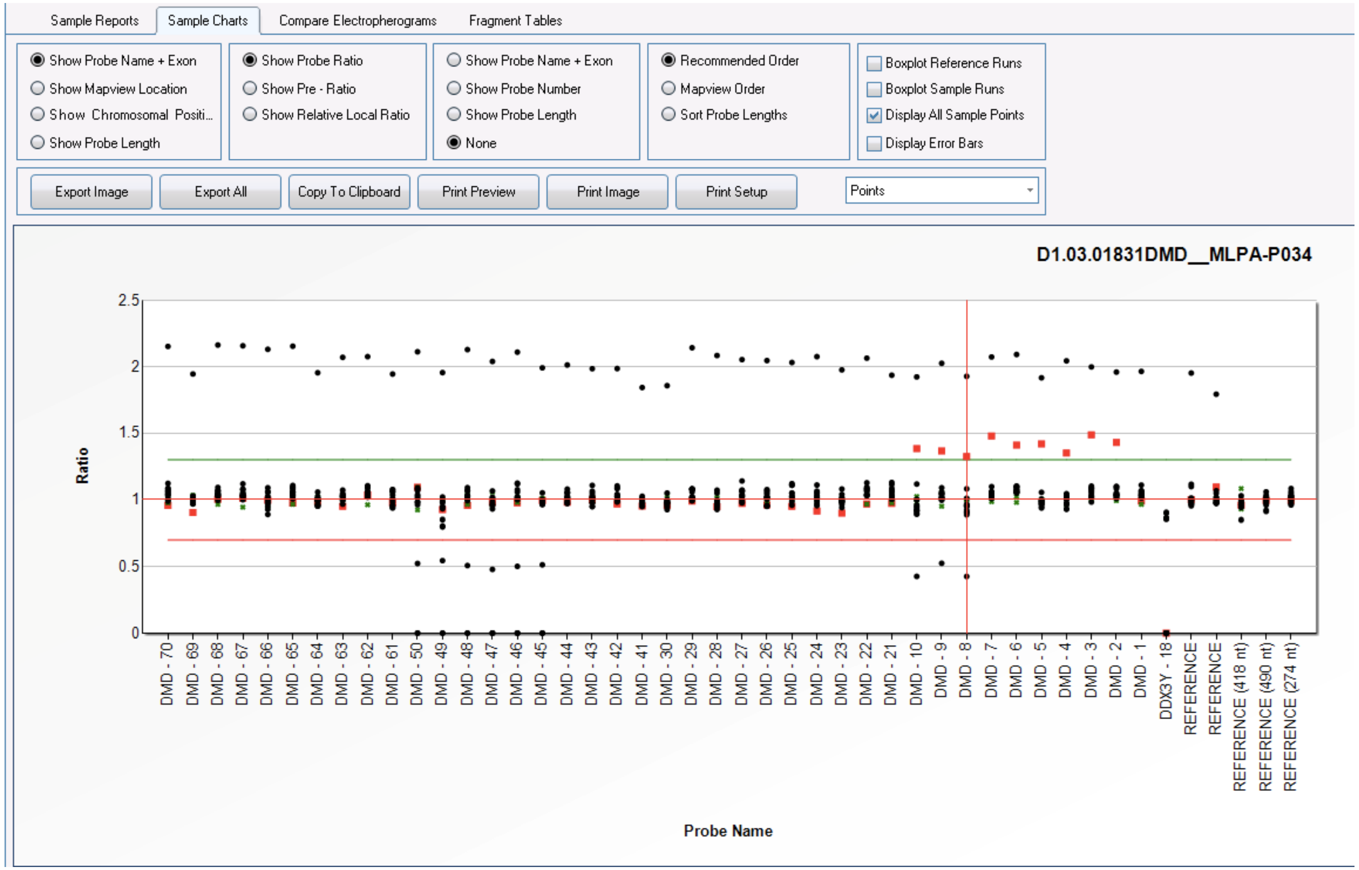
○ Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
● None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Reference Runs
☐ Boxplot Sample Runs
☐ Display All Sample Points
☐ Display Error Bars

Bar Chart

Comparing individual sample to the other sample ratio results



Sample Reports | Sample Charts | Compare Electropherograms | Fragment Tables

● Show Probe Name + Exon
○ Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

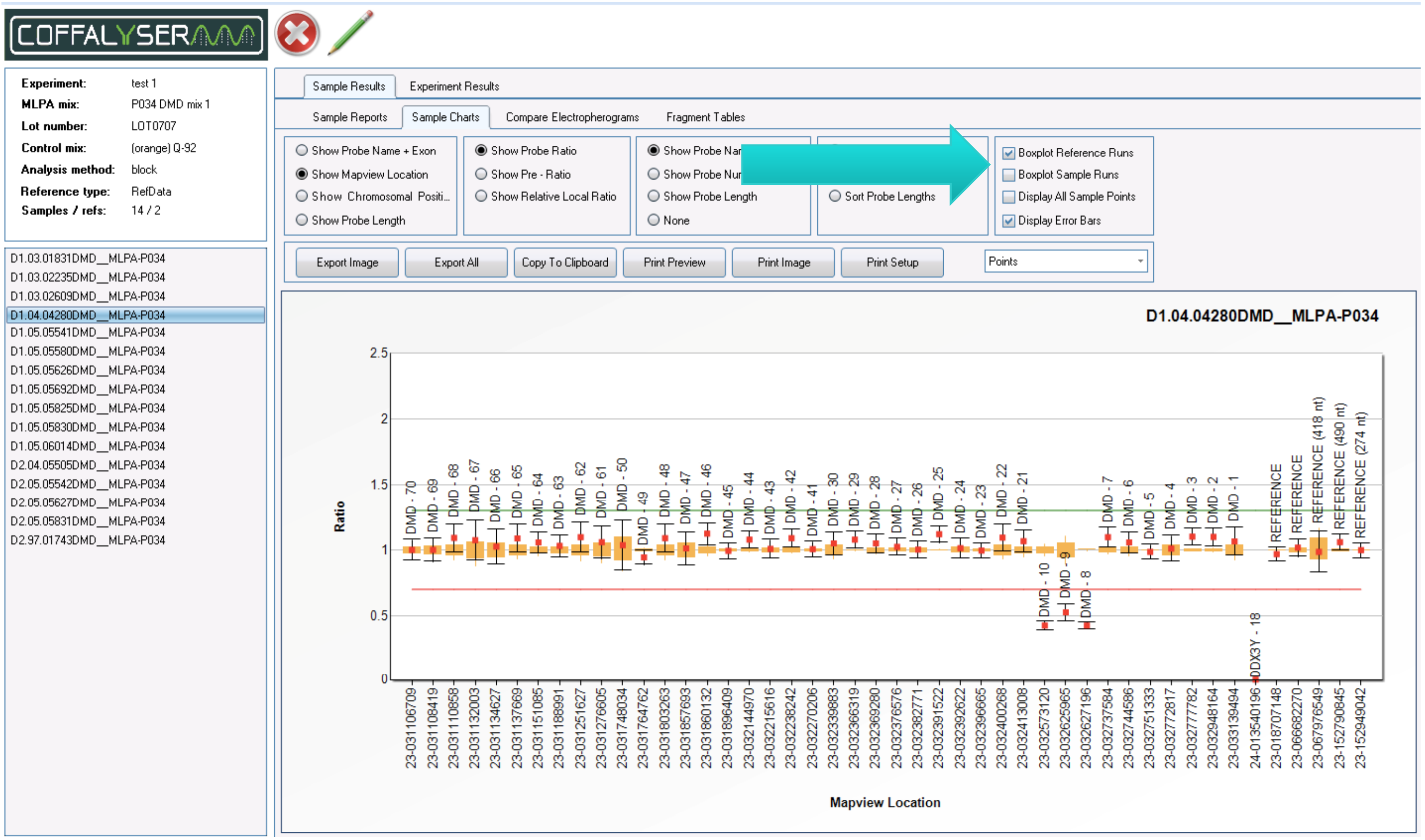
● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

○ Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
● None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Reference Runs
☐ Boxplot Sample Runs
☑ Display All Sample Points
☐ Display Error Bars

Adding estimated distributions over the different populations



Experiment: test 1
MLPA mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92
Analysis method: block
Reference type: RefData
Samples / refs: 14 / 2

D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034
D2.05.05627DMD__MLPA-P034
D2.05.05831DMD__MLPA-P034
D2.97.01743DMD__MLPA-P034

○ Show Probe Name + Exon
● Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

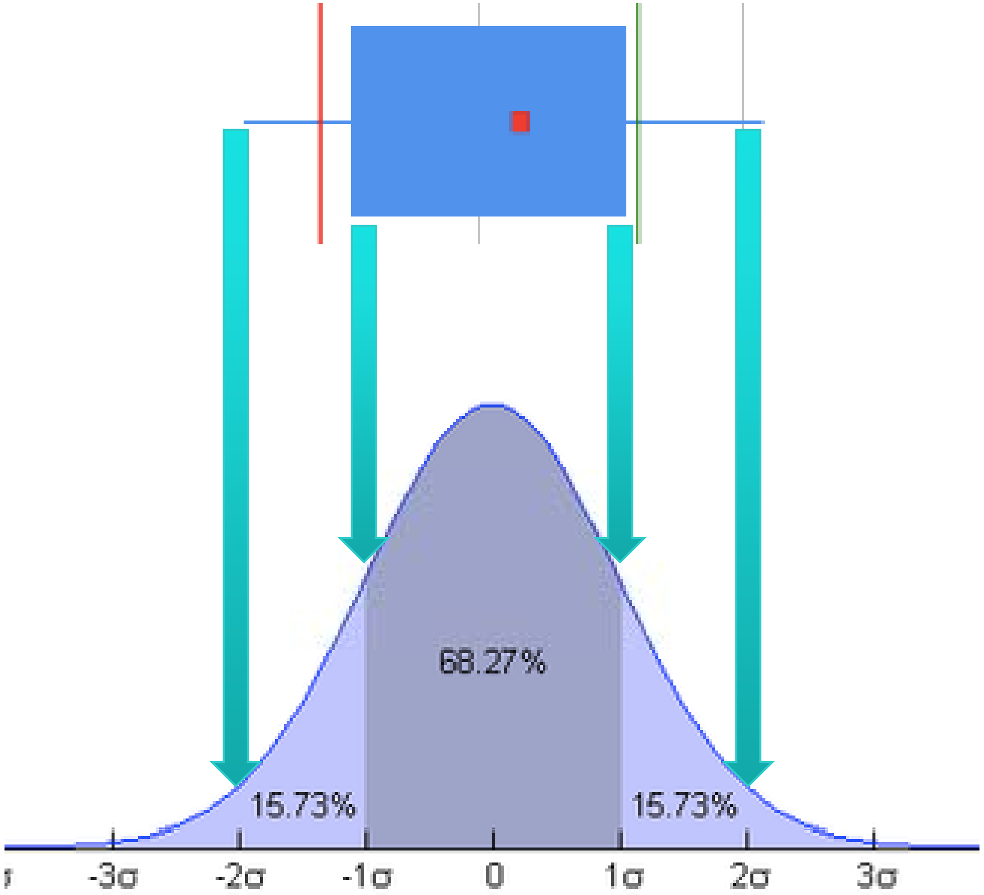
● Show Probe Nar
○ Show Probe Nur
○ Show Probe Length
○ None

○ Sort Probe Lengths

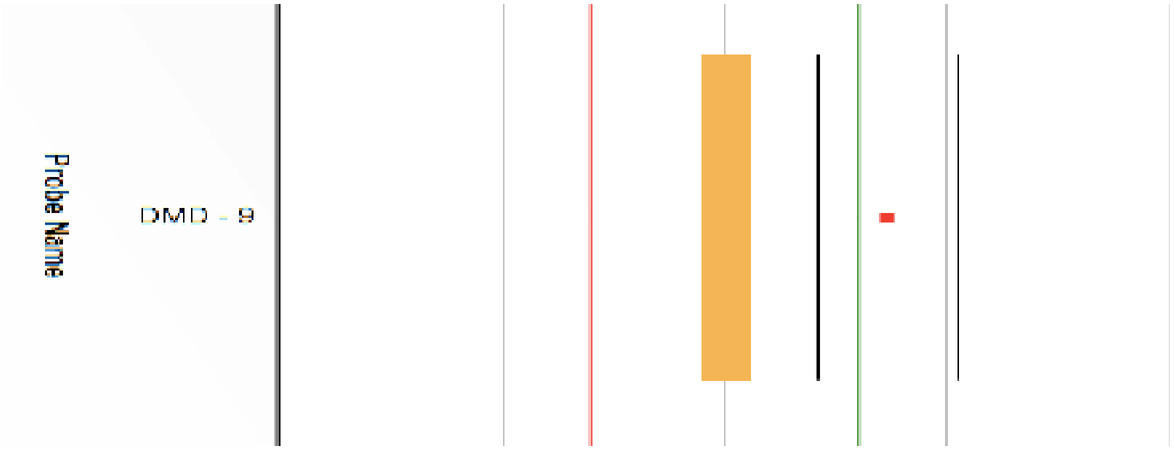
☑ Boxplot Reference Runs
☐ Boxplot Sample Runs
☐ Display All Sample Points
☑ Display Error Bars

Points

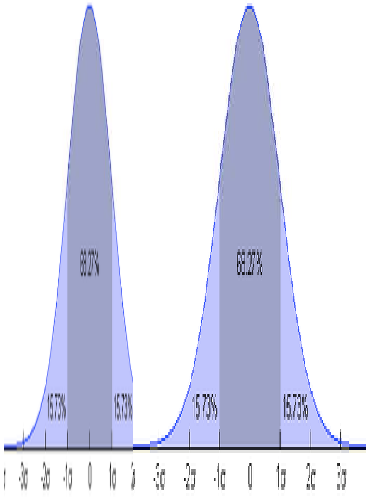
About the box-plots



Comparing sample probe ratio results to reference population distribution

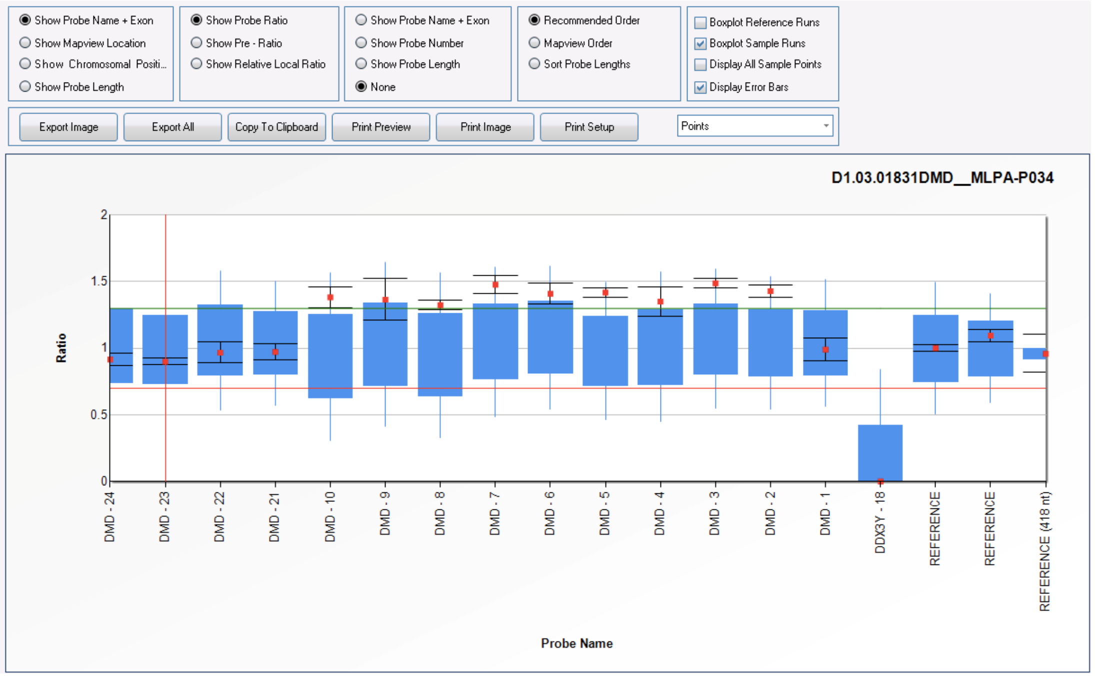


Two confidence intervals that do not overlap indicate that the estimated probe sample signal is significantly different from the reference population



Comparing sample probe ratio results to complete sample population distribution

The presence of too many aberrant samples in the sample population increases the distribution spread and thus does not allow conclusion of significant difference between individual sample and sample population



● Show Probe Name + Exon
○ Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

○ Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
● None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Reference Runs
☑ Boxplot Sample Runs
☐ Display All Sample Points
☑ Display Error Bars

Points

About the distributions

- Reference distribution give a good indication about the confidence interval of the ‘normal’ samples if:
  - At least 3 samples are used
  - These are properly spread over the experiment
  - These are treated equally to the test samples in the experiment
- Comparison of test samples to the complete sample population is possible but often does not provide enough statistical power to conclude that signal are significantly changed

Comparing electropherograms

Experiment: test 1
MLPA mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92
Analysis method: block
Reference type: RefData
Samples / refs: 14 / 2

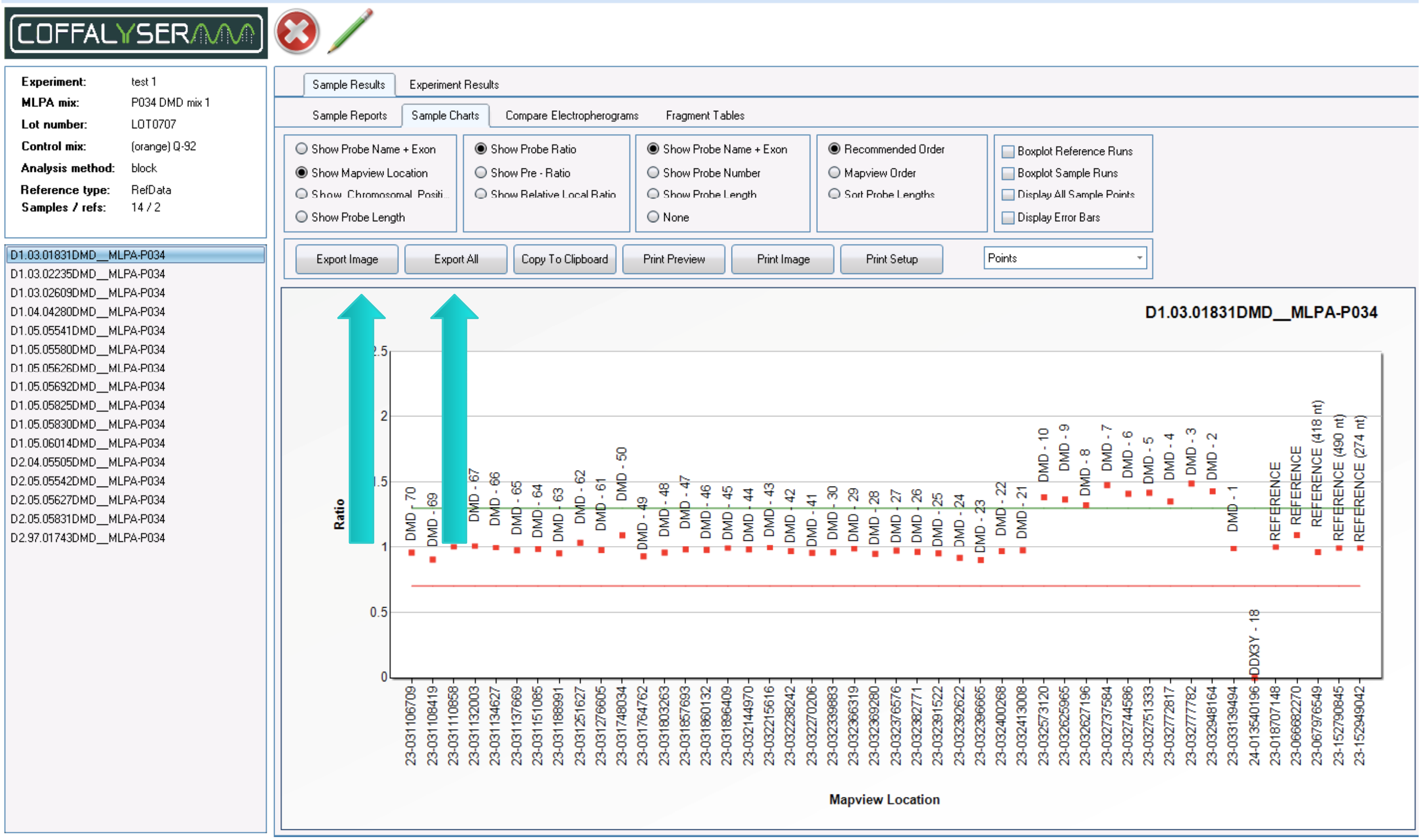
D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034
D2.05.05627DMD__MLPA-P034
D2.05.05831DMD__MLPA-P034
D2.97.01743DMD__MLPA-P034

○ Show Single Sample
○ Overlay Samples
● Stacked Graph
D1.03.02235DMD__MLPA-P034
Raw Data

● Show Probe Name_Exon
○ Show Mapview Location
○ Show Chromosomal Position
○ Show Probe Length

Confirmation of single probe aberrations and homozygous deletions on the raw electropherogram is always recommended

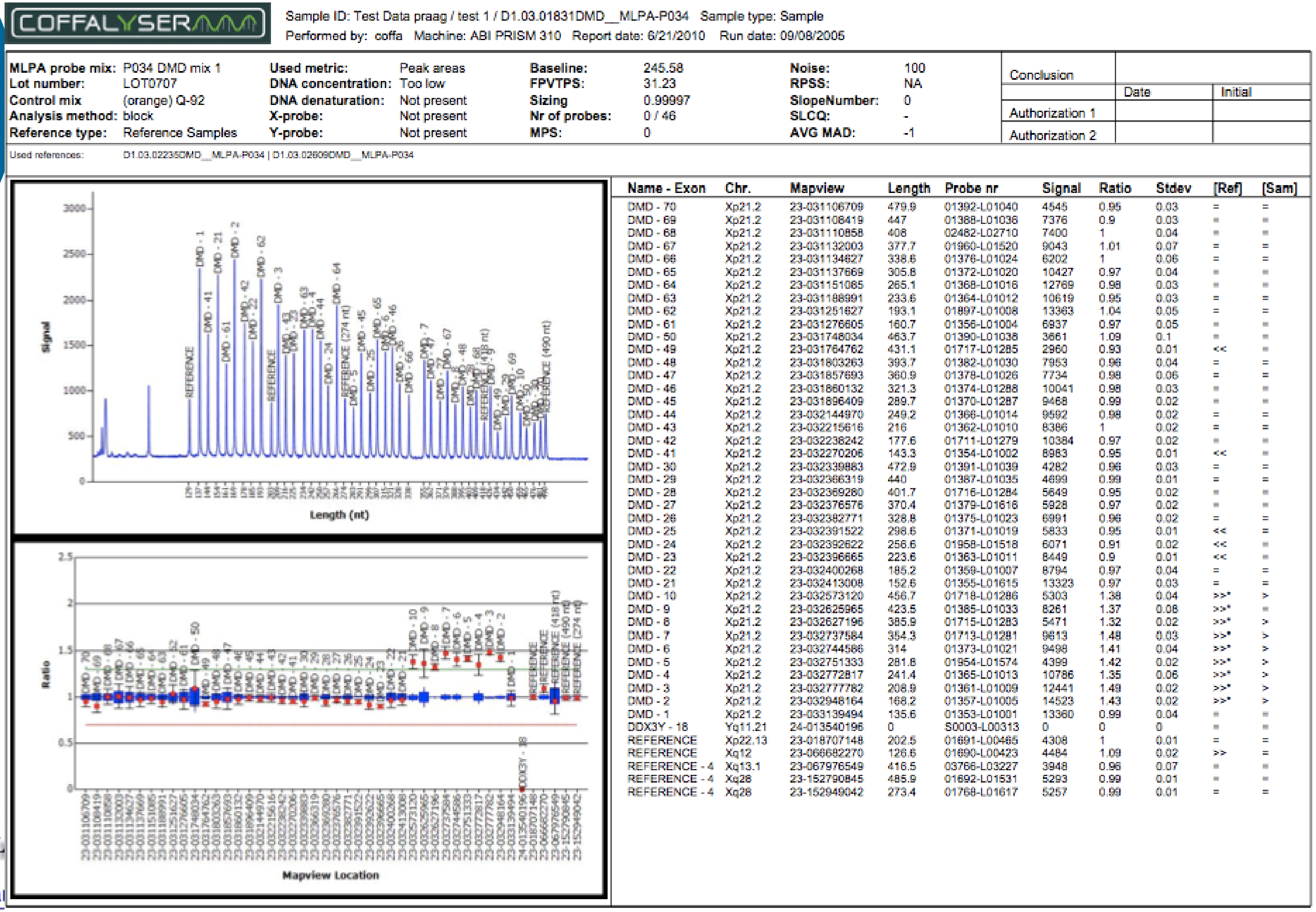
Exporting sample reports



Experiment: test 1
MLPA mix: P034 DMD mix 1
Lot number: LOT0707
Control mix: (orange) Q-92
Analysis method: block
Reference type: RefData
Samples / refs: 14 / 2

D1.03.01831DMD__MLPA-P034
D1.03.02235DMD__MLPA-P034
D1.03.02609DMD__MLPA-P034
D1.04.04280DMD__MLPA-P034
D1.05.05541DMD__MLPA-P034
D1.05.05580DMD__MLPA-P034
D1.05.05626DMD__MLPA-P034
D1.05.05692DMD__MLPA-P034
D1.05.05825DMD__MLPA-P034
D1.05.05830DMD__MLPA-P034
D1.05.06014DMD__MLPA-P034
D2.04.05505DMD__MLPA-P034
D2.05.05542DMD__MLPA-P034
D2.05.05627DMD__MLPA-P034
D2.05.05831DMD__MLPA-P034
D2.97.01743DMD__MLPA-P034

Sample report contents



Sample ID: Test Data praag / test 1 / D1.03.01831DMD__MLPA-P034 Sample type: Sample
Performed by: coffa Machine: ABI PRISM 310 Report date: 6/21/2010 Run date: 09/08/2005

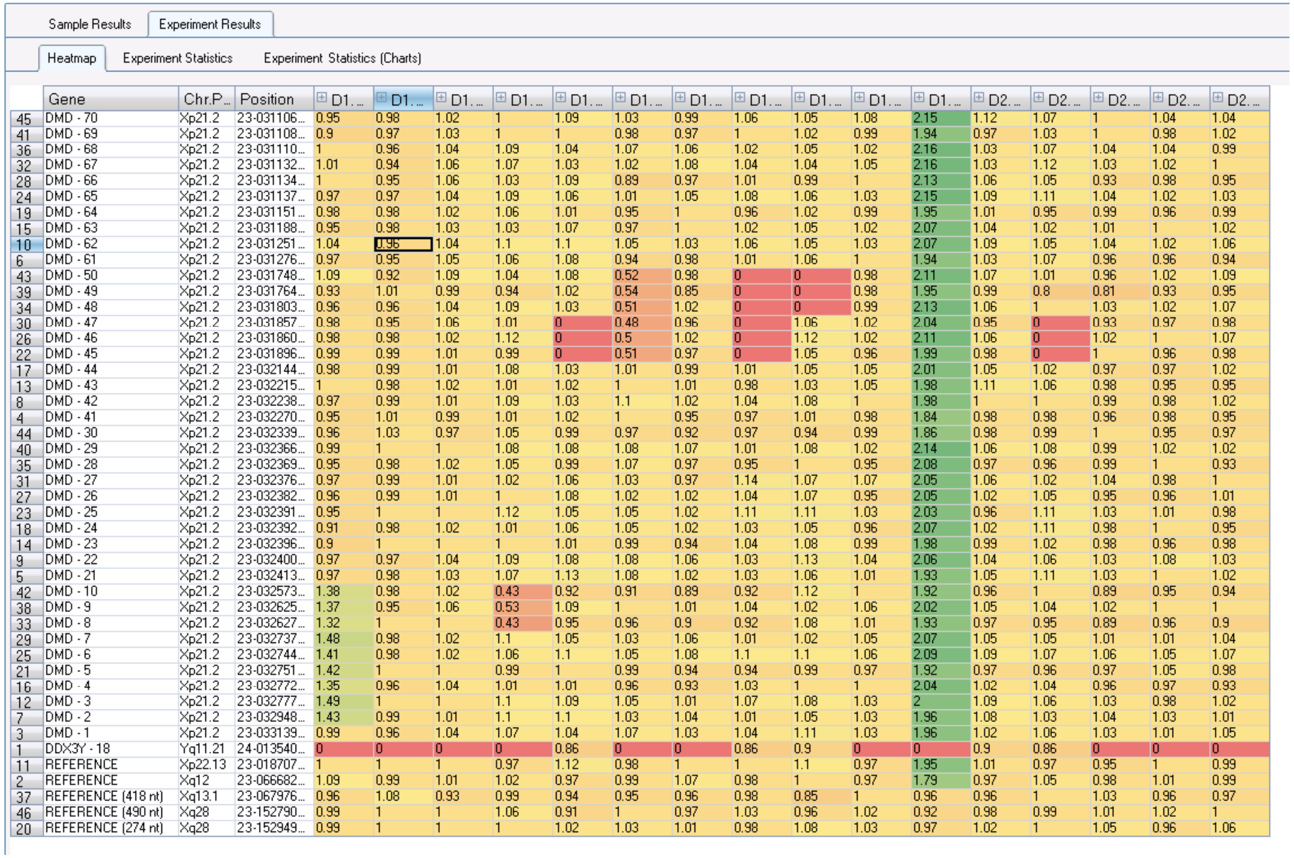
MLPA probe mix: P034 DMD mix 1
Lot number: LOT0707
Control mix (orange) Q-92
Analysis method: block
Reference type: Reference Samples
Used metric: Peak areas
DNA concentration: Too low
DNA denaturation: Not present
X-probe: Not present
Y-probe: Not present
Baseline: 245.58
FPVTPS: 31.23
Sizing 0.99997
Nr of probes: 0 / 46
MPS: 0
Noise: 100
RPSS: NA
SlopeNumber: 0
SLCQ: -
AVG MAD: -1

Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

Used references: D1.03.02235DMD__MLPA-P034 \| D1.03.02609DMD__MLPA-P034

Name - Exon	Chr.	Mapview	Length	Probe nr	Signal	Ratio	Stdev	[Ref]	[Sam]
DMD - 70	Xp21.2	23-031106709	479.9	01392-L01040	4545	0.95	0.03	=	=
DMD - 69	Xp21.2	23-031108419	447	01388-L01036	7376	0.9	0.03	=	=
DMD - 68	Xp21.2	23-031110858	408	02482-L02710	7400	1	0.04	=	=
DMD - 67	Xp21.2	23-031132003	377.7	01960-L01520	9043	1.01	0.07	=	=
DMD - 66	Xp21.2	23-031134627	338.6	01376-L01024	6202	1	0.06	=	=
DMD - 65	Xp21.2	23-031137669	305.8	01372-L01020	10427	0.97	0.04	=	=
DMD - 64	Xp21.2	23-031151065	265.1	01368-L01016	12769	0.98	0.03	=	=
DMD - 63	Xp21.2	23-031188991	233.6	01364-L01012	10619	0.95	0.03	=	=
DMD - 62	Xp21.2	23-031251627	193.1	01897-L01008	13363	1.04	0.05	=	=
DMD - 61	Xp21.2	23-031276605	160.7	01356-L01004	6937	0.97	0.05	=	=
DMD - 50	Xp21.2	23-031746034	463.7	01390-L01038	3661	1.09	0.1	=	=
DMD - 49	Xp21.2	23-031764762	431.1	01717-L01285	2960	0.93	0.01	<<	=
DMD - 48	Xp21.2	23-031803263	393.7	01382-L01030	7953	0.96	0.04	=	=
DMD - 47	Xp21.2	23-031857693	360.9	01378-L01026	7734	0.98	0.06	=	=
DMD - 46	Xp21.2	23-031860132	321.3	01374-L01288	10041	0.98	0.03	=	=
DMD - 45	Xp21.2	23-031896409	289.7	01370-L01287	9468	0.99	0.02	=	=
DMD - 44	Xp21.2	23-032144970	249.2	01366-L01014	9592	0.98	0.02	=	=
DMD - 43	Xp21.2	23-032215616	216	01362-L01010	8386	1	0.02	=	=
DMD - 42	Xp21.2	23-032238242	177.6	01711-L01279	10384	0.97	0.02	=	=
DMD - 41	Xp21.2	23-032270206	143.3	01354-L01002	8983	0.95	0.01	<<	=
DMD - 30	Xp21.2	23-032339883	472.9	01391-L01039	4282	0.96	0.03	=	=
DMD - 29	Xp21.2	23-032366319	440	01387-L01035	4699	0.99	0.01	=	=
DMD - 28	Xp21.2	23-032369280	401.7	01716-L01284	5649	0.95	0.02	=	=
DMD - 27	Xp21.2	23-032376576	370.4	01379-L01616	5928	0.97	0.02	=	=
DMD - 26	Xp21.2	23-032382771	328.8	01375-L01023	6991	0.96	0.02	=	=
DMD - 25	Xp21.2	23-032391522	298.6	01371-L01019	5833	0.95	0.01	<<	=
DMD - 24	Xp21.2	23-032392622	256.6	01958-L01518	6071	0.91	0.02	<<	=
DMD - 23	Xp21.2	23-032396665	223.6	01363-L01011	8449	0.9	0.01	<<	=
DMD - 22	Xp21.2	23-032400268	185.2	01359-L01007	8794	0.97	0.04	=	=
DMD - 21	Xp21.2	23-032413008	152.6	01355-L01615	13323	0.97	0.03	=	=
DMD - 10	Xp21.2	23-032573120	456.7	01718-L01286	5303	1.38	0.04	>>*	>
DMD - 9	Xp21.2	23-032625965	423.5	01385-L01033	8261	1.37	0.08	>>*	=
DMD - 8	Xp21.2	23-032627196	385.9	01715-L01283	5471	1.32	0.02	>>*	>
DMD - 7	Xp21.2	23-032737584	354.3	01713-L01281	9613	1.48	0.03	>>*	>
DMD - 6	Xp21.2	23-032744586	314	01373-L01021	9498	1.41	0.04	>>*	>
DMD - 5	Xp21.2	23-032751333	281.8	01954-L01574	4399	1.42	0.02	>>*	>
DMD - 4	Xp21.2	23-032772817	241.4	01365-L01013	10786	1.35	0.06	>>*	>
DMD - 3	Xp21.2	23-032777782	208.9	01361-L01009	12441	1.49	0.02	>>*	>
DMD - 2	Xp21.2	23-032948164	168.2	01357-L01005	14523	1.43	0.02	>>*	>
DMD - 1	Xp21.2	23-033139494	135.6	01353-L01001	13360	0.99	0.04	=	=
DDX3Y - 18	Yq11.21	24-013540196	0	S0003-L00313	0	0	0	=	=
REFERENCE	Xp22.13	23-018707148	202.5	01691-L00465	4308	1	0.01	=	=
REFERENCE	Xq12	23-066682270	126.6	01690-L00423	4484	1.09	0.02	>>	=
REFERENCE - 4	Xq13.1	23-067976549	416.5	03766-L03227	3948	0.96	0.07	=	=
REFERENCE - 4	Xq28	23-152790845	485.9	01692-L01531	5293	0.99	0.01	=	=
REFERENCE - 4	Xq28	23-152949042	273.4	01768-L01617	5257	0.99	0.01	=	=

Heat-mapping results

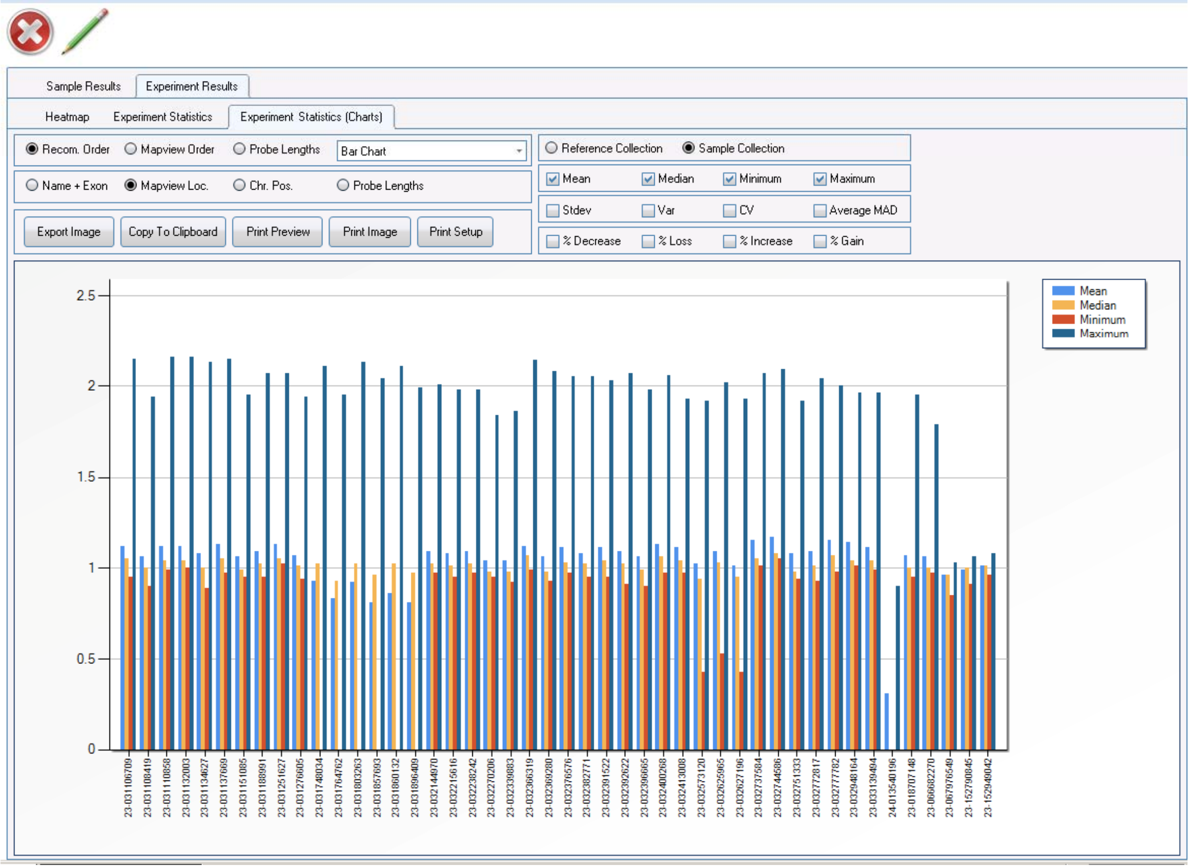


	Gene	Chr.P...	Position	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D1. ...	⊞ D2. ...	⊞ D2. ...	⊞ D2. ...	⊞ D2. ...	⊞ D2. ...
45	DMD - 70	Xp21.2	23-031106...	0.95	0.98	1.02	1	1.09	1.03	0.99	1.06	1.05	1.08	2.15	1.12	1.07	1	1.04	1.04
41	DMD - 69	Xp21.2	23-031108...	0.9	0.97	1.03	1	1	0.98	0.97	1	1.02	0.99	1.94	0.97	1.03	1	0.98	1.02
36	DMD - 68	Xp21.2	23-031110...	1	0.96	1.04	1.09	1.04	1.07	1.06	1.02	1.05	1.02	2.16	1.03	1.07	1.04	1.04	0.99
32	DMD - 67	Xp21.2	23-031132...	1.01	0.94	1.06	1.07	1.03	1.02	1.08	1.04	1.04	1.05	2.16	1.03	1.12	1.03	1.02	1
28	DMD - 66	Xp21.2	23-031134...	1	0.95	1.06	1.03	1.09	0.89	0.97	1.01	0.99	1	2.13	1.06	1.05	0.93	0.98	0.95
24	DMD - 65	Xp21.2	23-031137...	0.97	0.97	1.04	1.09	1.06	1.01	1.05	1.08	1.06	1.03	2.15	1.09	1.11	1.04	1.02	1.03
19	DMD - 64	Xp21.2	23-031151...	0.98	0.98	1.02	1.06	1.01	0.95	1	0.96	1.02	0.99	1.95	1.01	0.95	0.99	0.96	0.99
15	DMD - 63	Xp21.2	23-031188...	0.95	0.98	1.03	1.03	1.07	0.97	1	1.02	1.05	1.02	2.07	1.04	1.02	1.01	1	1.02
10	DMD - 62	Xp21.2	23-031251...	1.04	0.96	1.04	1.1	1.1	1.05	1.03	1.06	1.05	1.03	2.07	1.09	1.05	1.04	1.02	1.06
6	DMD - 61	Xp21.2	23-031276...	0.97	0.95	1.05	1.06	1.08	0.94	0.98	1.01	1.06	1	1.94	1.03	1.07	0.96	0.96	0.94
43	DMD - 50	Xp21.2	23-031748...	1.09	0.92	1.09	1.04	1.08	0.52	0.98	0	0	0.98	2.11	1.07	1.01	0.96	1.02	1.09
39	DMD - 49	Xp21.2	23-031764...	0.93	1.01	0.99	0.94	1.02	0.54	0.85	0	0	0.98	1.95	0.99	0.8	0.81	0.93	0.95
34	DMD - 48	Xp21.2	23-031803...	0.96	0.96	1.04	1.09	1.03	0.51	1.02	0	0	0.99	2.13	1.06	1	1.03	1.02	1.07
30	DMD - 47	Xp21.2	23-031857...	0.98	0.95	1.06	1.01	0	0.48	0.96	0	1.06	1.02	2.04	0.95	0	0.93	0.97	0.98
26	DMD - 46	Xp21.2	23-031860...	0.98	0.98	1.02	1.12	0	0.5	1.02	0	1.12	1.02	2.11	1.06	0	1.02	1	1.07
22	DMD - 45	Xp21.2	23-031896...	0.99	0.99	1.01	0.99	0	0.51	0.97	0	1.05	0.96	1.99	0.98	0	1	0.96	0.98
17	DMD - 44	Xp21.2	23-032144...	0.98	0.99	1.01	1.08	1.03	1.01	0.99	1.01	1.05	1.05	2.01	1.05	1.02	0.97	0.97	1.02
13	DMD - 43	Xp21.2	23-032215...	1	0.98	1.02	1.01	1.02	1	1.01	0.98	1.03	1.05	1.98	1.11	1.06	0.98	0.95	0.95
8	DMD - 42	Xp21.2	23-032238...	0.97	0.99	1.01	1.09	1.03	1.1	1.02	1.04	1.08	1	1.98	1	1	0.99	0.98	1.02
4	DMD - 41	Xp21.2	23-032270...	0.95	1.01	0.99	1.01	1.02	1	0.95	0.97	1.01	0.98	1.84	0.98	0.98	0.96	0.98	0.95
44	DMD - 30	Xp21.2	23-032339...	0.96	1.03	0.97	1.05	0.99	0.97	0.92	0.97	0.94	0.99	1.86	0.98	0.99	1	0.95	0.97
40	DMD - 29	Xp21.2	23-032366...	0.99	1	1	1.08	1.08	1.08	1.07	1.01	1.08	1.02	2.14	1.06	1.08	0.99	1.02	1.02
35	DMD - 28	Xp21.2	23-032369...	0.95	0.98	1.02	1.05	0.99	1.07	0.97	0.95	1	0.95	2.08	0.97	0.96	0.99	1	0.93
31	DMD - 27	Xp21.2	23-032376...	0.97	0.99	1.01	1.02	1.06	1.03	0.97	1.14	1.07	1.07	2.05	1.06	1.02	1.04	0.98	1
27	DMD - 26	Xp21.2	23-032382...	0.96	0.99	1.01	1	1.08	1.02	1.02	1.04	1.07	0.95	2.05	1.02	1.05	0.95	0.96	1.01
23	DMD - 25	Xp21.2	23-032391...	0.95	1	1	1.12	1.05	1.05	1.02	1.11	1.11	1.03	2.03	0.96	1.11	1.03	1.01	0.98
18	DMD - 24	Xp21.2	23-032392...	0.91	0.98	1.02	1.01	1.06	1.05	1.02	1.03	1.05	0.96	2.07	1.02	1.11	0.98	1	0.95
14	DMD - 23	Xp21.2	23-032396...	0.9	1	1	1	1.01	0.99	0.94	1.04	1.08	0.99	1.98	0.99	1.02	0.98	0.96	0.98
9	DMD - 22	Xp21.2	23-032400...	0.97	0.97	1.04	1.09	1.08	1.08	1.06	1.03	1.13	1.04	2.06	1.04	1.06	1.03	1.08	1.03
5	DMD - 21	Xp21.2	23-032413...	0.97	0.98	1.03	1.07	1.13	1.08	1.02	1.03	1.06	1.01	1.93	1.05	1.11	1.03	1	1.02
42	DMD - 10	Xp21.2	23-032573...	1.38	0.98	1.02	0.43	0.92	0.91	0.89	0.92	1.12	1	1.92	0.96	1	0.89	0.95	0.94
38	DMD - 9	Xp21.2	23-032625...	1.37	0.95	1.06	0.53	1.09	1	1.01	1.04	1.02	1.06	2.02	1.05	1.04	1.02	1	1
33	DMD - 8	Xp21.2	23-032627...	1.32	1	1	0.43	0.95	0.96	0.9	0.92	1.08	1.01	1.93	0.97	0.95	0.89	0.96	0.9
29	DMD - 7	Xp21.2	23-032737...	1.48	0.98	1.02	1.1	1.05	1.03	1.06	1.01	1.02	1.05	2.07	1.05	1.05	1.01	1.01	1.04
25	DMD - 6	Xp21.2	23-032744...	1.41	0.98	1.02	1.06	1.1	1.05	1.08	1.1	1.1	1.06	2.09	1.09	1.07	1.06	1.05	1.07
21	DMD - 5	Xp21.2	23-032751...	1.42	1	1	0.99	1	0.99	0.94	0.94	0.99	0.97	1.92	0.97	0.96	0.97	1.05	0.98
16	DMD - 4	Xp21.2	23-032772...	1.35	0.96	1.04	1.01	1.01	0.96	0.93	1.03	1	1	2.04	1.02	1.04	0.96	0.97	0.93
12	DMD - 3	Xp21.2	23-032777...	1.49	1	1	1.1	1.09	1.05	1.01	1.07	1.08	1.03	2	1.09	1.06	1.03	0.98	1.02
7	DMD - 2	Xp21.2	23-032948...	1.43	0.99	1.01	1.1	1.1	1.03	1.04	1.01	1.05	1.03	1.96	1.08	1.03	1.04	1.03	1.01
3	DMD - 1	Xp21.2	23-033139...	0.99	0.96	1.04	1.07	1.04	1.07	1.03	1.04	1.11	1.03	1.96	1.02	1.06	1.03	1.01	1.05
1	DDX3Y - 18	Yq11.21	24-013540...	0	0	0	0	0.86	0	0	0.86	0.9	0	0	0.9	0.86	0	0	0
11	REFERENCE	Xp22.13	23-018707...	1	1	1	0.97	1.12	0.98	1	1	1.1	0.97	1.95	1.01	0.97	0.95	1	0.99
2	REFERENCE	Xq12	23-066682...	1.09	0.99	1.01	1.02	0.97	0.99	1.07	0.98	1	0.97	1.79	0.97	1.05	0.98	1.01	0.99
37	REFERENCE (418 nt)	Xq13.1	23-067976...	0.96	1.08	0.93	0.99	0.94	0.95	0.96	0.98	0.85	1	0.96	0.96	1	1.03	0.96	0.97
46	REFERENCE (490 nt)	Xq28	23-152790...	0.99	1	1	1.06	0.91	1	0.97	1.03	0.96	1.02	0.92	0.98	0.99	1.01	1.02	1
20	REFERENCE (274 nt)	Xq28	23-152949...	0.99	1	1	1	1.02	1.03	1.01	0.98	1.08	1.03	0.97	1.02	1	1.05	0.96	1.06

Conditional format options

- Conditional formats
  - Heat-map experiment
  - Heat-map sample
  - Arbitrary colours
  - Gradient colors
- Grid fitting
- Export options

Exploring experiment statistics



● Recom. Order ○ Mapview Order ○ Probe Lengths Bar Chart

○ Name + Exon ● Mapview Loc. ○ Chr. Pos. ○ Probe Lengths

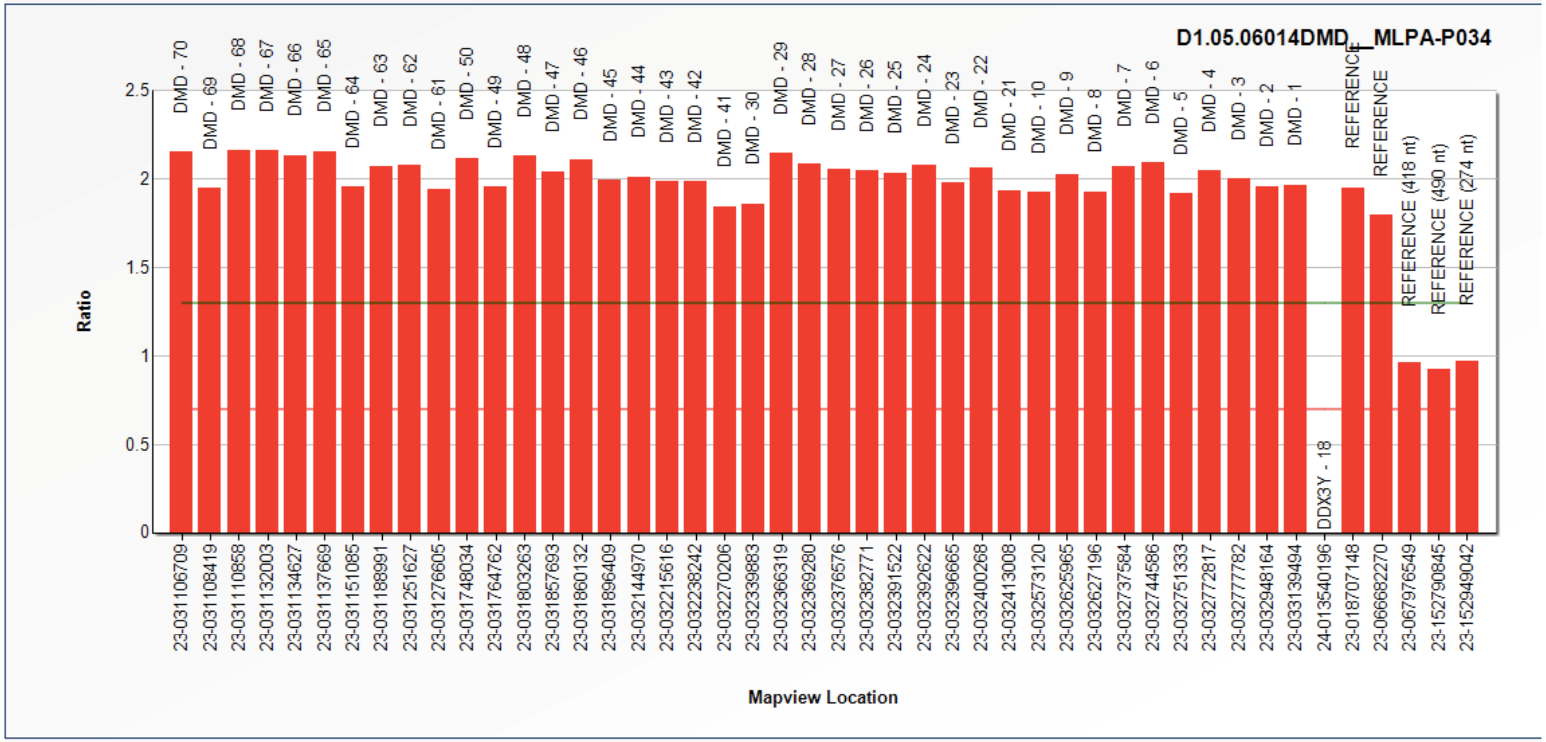
○ Reference Collection ● Sample Collection

☑ Mean ☑ Median ☑ Minimum ☑ Maximum

☐ Stdev ☐ Var ☐ CV ☐ Average MAD

☐ % Decrease ☐ % Loss ☐ % Increase ☐ % Gain

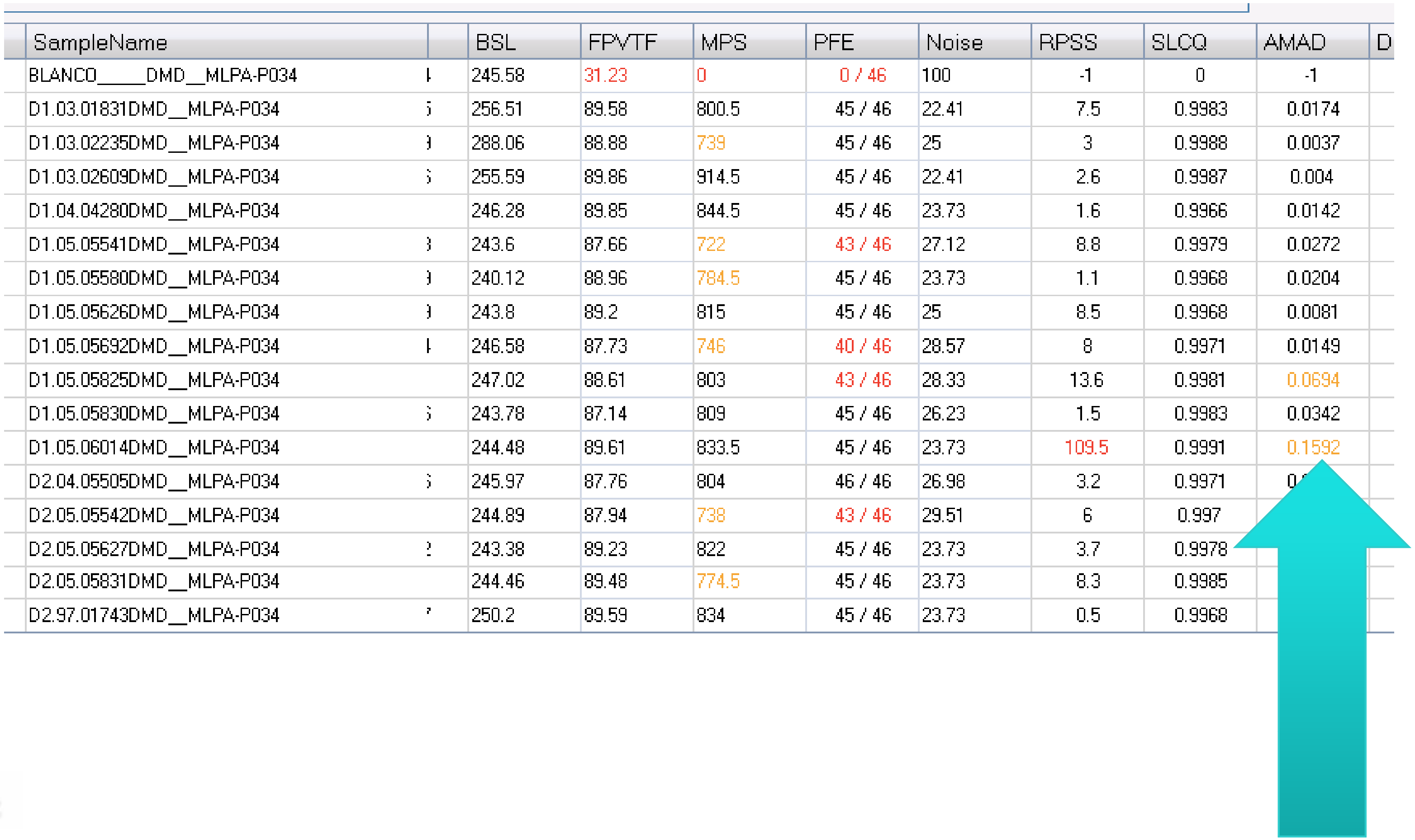
Sample 6014 Block analysis



OFFALYSER

MRC-Holland b.v.
MLPA

Increased AMAD indicated problem on analysis method

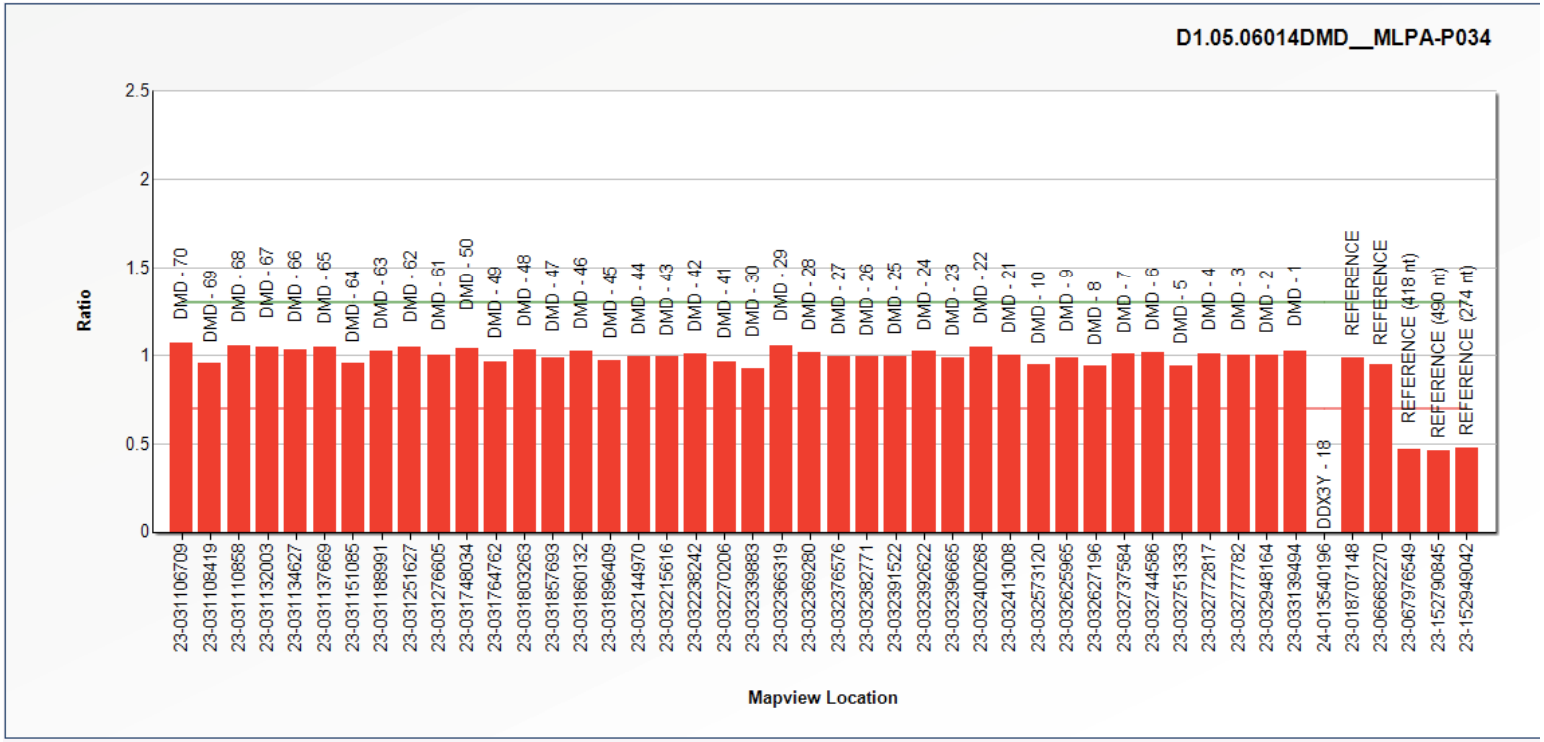


SampleName	BSL	FPVTF	MPS	PFE	Noise	RPSS	SLCQ	AMAD	D
BLANCO_____DMD__MLPA-P034	245.58	31.23	0	0 / 46	100	-1	0	-1	
D1.03.01831DMD__MLPA-P034	256.51	89.58	800.5	45 / 46	22.41	7.5	0.9983	0.0174	
D1.03.02235DMD__MLPA-P034	288.06	88.88	739	45 / 46	25	3	0.9988	0.0037	
D1.03.02609DMD__MLPA-P034	255.59	89.86	914.5	45 / 46	22.41	2.6	0.9987	0.004	
D1.04.04280DMD__MLPA-P034	246.28	89.85	844.5	45 / 46	23.73	1.6	0.9966	0.0142	
D1.05.05541DMD__MLPA-P034	243.6	87.66	722	43 / 46	27.12	8.8	0.9979	0.0272	
D1.05.05580DMD__MLPA-P034	240.12	88.96	784.5	45 / 46	23.73	1.1	0.9968	0.0204	
D1.05.05626DMD__MLPA-P034	243.8	89.2	815	45 / 46	25	8.5	0.9968	0.0081	
D1.05.05692DMD__MLPA-P034	246.58	87.73	746	40 / 46	28.57	8	0.9971	0.0149	
D1.05.05825DMD__MLPA-P034	247.02	88.61	803	43 / 46	28.33	13.6	0.9981	0.0694	
D1.05.05830DMD__MLPA-P034	243.78	87.14	809	45 / 46	26.23	1.5	0.9983	0.0342	
D1.05.06014DMD__MLPA-P034	244.48	89.61	833.5	45 / 46	23.73	109.5	0.9991	0.1592	
D2.04.05505DMD__MLPA-P034	245.97	87.76	804	46 / 46	26.98	3.2	0.9971	0.	
D2.05.05542DMD__MLPA-P034	244.89	87.94	738	43 / 46	29.51	6	0.997		
D2.05.05627DMD__MLPA-P034	243.38	89.23	822	45 / 46	23.73	3.7	0.9978		
D2.05.05831DMD__MLPA-P034	244.46	89.48	774.5	45 / 46	23.73	8.3	0.9985		
D2.97.01743DMD__MLPA-P034	250.2	89.59	834	45 / 46	23.73	0.5	0.9968		

COFFALYSER

MRC-Holland b.v.
MLPA

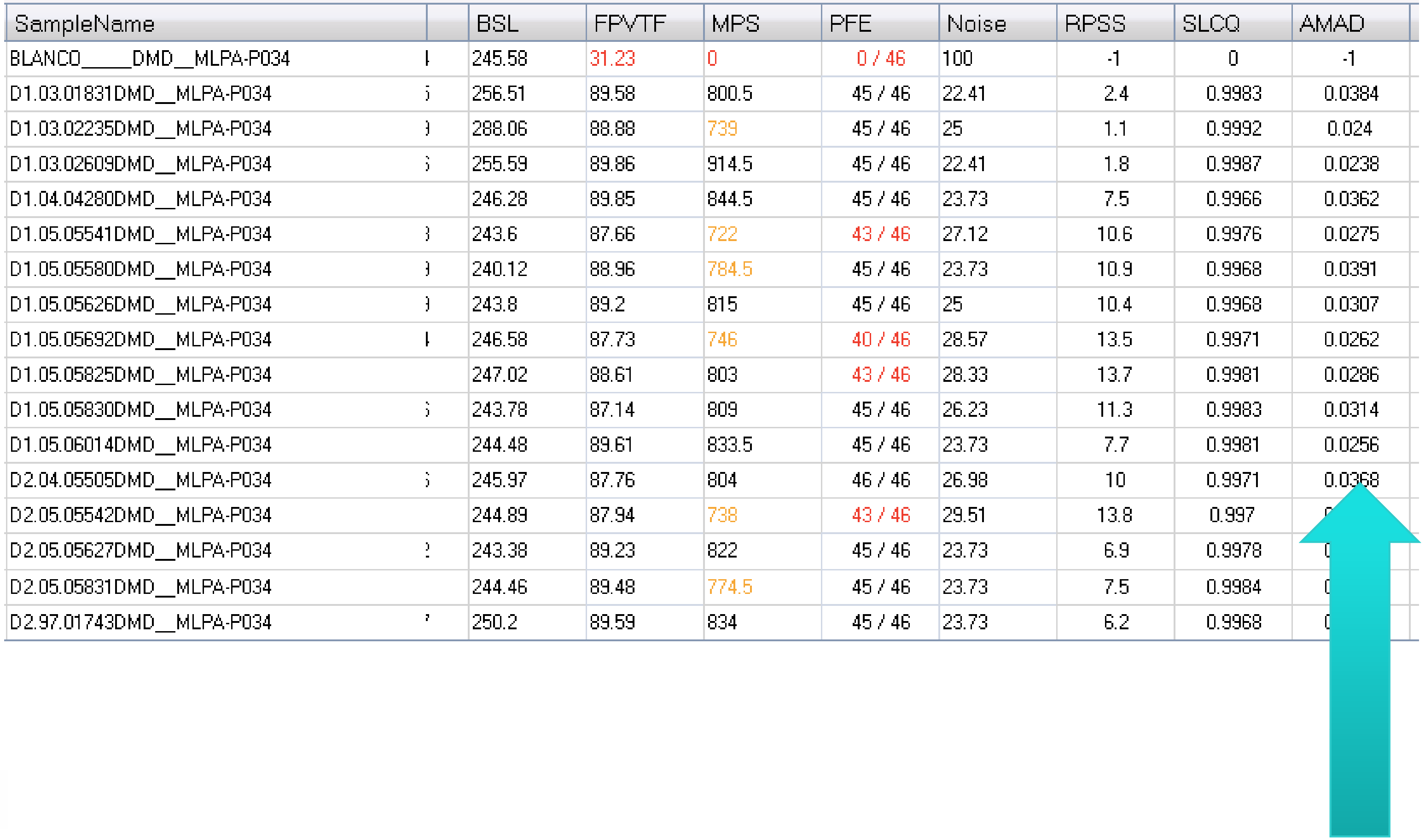
Sample 6014 Population



COFFALYSER

MRC-Holland b.v.
MLPA

AMAD ok

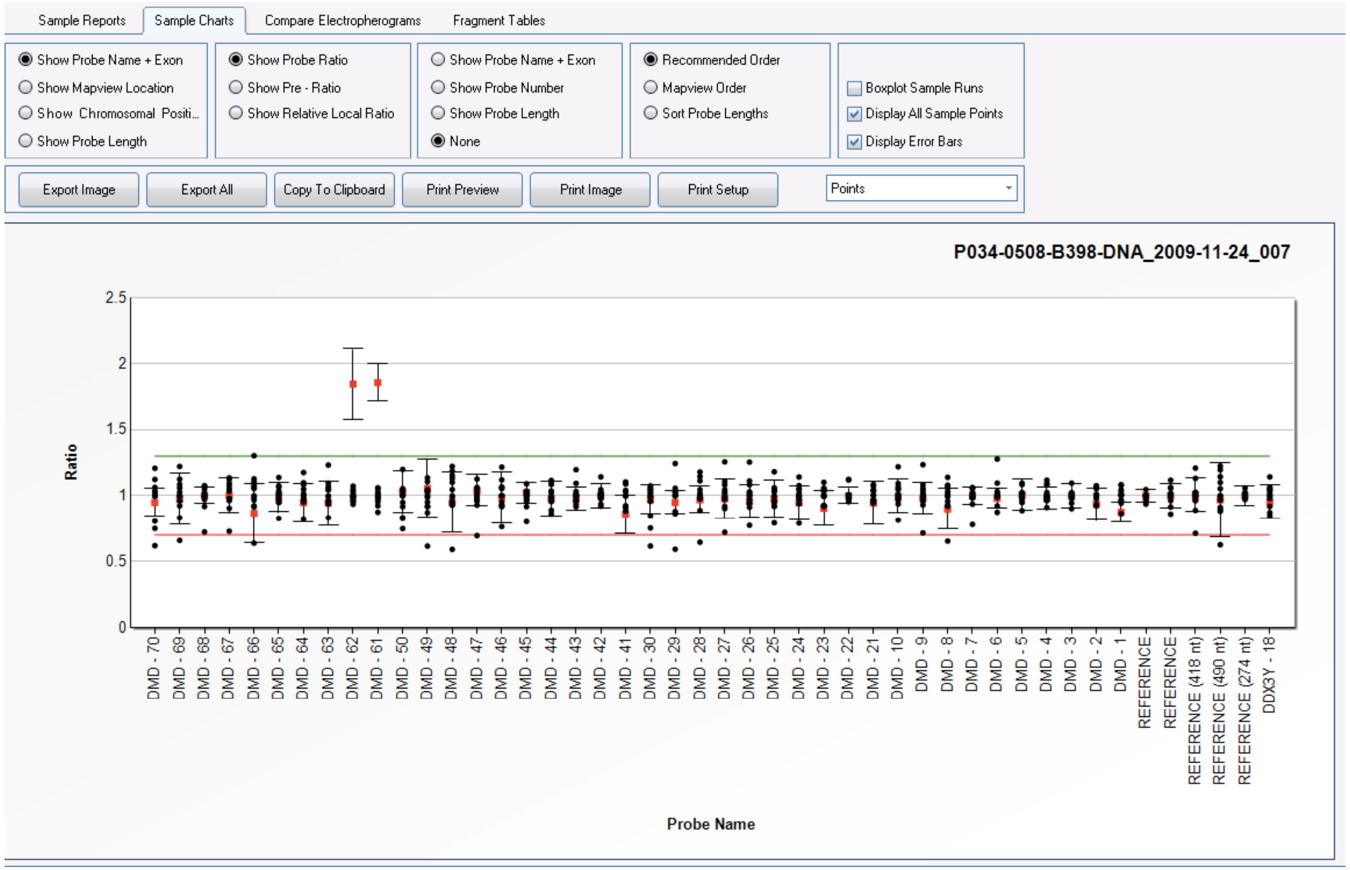


SampleName		BSL	FPVTF	MPS	PFE	Noise	RPSS	SLCQ	AMAD
BLANCO_____DMD__MLPA-P034		245.58	31.23	0	0 / 46	100	-1	0	-1
D1.03.01831DMD__MLPA-P034		256.51	89.58	800.5	45 / 46	22.41	2.4	0.9983	0.0384
D1.03.02235DMD__MLPA-P034		288.06	88.88	739	45 / 46	25	1.1	0.9992	0.024
D1.03.02609DMD__MLPA-P034		255.59	89.86	914.5	45 / 46	22.41	1.8	0.9987	0.0238
D1.04.04280DMD__MLPA-P034		246.28	89.85	844.5	45 / 46	23.73	7.5	0.9966	0.0362
D1.05.05541DMD__MLPA-P034		243.6	87.66	722	43 / 46	27.12	10.6	0.9976	0.0275
D1.05.05580DMD__MLPA-P034		240.12	88.96	784.5	45 / 46	23.73	10.9	0.9968	0.0391
D1.05.05626DMD__MLPA-P034		243.8	89.2	815	45 / 46	25	10.4	0.9968	0.0307
D1.05.05692DMD__MLPA-P034		246.58	87.73	746	40 / 46	28.57	13.5	0.9971	0.0262
D1.05.05825DMD__MLPA-P034		247.02	88.61	803	43 / 46	28.33	13.7	0.9981	0.0286
D1.05.05830DMD__MLPA-P034		243.78	87.14	809	45 / 46	26.23	11.3	0.9983	0.0314
D1.05.06014DMD__MLPA-P034		244.48	89.61	833.5	45 / 46	23.73	7.7	0.9981	0.0256
D2.04.05505DMD__MLPA-P034		245.97	87.76	804	46 / 46	26.98	10	0.9971	0.0368
D2.05.05542DMD__MLPA-P034		244.89	87.94	738	43 / 46	29.51	13.8	0.997	[illegible]
D2.05.05627DMD__MLPA-P034		243.38	89.23	822	45 / 46	23.73	6.9	0.9978	[illegible]
D2.05.05831DMD__MLPA-P034		244.46	89.48	774.5	45 / 46	23.73	7.5	0.9984	[illegible]
D2.97.01743DMD__MLPA-P034		250.2	89.59	834	45 / 46	23.73	6.2	0.9968	[illegible]

Test 2 Analysis wo references

- Only work with low incidence of aberrations

Single aberrant sample in population



● Show Probe Name + Exon
○ Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

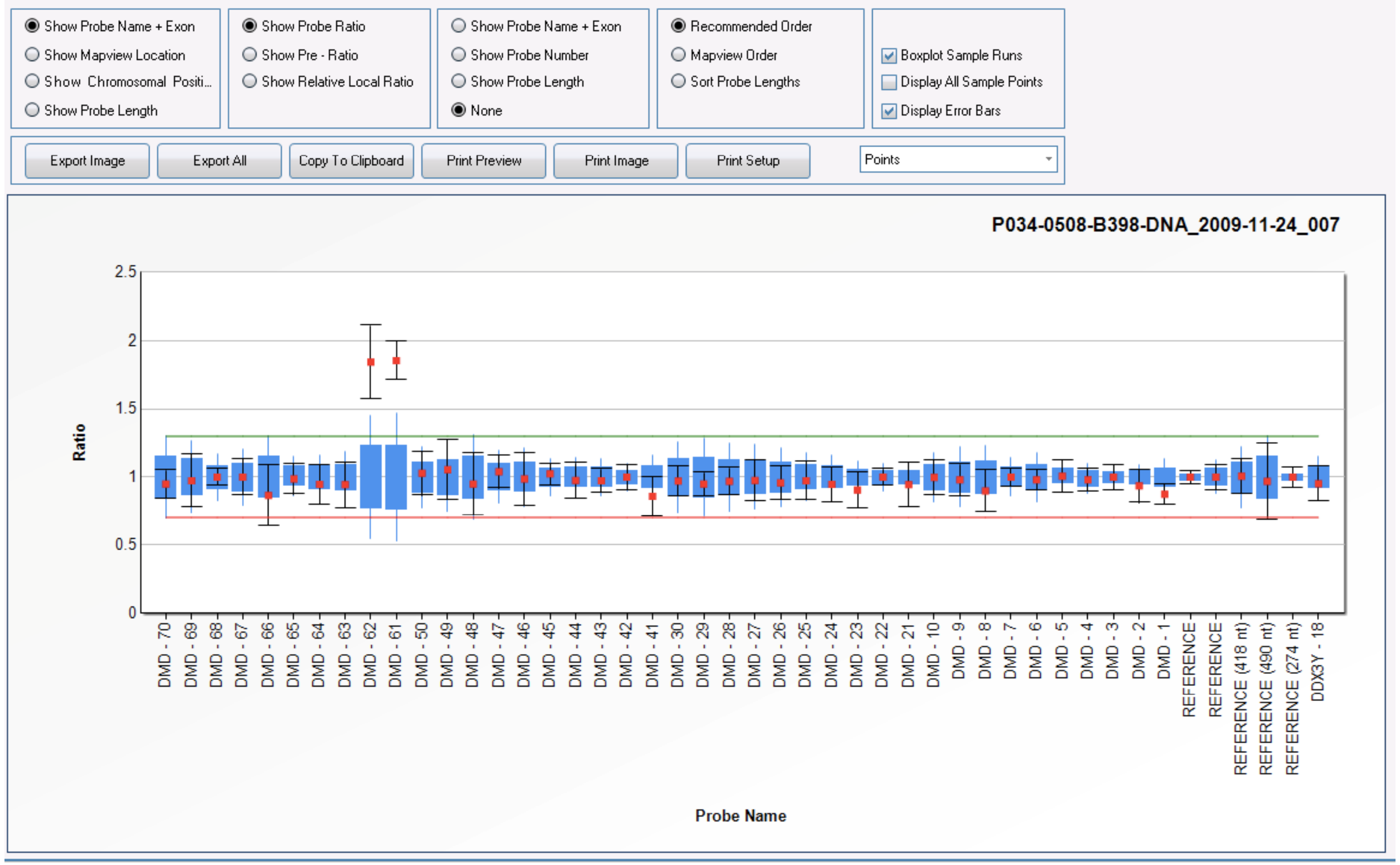
○ Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
● None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Sample Runs
☑ Display All Sample Points
☑ Display Error Bars

Points

Successful comparison to sample population



● Show Probe Name + Exon
○ Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

○ Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
● None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

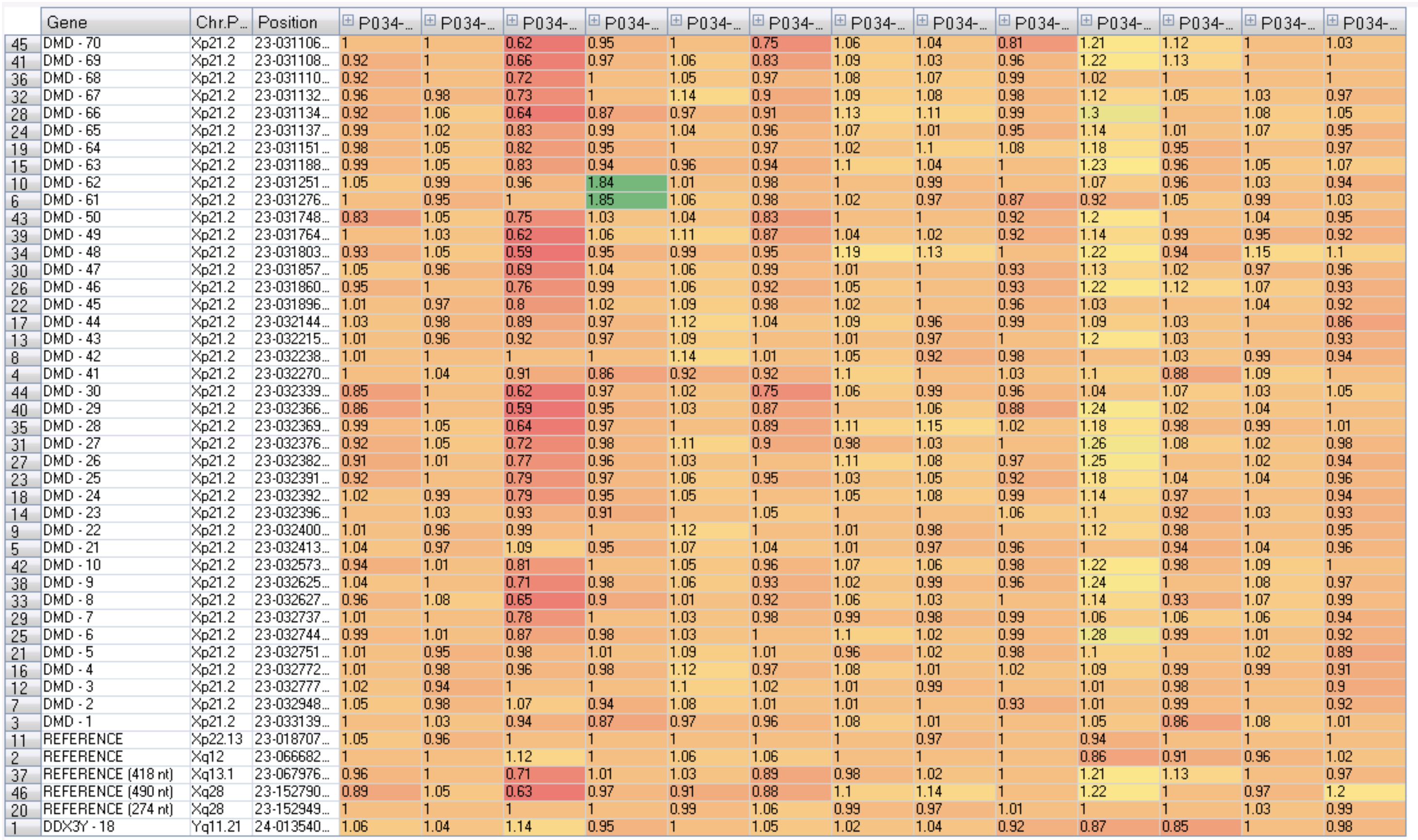
☑ Boxplot Sample Runs
☐ Display All Sample Points
☑ Display Error Bars

Points

COFFALYSER

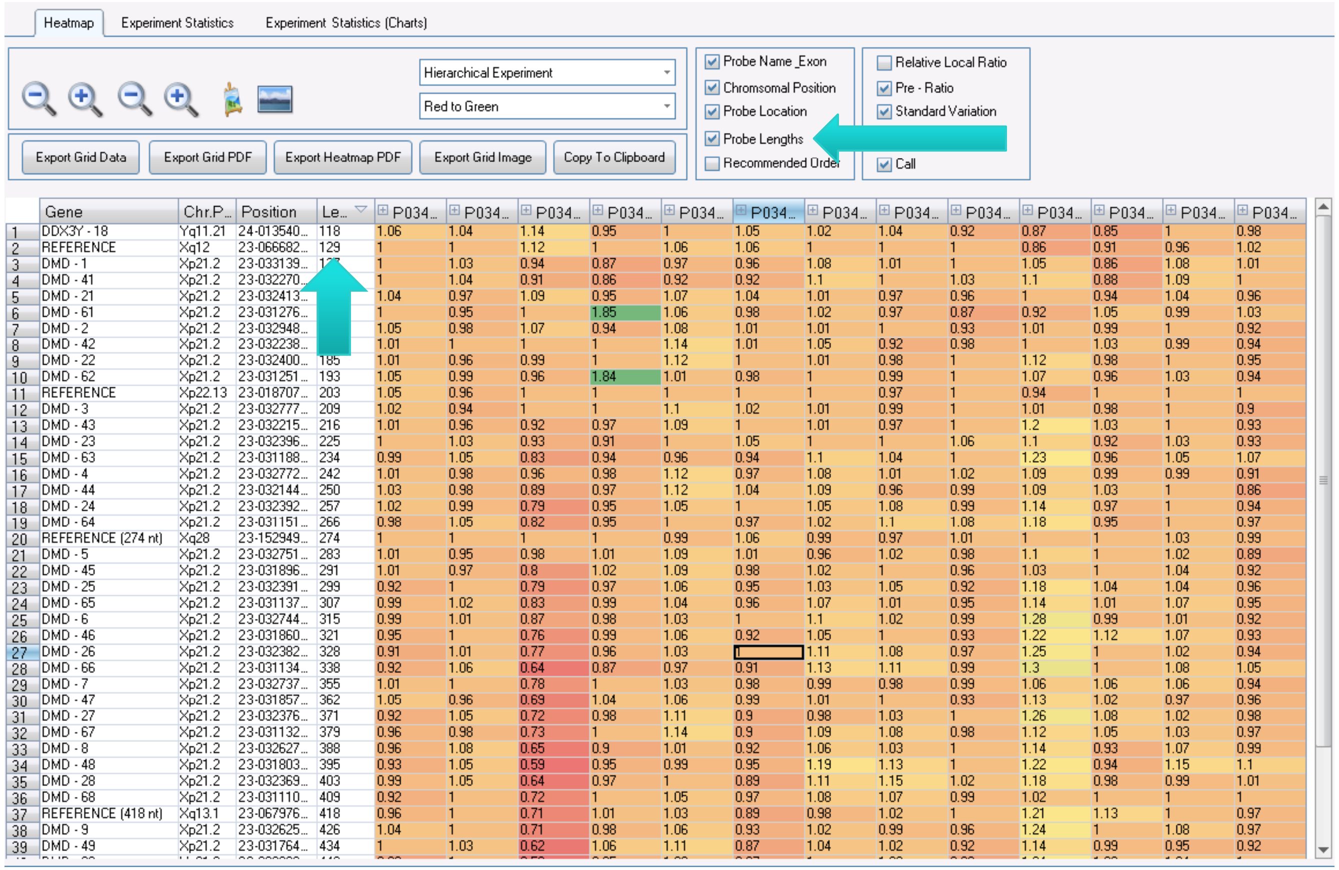
MRC-Holland b.v.
MLPA

Heatmap to slope corrected data



	Gene	Chr.P…	Position	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…	P034-…
45	DMD - 70	Xp21.2	23-031106…	1	1	0.62	0.95	1	0.75	1.06	1.04	0.81	1.21	1.12	1	1.03
41	DMD - 69	Xp21.2	23-031108…	0.92	1	0.66	0.97	1.06	0.83	1.09	1.03	0.96	1.22	1.13	1	1
36	DMD - 68	Xp21.2	23-031110…	0.92	1	0.72	1	1.05	0.97	1.08	1.07	0.99	1.02	1	1	1
32	DMD - 67	Xp21.2	23-031132…	0.96	0.98	0.73	1	1.14	0.9	1.09	1.08	0.98	1.12	1.05	1.03	0.97
28	DMD - 66	Xp21.2	23-031134…	0.92	1.06	0.64	0.87	0.97	0.91	1.13	1.11	0.99	1.3	1	1.08	1.05
24	DMD - 65	Xp21.2	23-031137…	0.99	1.02	0.83	0.99	1.04	0.96	1.07	1.01	0.95	1.14	1.01	1.07	0.95
19	DMD - 64	Xp21.2	23-031151…	0.98	1.05	0.82	0.95	1	0.97	1.02	1.1	1.08	1.18	0.95	1	0.97
15	DMD - 63	Xp21.2	23-031188…	0.99	1.05	0.83	0.94	0.96	0.94	1.1	1.04	1	1.23	0.96	1.05	1.07
10	DMD - 62	Xp21.2	23-031251…	1.05	0.99	0.96	1.84	1.01	0.98	1	0.99	1	1.07	0.96	1.03	0.94
6	DMD - 61	Xp21.2	23-031276…	1	0.95	1	1.85	1.06	0.98	1.02	0.97	0.87	0.92	1.05	0.99	1.03
43	DMD - 50	Xp21.2	23-031748…	0.83	1.05	0.75	1.03	1.04	0.83	1	1	0.92	1.2	1	1.04	0.95
39	DMD - 49	Xp21.2	23-031764…	1	1.03	0.62	1.06	1.11	0.87	1.04	1.02	0.92	1.14	0.99	0.95	0.92
34	DMD - 48	Xp21.2	23-031803…	0.93	1.05	0.59	0.95	0.99	0.95	1.19	1.13	1	1.22	0.94	1.15	1.1
30	DMD - 47	Xp21.2	23-031857…	1.05	0.96	0.69	1.04	1.06	0.99	1.01	1	0.93	1.13	1.02	0.97	0.96
26	DMD - 46	Xp21.2	23-031860…	0.95	1	0.76	0.99	1.06	0.92	1.05	1	0.93	1.22	1.12	1.07	0.93
22	DMD - 45	Xp21.2	23-031896…	1.01	0.97	0.8	1.02	1.09	0.98	1.02	1	0.96	1.03	1	1.04	0.92
17	DMD - 44	Xp21.2	23-032144…	1.03	0.98	0.89	0.97	1.12	1.04	1.09	0.96	0.99	1.09	1.03	1	0.86
13	DMD - 43	Xp21.2	23-032215…	1.01	0.96	0.92	0.97	1.09	1	1.01	0.97	1	1.2	1.03	1	0.93
8	DMD - 42	Xp21.2	23-032238…	1.01	1	1	1	1.14	1.01	1.05	0.92	0.98	1	1.03	0.99	0.94
4	DMD - 41	Xp21.2	23-032270…	1	1.04	0.91	0.86	0.92	0.92	1.1	1	1.03	1.1	0.88	1.09	1
44	DMD - 30	Xp21.2	23-032339…	0.85	1	0.62	0.97	1.02	0.75	1.06	0.99	0.96	1.04	1.07	1.03	1.05
40	DMD - 29	Xp21.2	23-032366…	0.86	1	0.59	0.95	1.03	0.87	1	1.06	0.88	1.24	1.02	1.04	1
35	DMD - 28	Xp21.2	23-032369…	0.99	1.05	0.64	0.97	1	0.89	1.11	1.15	1.02	1.18	0.98	0.99	1.01
31	DMD - 27	Xp21.2	23-032376…	0.92	1.05	0.72	0.98	1.11	0.9	0.98	1.03	1	1.26	1.08	1.02	0.98
27	DMD - 26	Xp21.2	23-032382…	0.91	1.01	0.77	0.96	1.03	1	1.11	1.08	0.97	1.25	1	1.02	0.94
23	DMD - 25	Xp21.2	23-032391…	0.92	1	0.79	0.97	1.06	0.95	1.03	1.05	0.92	1.18	1.04	1.04	0.96
18	DMD - 24	Xp21.2	23-032392…	1.02	0.99	0.79	0.95	1.05	1	1.05	1.08	0.99	1.14	0.97	1	0.94
14	DMD - 23	Xp21.2	23-032396…	1	1.03	0.93	0.91	1	1.05	1	1	1.06	1.1	0.92	1.03	0.93
9	DMD - 22	Xp21.2	23-032400…	1.01	0.96	0.99	1	1.12	1	1.01	0.98	1	1.12	0.98	1	0.95
5	DMD - 21	Xp21.2	23-032413…	1.04	0.97	1.09	0.95	1.07	1.04	1.01	0.97	0.96	1	0.94	1.04	0.96
42	DMD - 10	Xp21.2	23-032573…	0.94	1.01	0.81	1	1.05	0.96	1.07	1.06	0.98	1.22	0.98	1.09	1
38	DMD - 9	Xp21.2	23-032625…	1.04	1	0.71	0.98	1.06	0.93	1.02	0.99	0.96	1.24	1	1.08	0.97
33	DMD - 8	Xp21.2	23-032627…	0.96	1.08	0.65	0.9	1.01	0.92	1.06	1.03	1	1.14	0.93	1.07	0.99
29	DMD - 7	Xp21.2	23-032737…	1.01	1	0.78	1	1.03	0.98	0.99	0.98	0.99	1.06	1.06	1.06	0.94
25	DMD - 6	Xp21.2	23-032744…	0.99	1.01	0.87	0.98	1.03	1	1.1	1.02	0.99	1.28	0.99	1.01	0.92
21	DMD - 5	Xp21.2	23-032751…	1.01	0.95	0.98	1.01	1.09	1.01	0.96	1.02	0.98	1.1	1	1.02	0.89
16	DMD - 4	Xp21.2	23-032772…	1.01	0.98	0.96	0.98	1.12	0.97	1.08	1.01	1.02	1.09	0.99	0.99	0.91
12	DMD - 3	Xp21.2	23-032777…	1.02	0.94	1	1	1.1	1.02	1.01	0.99	1	1.01	0.98	1	0.9
7	DMD - 2	Xp21.2	23-032948…	1.05	0.98	1.07	0.94	1.08	1.01	1.01	1	0.93	1.01	0.99	1	0.92
3	DMD - 1	Xp21.2	23-033139…	1	1.03	0.94	0.87	0.97	0.96	1.08	1.01	1	1.05	0.86	1.08	1.01
11	REFERENCE	Xp22.13	23-018707…	1.05	0.96	1	1	1	1	1	0.97	1	0.94	1	1	1
2	REFERENCE	Xq12	23-066682…	1	1	1.12	1	1.06	1.06	1	1	1	0.86	0.91	0.96	1.02
37	REFERENCE (418 nt)	Xq13.1	23-067976…	0.96	1	0.71	1.01	1.03	0.89	0.98	1.02	1	1.21	1.13	1	0.97
46	REFERENCE (490 nt)	Xq28	23-152790…	0.89	1.05	0.63	0.97	0.91	0.88	1.1	1.14	1	1.22	1	0.97	1.2
20	REFERENCE (274 nt)	Xq28	23-152949…	1	1	1	1	0.99	1.06	0.99	0.97	1.01	1	1	1.03	0.99
1	DDX3Y - 18	Yq11.21	24-013540…	1.06	1.04	1.14	0.95	1	1.05	1.02	1.04	0.92	0.87	0.85	1	0.98

Slope corrected / uncorrected results



☑ Probe Name_Exon
☑ Chromsomal Position
☑ Probe Location
☑ Probe Lengths
☐ Recommended Order

☐ Relative Local Ratio
☑ Pre - Ratio
☑ Standard Variation
☑ Call

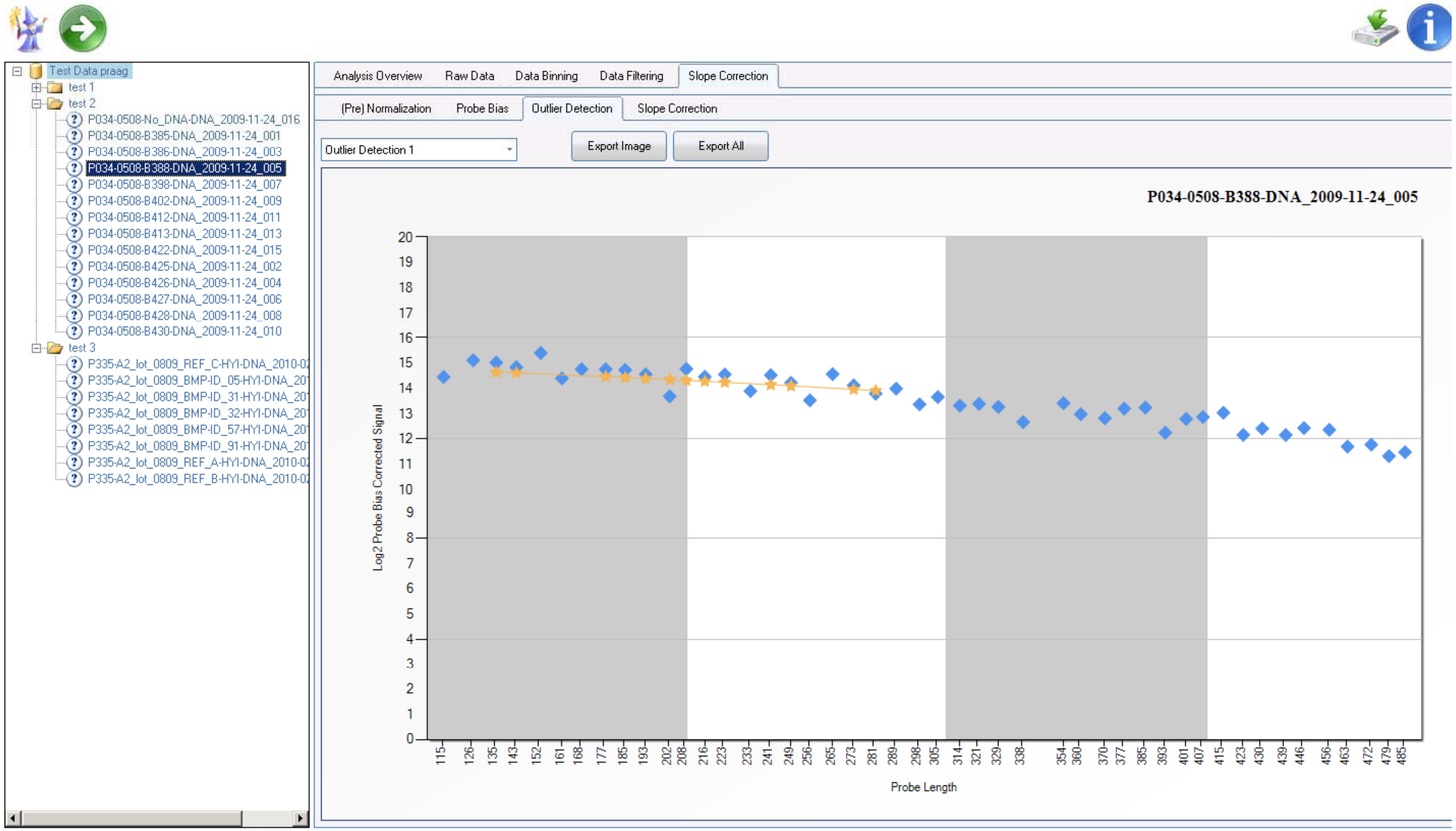
	Gene	Chr.P...	Position	Le...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...	P034...
1	DDX3Y - 18	Yq11.21	24-013540...	118	1.06	1.04	1.14	0.95	1	1.05	1.02	1.04	0.92	0.87	0.85	1	0.98
2	REFERENCE	Xq12	23-066682...	129	1	1	1.12	1	1.06	1.06	1	1	1	0.86	0.91	0.96	1.02
3	DMD - 1	Xp21.2	23-033139...	[illegible]	1	1.03	0.94	0.87	0.97	0.96	1.08	1.01	1	1.05	0.86	1.08	1.01
4	DMD - 41	Xp21.2	23-032270...	[illegible]	1	1.04	0.91	0.86	0.92	0.92	1.1	1	1.03	1.1	0.88	1.09	1
5	DMD - 21	Xp21.2	23-032413...	[illegible]	1.04	0.97	1.09	0.95	1.07	1.04	1.01	0.97	0.96	1	0.94	1.04	0.96
6	DMD - 61	Xp21.2	23-031276...	[illegible]	1	0.95	1	1.85	1.06	0.98	1.02	0.97	0.87	0.92	1.05	0.99	1.03
7	DMD - 2	Xp21.2	23-032948...	[illegible]	1.05	0.98	1.07	0.94	1.08	1.01	1.01	1	0.93	1.01	0.99	1	0.92
8	DMD - 42	Xp21.2	23-032238...	[illegible]	1.01	1	1	1	1.14	1.01	1.05	0.92	0.98	1	1.03	0.99	0.94
9	DMD - 22	Xp21.2	23-032400...	185	1.01	0.96	0.99	1	1.12	1	1.01	0.98	1	1.12	0.98	1	0.95
10	DMD - 62	Xp21.2	23-031251...	193	1.05	0.99	0.96	1.84	1.01	0.98	1	0.99	1	1.07	0.96	1.03	0.94
11	REFERENCE	Xp22.13	23-018707...	203	1.05	0.96	1	1	1	1	1	0.97	1	0.94	1	1	1
12	DMD - 3	Xp21.2	23-032777...	209	1.02	0.94	1	1	1.1	1.02	1.01	0.99	1	1.01	0.98	1	0.9
13	DMD - 43	Xp21.2	23-032215...	216	1.01	0.96	0.92	0.97	1.09	1	1.01	0.97	1	1.2	1.03	1	0.93
14	DMD - 23	Xp21.2	23-032396...	225	1	1.03	0.93	0.91	1	1.05	1	1	1.06	1.1	0.92	1.03	0.93
15	DMD - 63	Xp21.2	23-031188...	234	0.99	1.05	0.83	0.94	0.96	0.94	1.1	1.04	1	1.23	0.96	1.05	1.07
16	DMD - 4	Xp21.2	23-032772...	242	1.01	0.98	0.96	0.98	1.12	0.97	1.08	1.01	1.02	1.09	0.99	0.99	0.91
17	DMD - 44	Xp21.2	23-032144...	250	1.03	0.98	0.89	0.97	1.12	1.04	1.09	0.96	0.99	1.09	1.03	1	0.86
18	DMD - 24	Xp21.2	23-032392...	257	1.02	0.99	0.79	0.95	1.05	1	1.05	1.08	0.99	1.14	0.97	1	0.94
19	DMD - 64	Xp21.2	23-031151...	266	0.98	1.05	0.82	0.95	1	0.97	1.02	1.1	1.08	1.18	0.95	1	0.97
20	REFERENCE (274 nt)	Xq28	23-152949...	274	1	1	1	1	0.99	1.06	0.99	0.97	1.01	1	1	1.03	0.99
21	DMD - 5	Xp21.2	23-032751...	283	1.01	0.95	0.98	1.01	1.09	1.01	0.96	1.02	0.98	1.1	1	1.02	0.89
22	DMD - 45	Xp21.2	23-031896...	291	1.01	0.97	0.8	1.02	1.09	0.98	1.02	1	0.96	1.03	1	1.04	0.92
23	DMD - 25	Xp21.2	23-032391...	299	0.92	1	0.79	0.97	1.06	0.95	1.03	1.05	0.92	1.18	1.04	1.04	0.96
24	DMD - 65	Xp21.2	23-031137...	307	0.99	1.02	0.83	0.99	1.04	0.96	1.07	1.01	0.95	1.14	1.01	1.07	0.95
25	DMD - 6	Xp21.2	23-032744...	315	0.99	1.01	0.87	0.98	1.03	1	1.1	1.02	0.99	1.28	0.99	1.01	0.92
26	DMD - 46	Xp21.2	23-031860...	321	0.95	1	0.76	0.99	1.06	0.92	1.05	1	0.93	1.22	1.12	1.07	0.93
27	DMD - 26	Xp21.2	23-032382...	328	0.91	1.01	0.77	0.96	1.03	1	1.11	1.08	0.97	1.25	1	1.02	0.94
28	DMD - 66	Xp21.2	23-031134...	338	0.92	1.06	0.64	0.87	0.97	0.91	1.13	1.11	0.99	1.3	1	1.08	1.05
29	DMD - 7	Xp21.2	23-032737...	355	1.01	1	0.78	1	1.03	0.98	0.99	0.98	0.99	1.06	1.06	1.06	0.94
30	DMD - 47	Xp21.2	23-031857...	362	1.05	0.96	0.69	1.04	1.06	0.99	1.01	1	0.93	1.13	1.02	0.97	0.96
31	DMD - 27	Xp21.2	23-032376...	371	0.92	1.05	0.72	0.98	1.11	0.9	0.98	1.03	1	1.26	1.08	1.02	0.98
32	DMD - 67	Xp21.2	23-031132...	379	0.96	0.98	0.73	1	1.14	0.9	1.09	1.08	0.98	1.12	1.05	1.03	0.97
33	DMD - 8	Xp21.2	23-032627...	388	0.96	1.08	0.65	0.9	1.01	0.92	1.06	1.03	1	1.14	0.93	1.07	0.99
34	DMD - 48	Xp21.2	23-031803...	395	0.93	1.05	0.59	0.95	0.99	0.95	1.19	1.13	1	1.22	0.94	1.15	1.1
35	DMD - 28	Xp21.2	23-032369...	403	0.99	1.05	0.64	0.97	1	0.89	1.11	1.15	1.02	1.18	0.98	0.99	1.01
36	DMD - 68	Xp21.2	23-031110...	409	0.92	1	0.72	1	1.05	0.97	1.08	1.07	0.99	1.02	1	1	1
37	REFERENCE (418 nt)	Xq13.1	23-067976...	418	0.96	1	0.71	1.01	1.03	0.89	0.98	1.02	1	1.21	1.13	1	0.97
38	DMD - 9	Xp21.2	23-032625...	426	1.04	1	0.71	0.98	1.06	0.93	1.02	0.99	0.96	1.24	1	1.08	0.97
39	DMD - 49	Xp21.2	23-031764...	434	1	1.03	0.62	1.06	1.11	0.87	1.04	1.02	0.92	1.14	0.99	0.95	0.92

COFFALYSER

MRC-Holland b.v.
MLPA

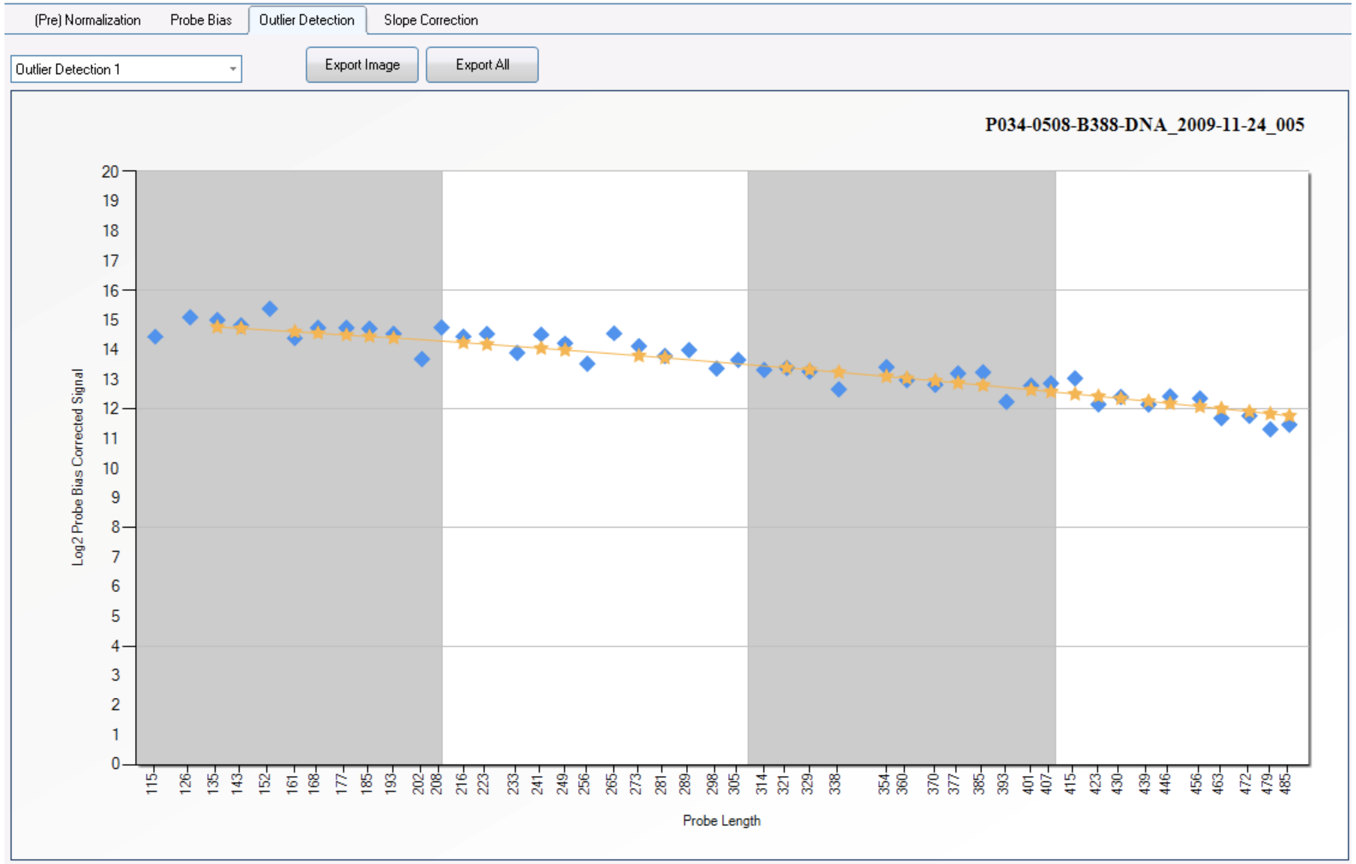
RPSS = 44%

Incorrect slope correction on block method all



- Test Data praag
  - test 1
  - test 2
    - P034-0508-No_DNA-DNA_2009-11-24_016
    - P034-0508-B385-DNA_2009-11-24_001
    - P034-0508-B386-DNA_2009-11-24_003
    - P034-0508-B388-DNA_2009-11-24_005
    - P034-0508-B398-DNA_2009-11-24_007
    - P034-0508-B402-DNA_2009-11-24_009
    - P034-0508-B412-DNA_2009-11-24_011
    - P034-0508-B413-DNA_2009-11-24_013
    - P034-0508-B422-DNA_2009-11-24_015
    - P034-0508-B425-DNA_2009-11-24_002
    - P034-0508-B426-DNA_2009-11-24_004
    - P034-0508-B427-DNA_2009-11-24_006
    - P034-0508-B428-DNA_2009-11-24_008
    - P034-0508-B430-DNA_2009-11-24_010
  - test 3
    - P335-A2_lot_0809_REF_C-HYI-DNA_2010-0
    - P335-A2_lot_0809_BMP-ID_05-HYI-DNA_20
    - P335-A2_lot_0809_BMP-ID_31-HYI-DNA_20
    - P335-A2_lot_0809_BMP-ID_32-HYI-DNA_20
    - P335-A2_lot_0809_BMP-ID_57-HYI-DNA_20
    - P335-A2_lot_0809_BMP-ID_91-HYI-DNA_20
    - P335-A2_lot_0809_REF_A-HYI-DNA_2010-0
    - P335-A2_lot_0809_REF_B-HYI-DNA_2010-0

Correct slope correction on population method local



OFFALYSER

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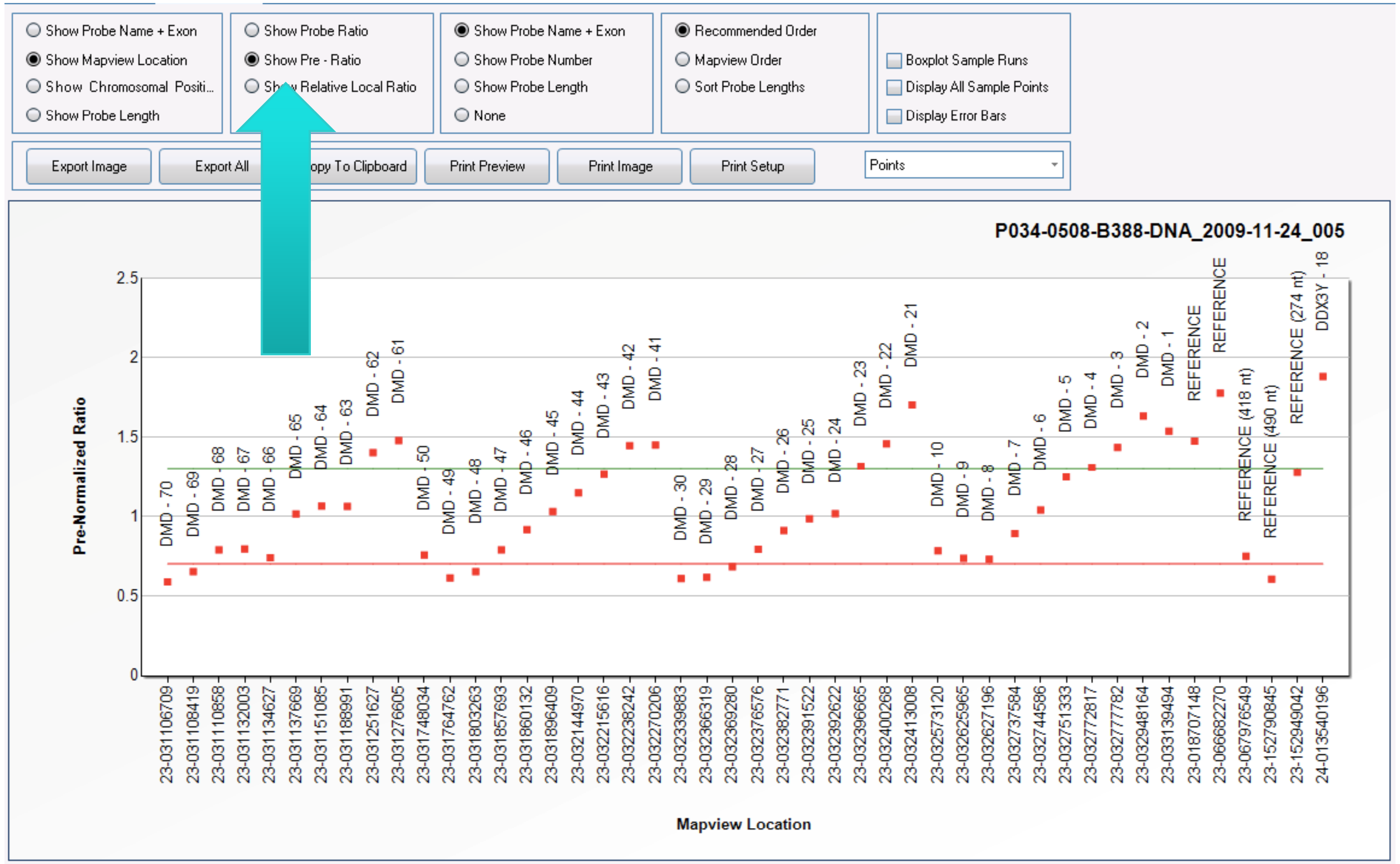
Checking uncorrected data



○ Show Probe Name + Exon
● Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

○ Show Probe Ratio
● Show Pre - Ratio
○ Sh w Relative Local Ratio

● Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
○ None

● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Sample Runs
☐ Display All Sample Points
☐ Display Error Bars

Points

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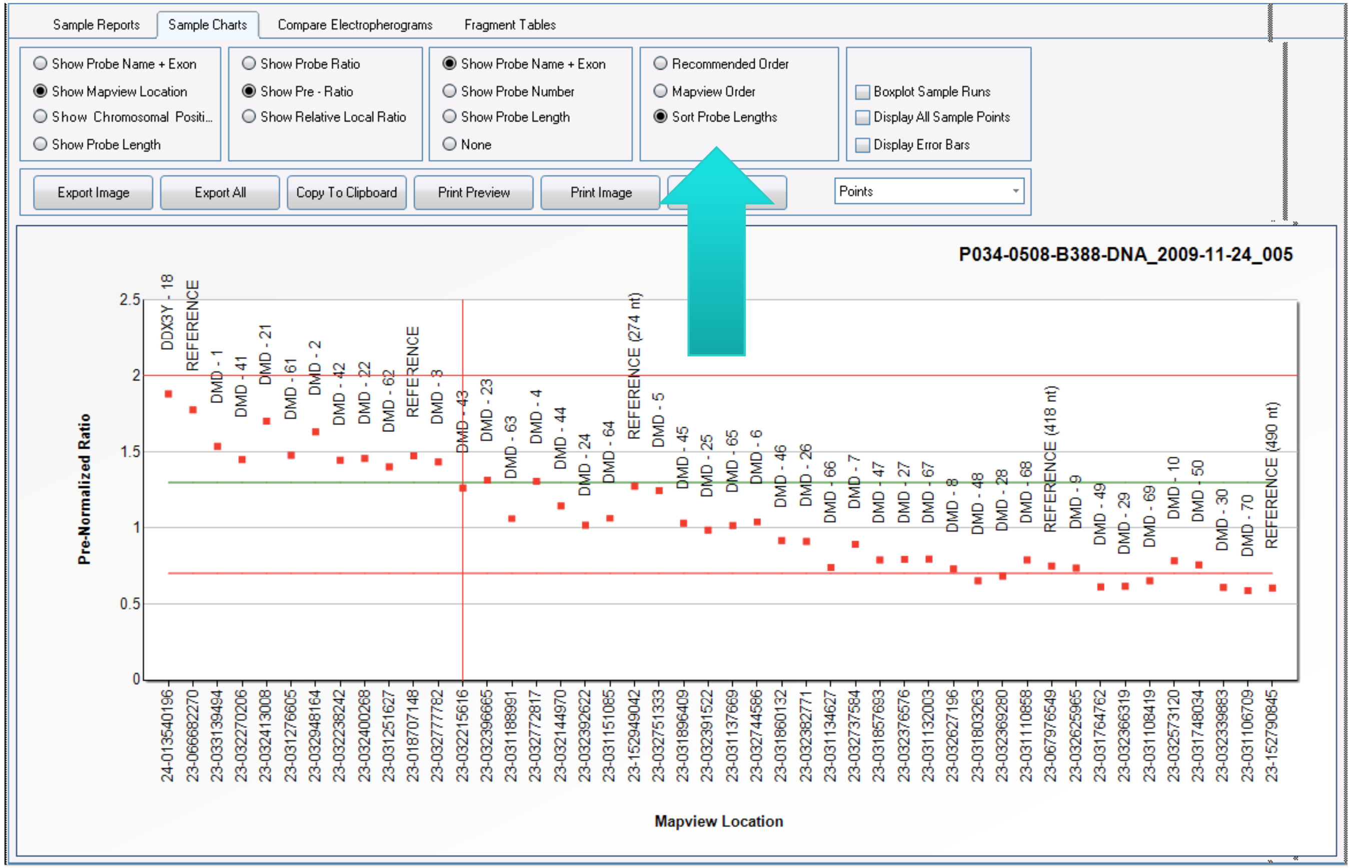
MLPA

Sort on probe length



○ Show Probe Name + Exon
● Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

○ Show Probe Ratio
● Show Pre - Ratio
○ Show Relative Local Ratio

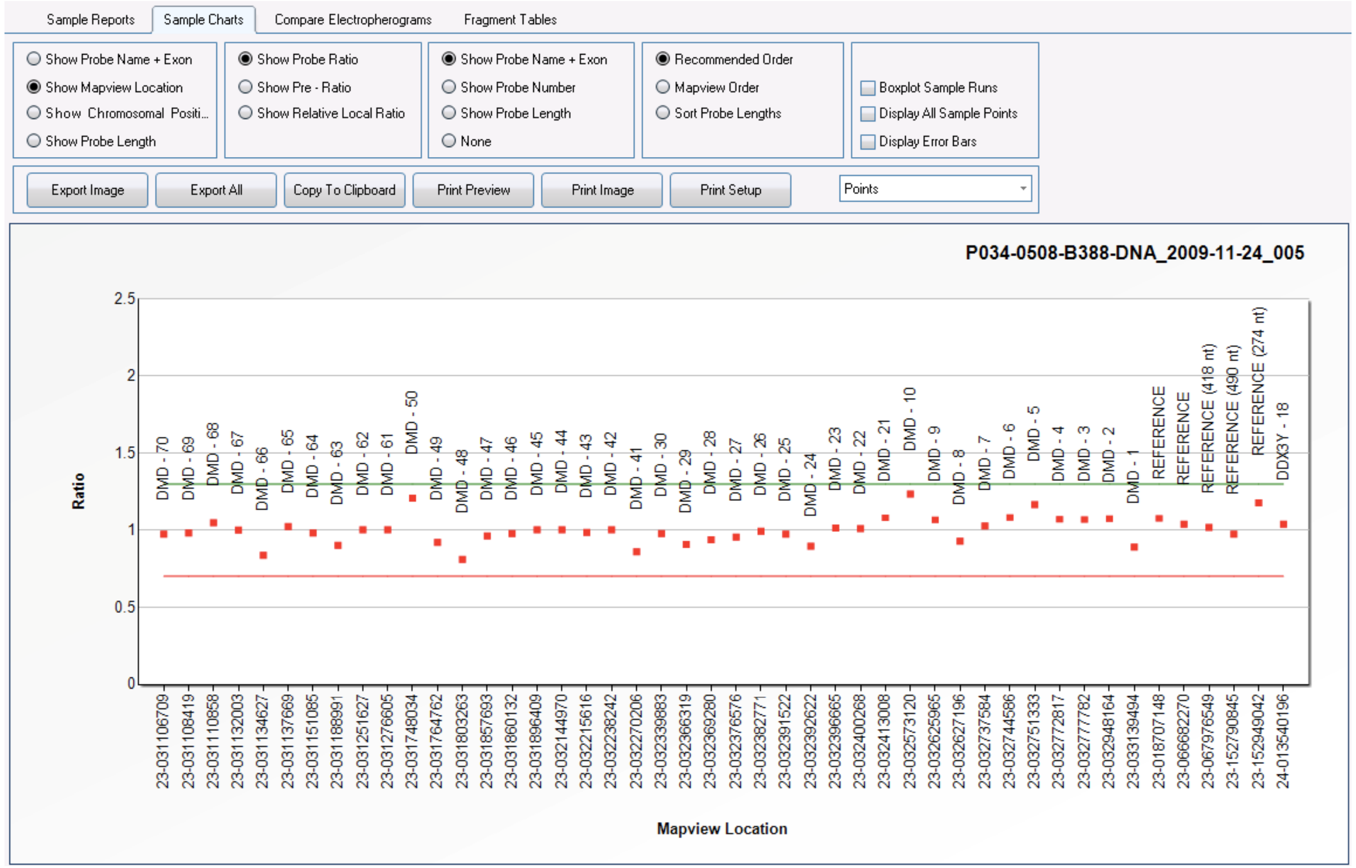
● Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
○ None

○ Recommended Order
○ Mapview Order
● Sort Probe Lengths

☐ Boxplot Sample Runs
☐ Display All Sample Points
☐ Display Error Bars

Points

Corrected data



○ Show Probe Name + Exon
● Show Mapview Location
○ Show Chromosomal Positi...
○ Show Probe Length

● Show Probe Ratio
○ Show Pre - Ratio
○ Show Relative Local Ratio

● Show Probe Name + Exon
○ Show Probe Number
○ Show Probe Length
○ None

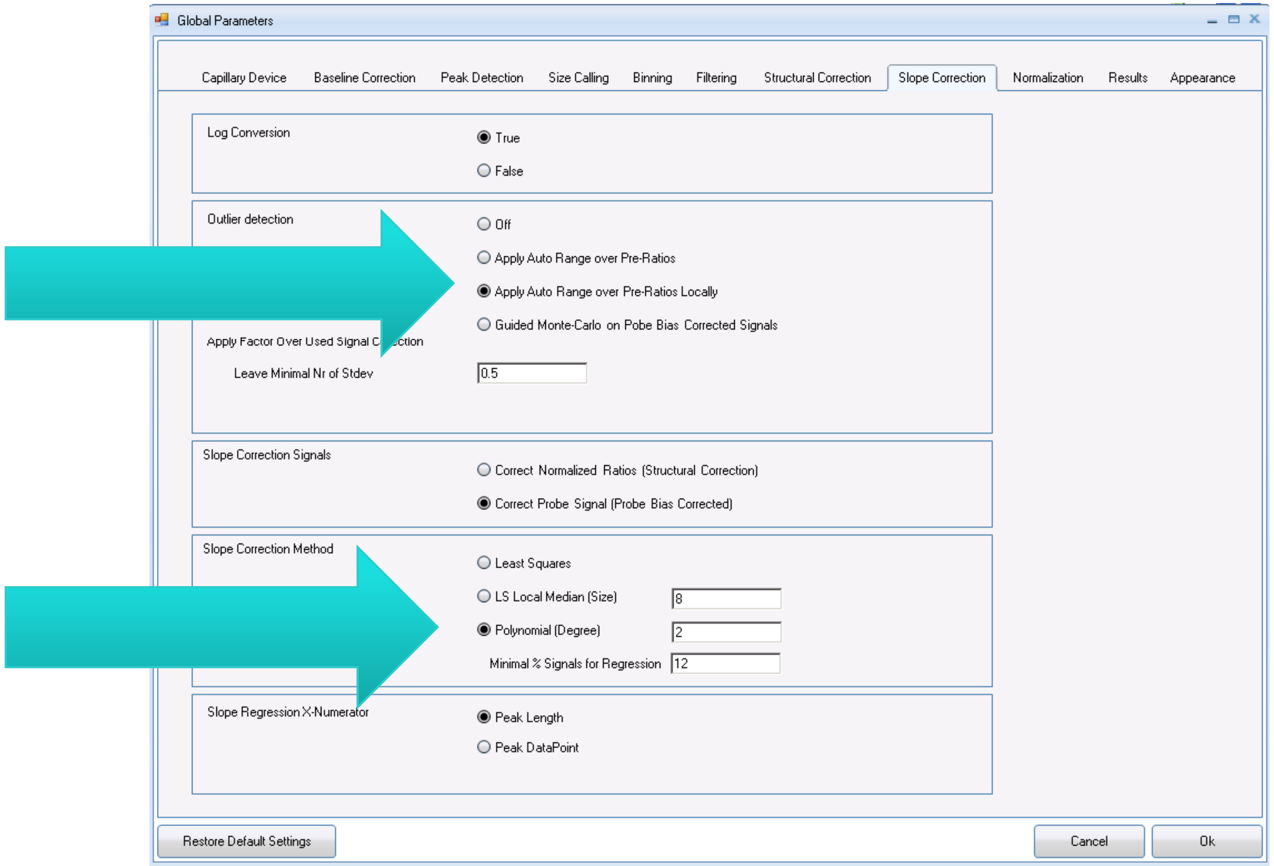
● Recommended Order
○ Mapview Order
○ Sort Probe Lengths

☐ Boxplot Sample Runs
☐ Display All Sample Points
☐ Display Error Bars

Points

COFFALYSER
MRC-Holland b.v.
MLPA

Slope correction methods



Log Conversion
● True
○ False

Outlier detection
○ Off
○ Apply Auto Range over Pre-Ratios
● Apply Auto Range over Pre-Ratios Locally
○ Guided Monte-Carlo on Pobe Bias Corrected Signals

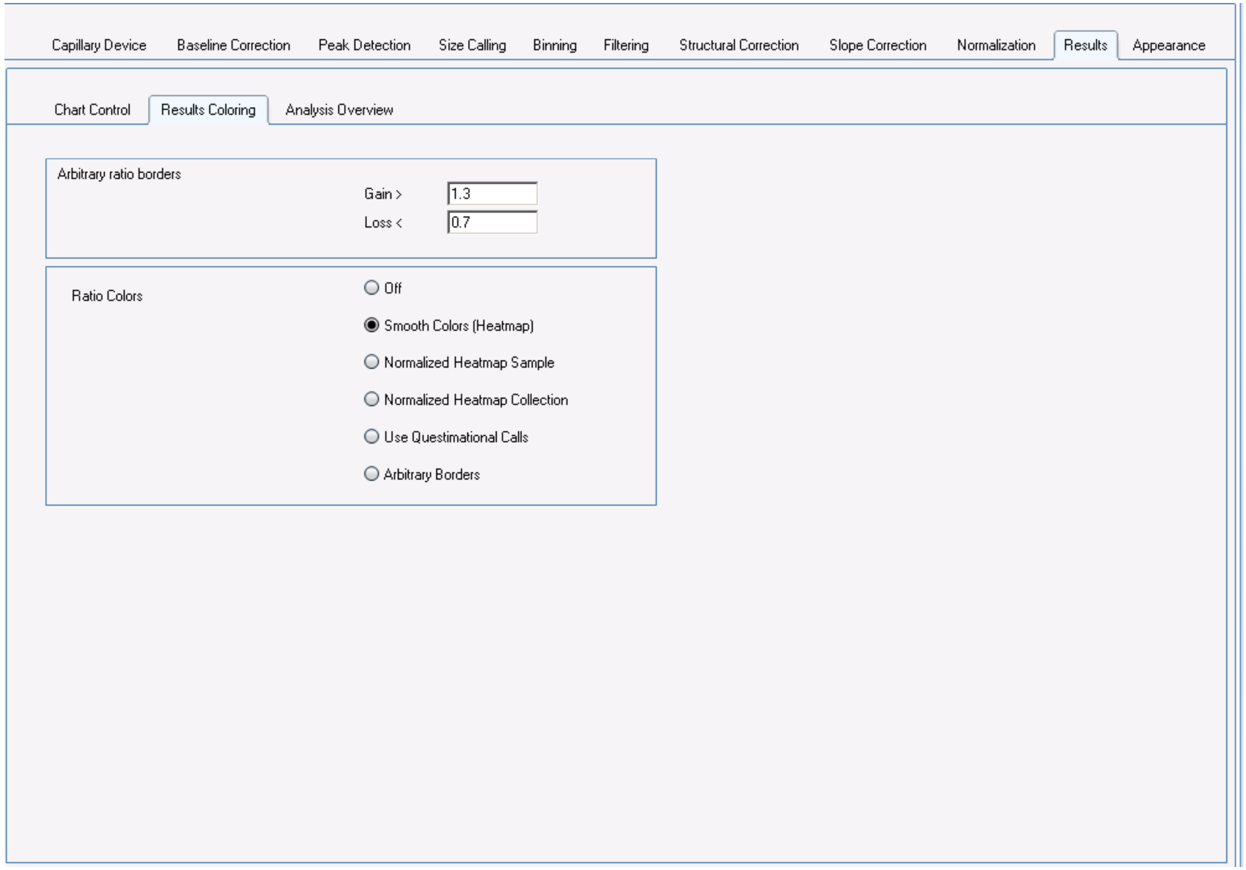
Apply Factor Over Used Signal C ection
Leave Minimal Nr of Stdev: 0.5

Slope Correction Signals
○ Correct Normalized Ratios (Structural Correction)
● Correct Probe Signal (Probe Bias Corrected)

Slope Correction Method
○ Least Squares
○ LS Local Median (Size): 8
● Polynomial (Degree): 2
Minimal % Signals for Regression: 12

Slope Regression X-Numerator
● Peak Length
○ Peak DataPoint

Changing arbitrary borders



Arbitrary ratio borders

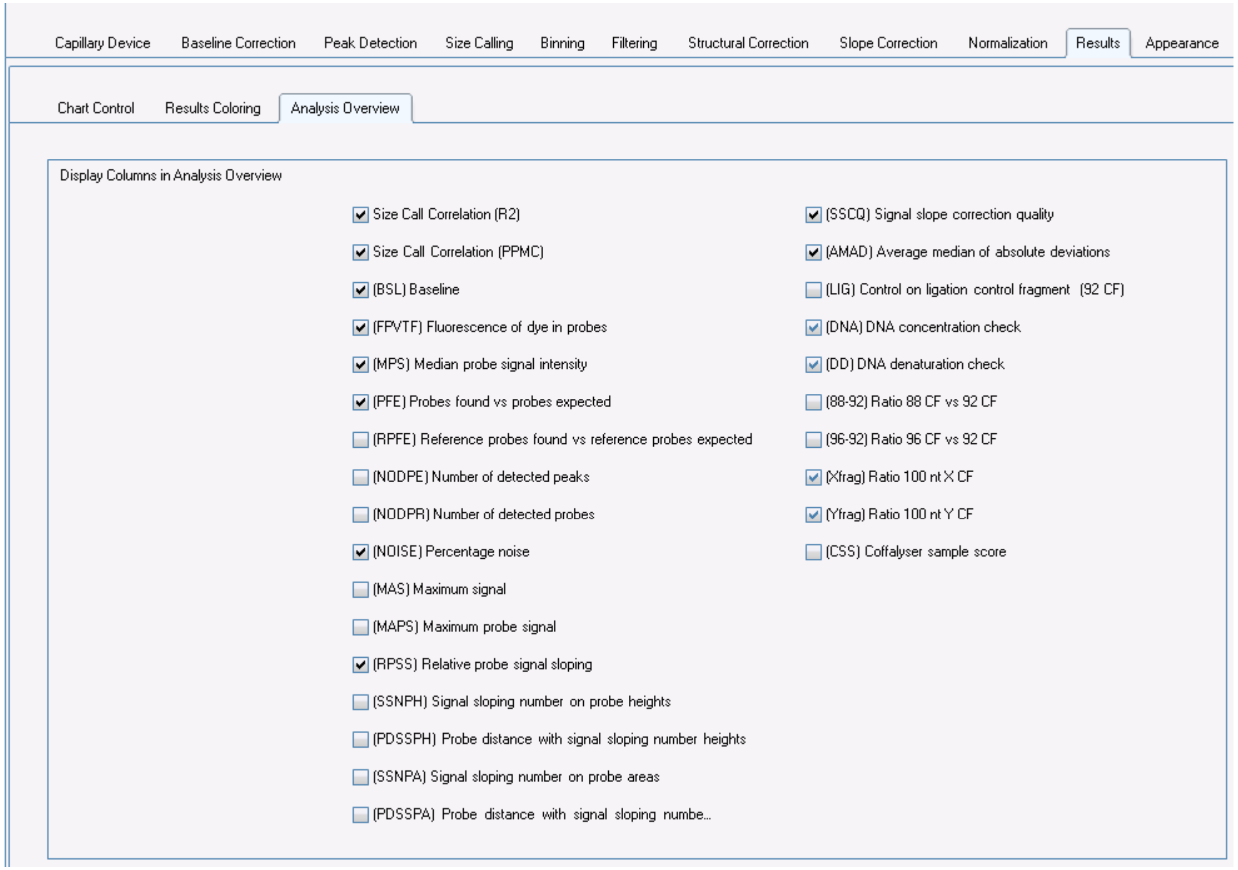
Gain > 1.3

Loss < 0.7

Ratio Colors

○ Off
● Smooth Colors (Heatmap)
○ Normalized Heatmap Sample
○ Normalized Heatmap Collection
○ Use Questimational Calls
○ Arbitrary Borders

Changing raw data columns



Display Columns in Analysis Overview

- ☑ Size Call Correlation (R2)
- ☑ Size Call Correlation (PPMC)
- ☑ (BSL) Baseline
- ☑ (FPVTF) Fluorescence of dye in probes
- ☑ (MPS) Median probe signal intensity
- ☑ (PFE) Probes found vs probes expected
- ☐ (RPFE) Reference probes found vs reference probes expected
- ☐ (NODPE) Number of detected peaks
- ☐ (NODPR) Number of detected probes
- ☑ (NOISE) Percentage noise
- ☐ (MAS) Maximum signal
- ☐ (MAPS) Maximum probe signal
- ☑ (RPSS) Relative probe signal sloping
- ☐ (SSNPH) Signal sloping number on probe heights
- ☐ (PDSSPH) Probe distance with signal sloping number heights
- ☐ (SSNPA) Signal sloping number on probe areas
- ☐ (PDSSPA) Probe distance with signal sloping numbe...
- ☑ (SSCQ) Signal slope correction quality
- ☑ (AMAD) Average median of absolute deviations
- ☐ (LIG) Control on ligation control fragment (92 CF)
- ☑ (DNA) DNA concentration check
- ☑ (DD) DNA denaturation check
- ☐ (88-92) Ratio 88 CF vs 92 CF
- ☐ (96-92) Ratio 96 CF vs 92 CF
- ☑ (Xfrag) Ratio 100 nt X CF
- ☑ (Yfrag) Ratio 100 nt Y CF
- ☐ (CSS) Coffalyser sample score

Test 3 P335 tumor data

- Difficult data
- Multiple copy numbers
- Difference in sample tissue type

Questions