Elucigene® QST*R-PL (Pregnancy Loss) Assay Instructions for Use

Cat Code: AN6XYB1 – 25 tests

IVD For In-Vitro Diagnostic Use

CE

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Elucigene Diagnostics is the trading name of Delta Diagnostics (UK) Limited., a company registered in England and Wales, registration number 8696299.

Elucigene QST*R-PL

Intended Use

For the routine *in vitro* diagnosis of the six most common autosomal trisomies associated with pregnancy loss: trisomy 13 (Patau syndrome), trisomy 15, trisomy 16, trisomy 18 (Edwards' syndrome), trisomy 21 (Down syndrome) and trisomy 22. The kit also includes X and Y chromosome markers and the TAF9L marker for the determination of sex status. The method employed by the Elucigene QST*R-PL kit is the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. The device is intended to be used on DNA extracted from either fetal material obtained post-miscarriage or whole blood (of fetal origin). The intended target population is individuals who have experienced a spontaneous miscarriage. The results obtained from QST*R-PL kit can help determine the aneuploidy status of the fetus and is intended to be used in conjunction with other diagnostic procedures to support or discount the proposed clinical diagnosis. The device is for Professional Use only within a molecular or cytogenetics laboratory environment.

Summary and Explanation

Statistically, 10-20% of all pregnancies end in spontaneous abortion (miscarriage), the majority of which occur towards the end of the first trimester. Of these, over 50% of cases have been shown to be caused by a chromosome abnormality (1), primarily aneuploidy; the most commonly noted are trisomies, which account for 60% of all chromosome abnormalities in miscarriage. The most frequent trisomy found in products of conception (POC) is trisomy for chromosome 16 however trisomies for chromosomes 13, 15, 18, 21 and 22 are also amongst the most common. Other aneuploidies commonly seen include monosomy X and triploidy which account for approximately 20% and 15% of all abnormalities, respectively. These data are represented in Figure 1 below (2).

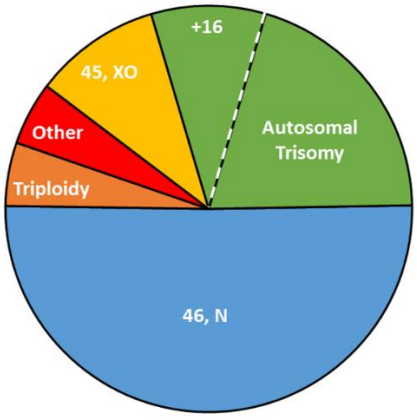


Figure 1: Showing the chromosomal findings in products of conception with 46N representing normal results.

Principles of the procedure

The method employed by Elucigene QST*R kits uses the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique (3-6). Using PCR amplification, fluorescent dye labelled primers target highly polymorphic regions of DNA sequence called short tandem repeats (STRs) that are located on the chromosomes of interest. Each targeted STR marker is specific to the chromosome on which it is located, thus the copy number of the STR marker can be diagnostic of the copy number of the chromosome. Informative STR markers have been selected that exhibit a high heterogeneity so that copy number can be easily determined. A normal diploid sample has the normal complement of two of each of the somatic chromosomes, thus two alleles of a chromosome specific STR are determined by the QF-PCR technique as two peaks in a 1:1 ratio. The observation of an extra STR allele as either a three peak pattern in a 1:1:1 ratio or two peak pattern in a 2:1 or 1:2 peak ratio is diagnostic of the presence of an additional sequence which in turn may represent an additional chromosome, as in the case of a trisomy.

Amplified products of the QF-PCR technique are analysed quantitatively on a capillary electrophoresis Genetic Analyzer to determine the copy number of the analysed STR markers.

Warnings and Precautions

1. The normal DNA Control provided in the kits has been independently tested and found to be negative for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) 1 and 2.

2. Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that HBV, HCV, HIV or other infectious agents are absent.

3. Handling of samples and test components, their use, storage and disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation.

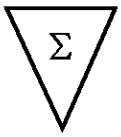
4. In line with current good laboratory practice, laboratories should process their own internal QC samples of known type in each assay, so that the validity of the procedure can be assessed.

5. If kit box is damaged, there may be damage to the contents, do not use the kit, contact Customer Service.

Symbols used on labels

The symbols used on all labels and packaging conform to the harmonised standard ISO 15223

Manufacturer



Number of tests

See Instructions for Use

Store below temperature shown

Use before date shown

Catalogue code

Lot or batch number

In Vitro Diagnostic Medical Device

Materials Provided

Store all components below -20°C

The Elucigene QST*R-PL IVD kit contains:

450089 1 x 250µl Reaction Mix (TA)

404485, 1 x 50µl Control DNA (DC)

Sufficient for 25 tests.

Kit Preparation and Storage

Upon opening the kit it is recommended that the reaction mix be dispensed into 0.2ml PCR vials in 10µl volumes and frozen at -20°C. Ensure that vial contents are thoroughly thawed and mixed before dispensing.

The Control DNA should be frozen at -20°C.

Materials required but not provided

General

Laboratory consumables – gloves; screw-capped microfuge tubes; 0.2ml PCR vials or microtitre plates recommended by the manufacturer of the thermal cycler used; pipette tips.

Laboratory equipment – precision pipettes (2 sets: 1 for pre-amplification and 1 for post-amplification handling; preferably positive displacement pipettes); protective clothing; vortex mixer; microfuge; 96-well microtitre plate centrifuge.

PCR Amplification

Thermal cycler to accommodate 96-well microtitre plates or 0.2ml vials with a temperature accuracy of +/-1°C between 33°C and 100°C and static temperature uniformity of +/-1°C. QST*R-PL has been validated and shown to perform to specification on the following recommended Thermal Cycler platforms:

- Life Technologies GeneAmp 9700
- Life Technologies Veriti Dx (Running in Standard mode)
- Life Technologies Veriti Dx (Running in 9700-simulation mode)
- Life Technologies Proflex (Running in Standard mode)
- Life Technologies Proflex (Running in 9700-simulation mode)

Note: *Peak signal intensities may increase when using the Veriti and Proflex thermal cycler platforms compared to the GeneAmp 9700 platform.*

Capillary Electrophoresis

Capillary Electrophoresis –GeneScan 500 LIZ size standard (ABI Cat No 4322682), POP-7 Polymer (ABI Cat No 4352759), DS-33 (dye set G5) matrix standard (ABI Cat No 4345833), 10x Genetic Analyzer Buffer (ABI Cat No 402824) and Hi-Di Formamide (ABI Cat No 4311320).

Applied Biosystems ABI 3130 and 3500 Genetic Analyzers (with current Data Collection software), 36cm capillary array (50cm capillary array for 3500 Genetic Analyzer), 96-well optical plates, 96-well septa, 96-well cassettes.

Data Analysis

One of the following data analysis software packages is required: GeneMapper 4.1 (Applied Biosystems Inc.) or above or GeneMarker 1.65 (SoftGenetics LLC) or above.

Additional Elucigene QST*R Documentation

These Instructions for Use include a basic section on interpretation of the results obtained in addition to a guide to software analysis with both the GeneMapper and GeneMarker packages. A supplemental Guide to Interpretation with examples and glossary are available from the Elucigene website: www.elucigene.com.

DNA Extraction

A DNA Extraction method to yield PCR amplifiable quality DNA at a concentration of 0.5ng/µl to 4ng ng/µl.

DNA Concentration

Using the recommended PCR conditions and sample injection settings* stated in the capillary column run module (page 9), optimal results are obtained with an input DNA amount of 2.5ng. However interpretable results are obtained with input DNA range of 1.25ng to 10ng.

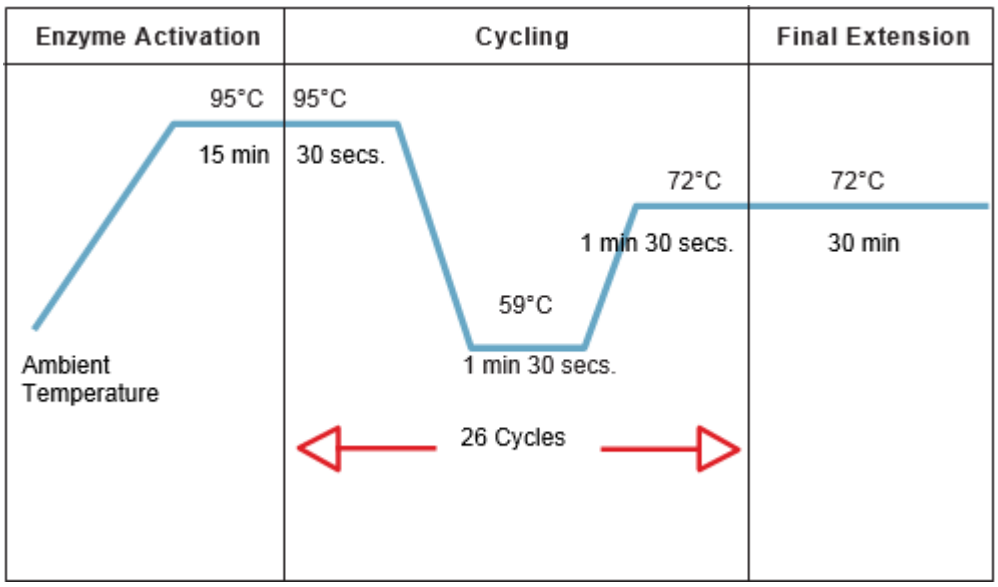
***Note:** sample injection settings can be modified to suit the amount of amplicon produced during the PCR reaction which can vary due to amount of input genomic DNA added. Less amplicon can be applied to the column for analysis by reducing time of injection. Conversely, more amplicon can be applied to the column for analysis by increasing either time or voltage of injection. Previously amplified samples can be re-injected multiple times for re-analysis.*

Test Protocol

Amplification Procedure

Note: *to minimise the risk of contamination, steps 3 - 5 must be carried out in an area free from DNA. Steps should also be taken to avoid contamination with PCR product.*

1. Program the thermal cycler for a single step cycle to activate the DNA polymerase at 95°C for 15 minutes linked to an amplification cycling program of 30 seconds at 95°C (denaturation), 1 minute and 30 seconds at 59°C (annealing) and 1 minute and 30 seconds at 72°C (extension) for 26 cycles. This should be linked to a 30 minutes time-delay file at 72°C (extension) on the final cycle



2. A negative (water) control must be included in each PCR run. It may also be considered appropriate to include other controls, e.g. positive normal (DNA control supplied) and positive trisomy control (DNA not supplied).

3. Thaw sufficient vials of pre-aliquoted QST*R-PL reaction mix for the number of samples and controls to be run (see note under Materials Provided) and centrifuge the vials at 12,000g for 10 seconds.

4. Using separate pipette tips, add 2.5µl of test DNA to a sample vial containing 10µl QST*R-PL reaction mix and mix by pipetting up and down. Do this for all samples to be tested.

5. Do not add DNA to the PCR vial for the negative control; instead add 2.5µl of sterile distilled water.

6. Briefly centrifuge the vials until all liquid is at the bottom of each vial.

7. Place all vials firmly in the thermal cycler block. Initiate the 95°C activation program followed by the amplification program (see step 1).

8. On completion of the amplification program the samples may be stored at room temperature overnight or at 2-8°C for up to 7 days before analysis by capillary electrophoresis.

Capillary Electrophoresis

It is recommended that each user ensure that the chosen equipment is used according to the manufacturer's instructions and is compatible with this test. In this context the key parameters are the polymer and the capillary array. Optimal results can be obtained using the following capillary electrophoresis conditions on an ABI3130 or ABI3500 Genetic Analyzer.

1. Combine 6.85µl of size standard with 250µl Hi-Di Formamide and mix thoroughly (sufficient mix for 16 wells). Dispense 15µl of the mix into the required number of wells of a 96 well optical plate*.

2. Add 3µl of test sample PCR product to the size standard mix (from step 1) already dispensed into the plate and mix using the pipette. Seal the plate.

3. Denature the PCR product dispensed into the optical plate on a thermal cycler using the following parameters: 94°C for 3 minutes linked to 4°C for 30 seconds.

4. Centrifuge the plate at 1,000g for 10 seconds to remove any bubbles in the wells and load onto the Genetic Analyzer.

*__Note__: *It is essential that unused wells (i.e. wells in which No DNA sample is loaded) are still loaded with Hi-Di Formamide to ensure that the capillaries do not dry out.*

Post-PCR Data Analysis

ABI3130 GENETIC ANALYZER

Create a sample sheet using the 3130 data collection software with the following settings:

• Sample Name: this must be the same sample specific name or number.

• Run owner: select the default owner for lab.

• Run Protocol: QSTR (contains QST*R 3130 run module – see below)*.

****Note:** It is necessary to create a run module detailing the instrument settings and subsequently assign this to a Run protocol in which Dye set G5 has been selected. For more information on creating run modules please refer to your instrument user manual.*

3130 RUN MODULE

FOR POP7 POLYMER

36cm Capillary Module: QSTR

#	Parameter Name	Value	Range
1	Oven Temperature	60	int 18…65 Deg.C
2	Poly_fill_Vol.	6500	6500…38000 steps
3	Current Stability	5.0	int 0…2000 uAmps
4	PreRun_Voltage	15.0	0…15 kvolts
5	Pre_Run_Time	180	1…1000 sec.
6	Injection_Voltage	3.0	1…15 kvolts
7	Injection_Time	15	1…600 sec.
8	Voltage_Number_of_Steps	20	1…100 nk
9	Voltage_Step_Interval	15	1…60 sec.
10	Data_Delay_Time	60	1…3600 sec.
11	Run_Voltage	15.0	0…15 kvolts
12	Run_Time	1200	300…14000 sec.

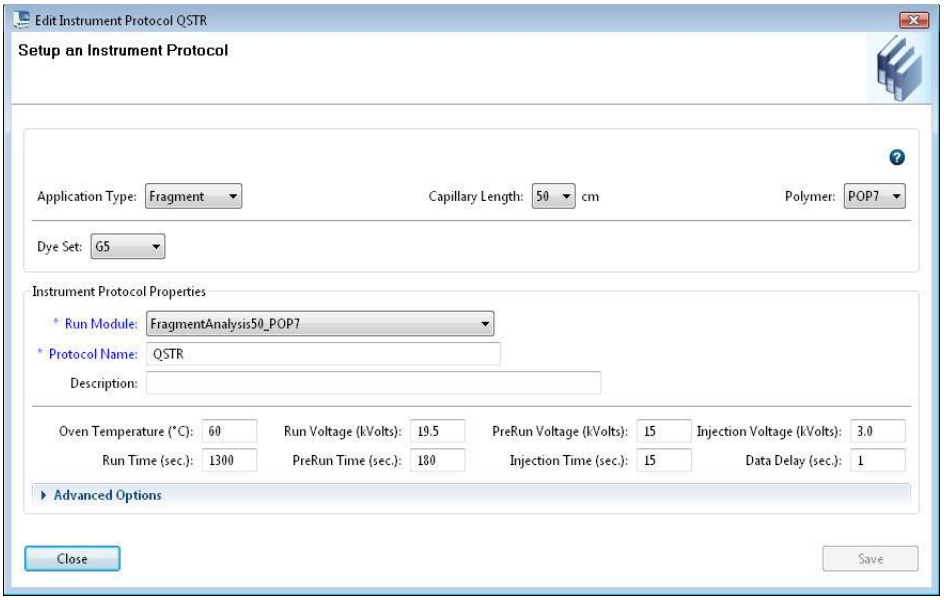
ABI3500 GENETIC ANALYZER

A QST*R Instrument Protocol needs to be created which can then be used for each QST*R run. Create the QST*R Instrument Protocol through the 3500 Instrument Protocols library.

Ensure the following are selected:

• Run Module: FragmentAnalysis50_POP7

• Enter the settings detailed in the image below:



To run the samples create a sample plate by clicking on ‘Create Plate from Template’ in the ‘Dashboard’, ensure the correct Instrument Protocol for QST*R has been assigned (see above).

Analysis and Interpretation of Results

It is recommended that each laboratory develops its own interpretation and reporting procedures and criteria. Best practice guidelines for QF-PCR have been documented by the UK's Association for Clinical Genetic Science and are available for reference at:

www.acgs.uk.com

PCR products are observed as a 5 dye labelled system using filter set G5. Filter set G5 detects the 6-FAM (blue), VIC (green), NED (yellow) and PET (red) labelled fragments plus the Size Standard marker labelled with LIZ (orange) on an electrophoretogram and in the GeneMapper or GeneMarker program.

A QST*R Guide to Interpretation is available from the Elucigene website: www.elucigene.com.

Important Note: *different combinations of instrument, polymer and size standard may cause the size calling to vary slightly. During validation of the kit, users should check that the default bin settings result in accurate peak labelling and adjust if necessary. In case of any difficulty, please contact Technical Support (techsupport@elucigene.com) for advice.*

General analysis guidelines for QST*R-PL

1. The negative control should show no sharp peaks within the read range of 100 to 510bp.

2. The positive control must show the expected results and all peaks must meet the criteria below.

3. For analysis of DNA samples at least 1 peak should be observed for each marker tested. The acceptable range for marker peaks analysed on the 3130 Genetic Analyzer is between 50 and 6000 relative fluorescent units (rfus) and for the 3500 Genetic Analyzers is between 175 and 32000 rfus. Peak heights falling outside this range must not be analysed.

4. Electrophoretograms of poor quality due to excessive bleed-through between dye colours (also known as 'pull-up') or 'electrophoretic spikes' (sharp peaks present in more than one dye) should not be interpreted. The PCR products should be re-injected and re-analysed.

5. Analysis is performed by assessment of peak ratios (A1/A2), where A1 is the peak area of the shorter length fragment and A2 is the peak area of the longer length fragment. The resulting ratio is indicative of locus copy number. For disomic chromosomes heterozygous markers should show two peaks with similar heights. A complete analysis of chromosome copy number status is performed by comparison of peak area ratios.

6. Heterozygous di-allelic (i.e. two alleles) markers should fall within a ratio window of 0.8 to 1.4. However, for two alleles separated by more than 24bp in size a ratio of up to 1.5 is acceptable. Any values falling within this region are referred to as having a ratio of 1:1. If the ratio balance falls out of this window then it may be due to a number of factors, such as:-

    - Whole chromosome trisomy
    - Partial chromosome trisomy (including sub-microscopic duplications)
    - Mosaicism
    - Contaminating second genotype (e.g. maternal, twin, external)
    - Stutters causing skewing
    - Preferential amplification of one allele causing skewing
    - Primer site polymorphisms
    - Somatic microsatellite mutations

The **Guide to Interpretation** gives examples of typical profiles for many of these. Homozygous markers are uninformative since a ratio cannot be determined.

7. To interpret a result as abnormal (i.e. trisomy present), at least two informative markers consistent with a tri-allelic genotype are required with all other markers being uninformative. It is not recommended to interpret a result as abnormal based on information from only one marker. If required, follow-up testing with the single chromosome kits (i.e. Elucigene QST*R-13, Elucigene QST*R-18, Elucigene QST*R-21) may provide sufficient information for interpretation.

   Trisomy is determined by either:-

   7.1. Two peaks of uneven height due to one of the peaks representing two alleles which are common to one or both parents. In this case the ratio between the two peaks will be classed as 2:1 or 1:2 such that A1/A2 will give a result in the region of 1.8 to 2.4 when the peak representing the shorter length allele is greater in area than the peak representing the longer length allele, or where A1/A2 will give a result in the region of 0.45 to 0.65 when the peak representing the shorter length allele is smaller in area than the peak representing the longer length allele.

   7.2. Three peaks of comparable height present. The ratio of the peaks will be classed as 1:1:1 and their values fall within the normal range of 0.8 – 1.4 (although for alleles separated by more than 24bp an allele ratio of up to 1.5 is acceptable). If this does not occur then it may be due to one of the factors mentioned in step 6.

8. To interpret a result as normal, at least two informative markers consistent with a di-allelic genotype are required with all other markers being uninformative. A normal result indicates the normal complement of two for the chromosome tested.

9. Peak area ratios that fall between the normal and abnormal ranges are classed as inconclusive. Inconclusive results may be resolved by using the single chromosome kits.

10. If both normal and abnormal allele patterns are obtained for a single chromosome then it is recommended that follow-up studies are carried out to identify the reason for the discrepant results prior to any conclusions being reached.

11. In rare cases allele size ranges for markers may overlap. If this is suspected, analysis with the single chromosome kits may resolve this.

Analysis of Sex chromosome markers AMEL, TAF9 and SRY:-

1. The AMEL marker amplifies non-polymorphic sequences on the X (104bp) and Y (110bp) chromosomes and can be used to determine the presence or absence of a Y chromosome and represents the relative amount of X to Y sequence. Please note that on rare occasions amplification failure due to mutation of the AMEL-Y sequence has been reported.

2. TAF9L is an invariant paralogous marker with sequences on chromosomes 3 and X. The chromosome 3 specific peak (116bp, representing 2 copies of chromosome 3) can therefore be used as a reference peak to assist in the determination of the number of X chromosomes present (121bp peak). Analysed in combination with Amelogenin and the other sex chromosomes markers, it is particularly useful in the detection of sex chromosome aneuploidy. In a normal female the markers should fall within a ratio window of 0.8 to 1.4. In a normal male the markers will give a ratio ≥1.8. Further details on the interpretation of the TAF9L marker can be found in the Guide to Interpretation.

3. The Y-specific marker, SRY, will give a single peak in normal males and will not amplify in normal females.

Performance Characteristics

INTERNAL VALIDATION

125 fetally derived tissue samples were tested using Elucigene QST*R-PL. Of these, 34 were normal/XY, 40 were normal/XX, 5 were T22/XY, 1 was T22/XX, 3 were T21/XY, 4 were T21/ XX, 3 were T18/XY, 5 were T18/XX, 6 were T16/XY, 2 were T16/XX, 1 was T15/XY, 1 was T15/XX, 2 were T13/XY, 2 were T13/XX, 7 were Monosomy X, 8 were triploid for all chromosomes tested. One sample gave an uninformative result due to homozygosity within the marker set. This sample was subsequently analysed using QST*R-18 and was shown to be T18/XX. All analysable results showed 100% concordance with results previously obtained by alternative, established methods.

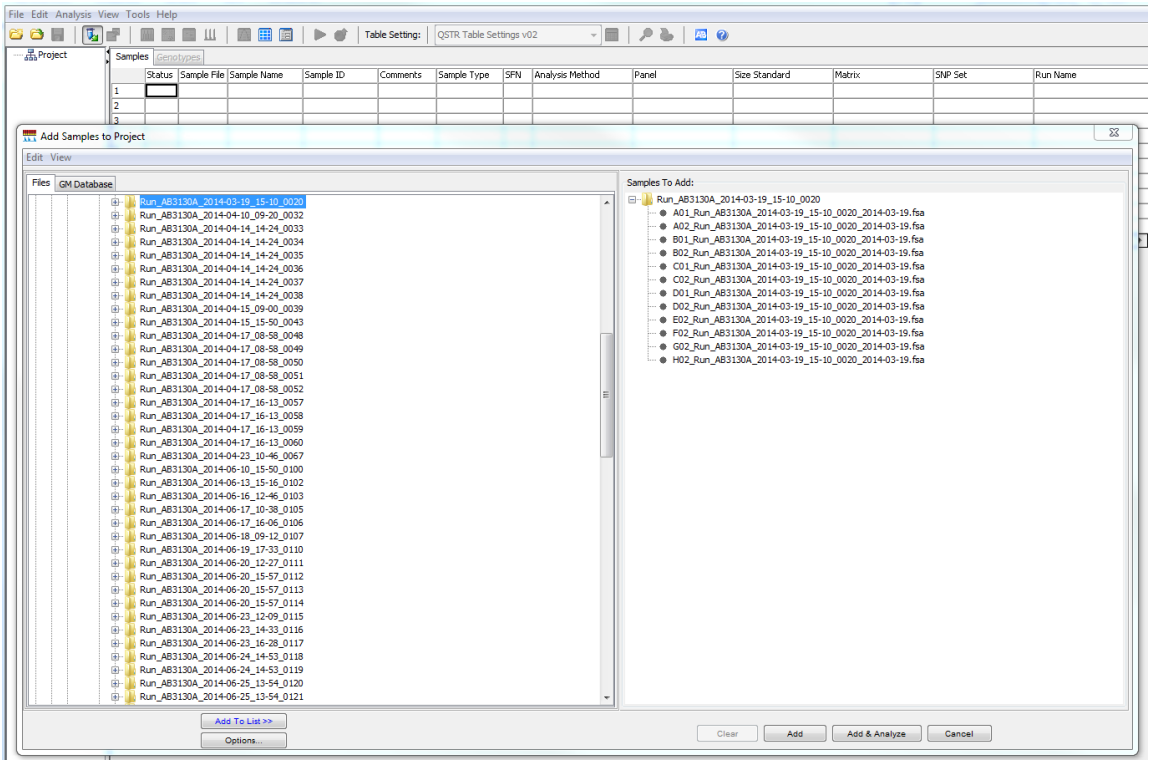
GeneMapper Analysis Guide

Note: *The following GeneMapper screen shots are taken from GeneMapper v5.0*

Importing and Analysing QST*R Files

1. Open the GeneMapper Program file
2. Click  in order to add data files to a new project. Navigate to where the raw .fsa data files are stored, highlight the appropriate data files and click the '**Add to List>>**' button.
3. The run folder will now appear in the '**Samples to Add**' window. Double clicking on the run folder icon in this window will show each .fsa file to be imported. Samples are then added by clicking  at the bottom of the screen. The data files now appear within the GeneMapper main screen (figure 2)

Figure 2: Samples ready to add to project



Importing QST*R GeneMapper Analysis Settings in GeneMapper Manager

It is necessary to import the QST*R settings for GeneMapper. This process is controlled through the '**GeneMapper Manager**' interface. QST*R GeneMapper settings are available from the Elucigene website: www.elucigene.com/product-category/rapid-aneuploidy-analysis/

1. Open '**GeneMapper Manager**' by clicking on the  icon.
2. Select the 'Analysis Methods' tab and then click the import button
3. Navigate to and import the required QST*R Analysis Settings file(s) (3130 or 3500)
4. Repeat the process, selecting the appropriate tab and importing the corresponding file for:
    - Table Settings
    - Plot Settings
    - Size Standards

Note: *Cluster Plot Settings, Matrices, SNP Sets and Report Settings do not require file imports.*

Importing QST*R-PL Panel settings in Panel Manager

It is necessary to import the QST*R-PL panel and bin settings for GeneMapper. This process is controlled through the '**Panel Manager**' interface.

QST*R-PL specific GeneMapper panel and bin settings files are available from the Elucigene website: www.elucigene.com/product-category/rapid-aneuploidy-analysis/

1. Open Panel Manager Program by clicking on the  icon.
2. Click '**Panel Manager**' on the left navigation window. Panel Manager will now appear highlighted in blue.
3. Select '**File/Import Panels**'. Navigate to and import the GeneMapper panel file '**QSTRPL Panel.txt**' (Figure 3).
4. The panel file will now be displayed on the left navigation window. Click on the panel file ensuring that it is highlighted blue.
5. Select '**File/Import Bin Set**'. Navigate to and import the GeneMapper bin file '**QSTRPL Bins.txt**' (Figure 4).
6. Click '**Apply**' then click '**OK**'.

Figure 3: Importing QST*R-PL Panel File

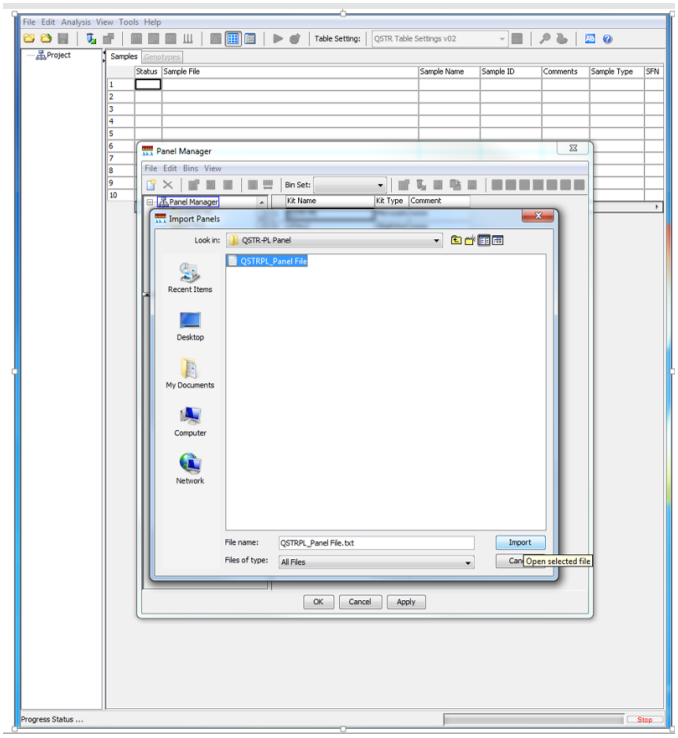
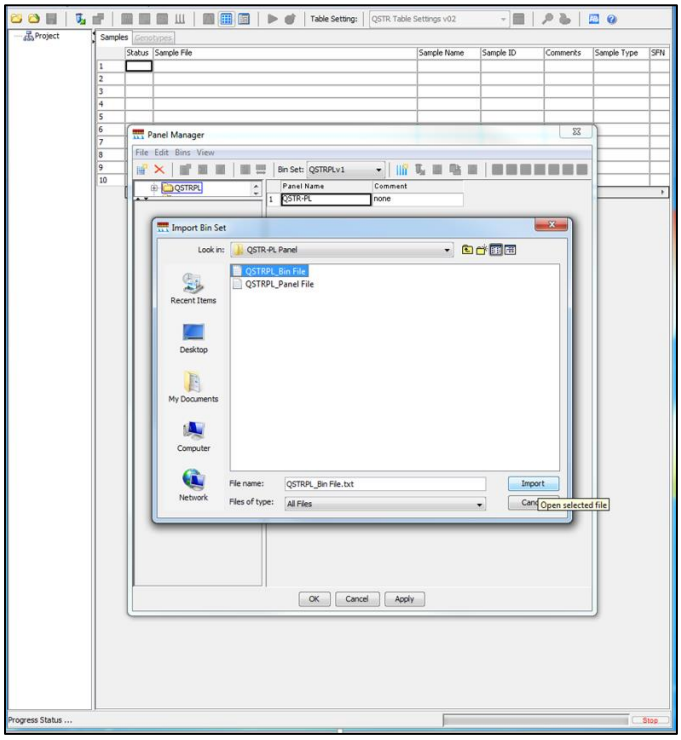


Figure 4: Importing QST*R-PL Bin File



Modifying the Analysis Parameter file

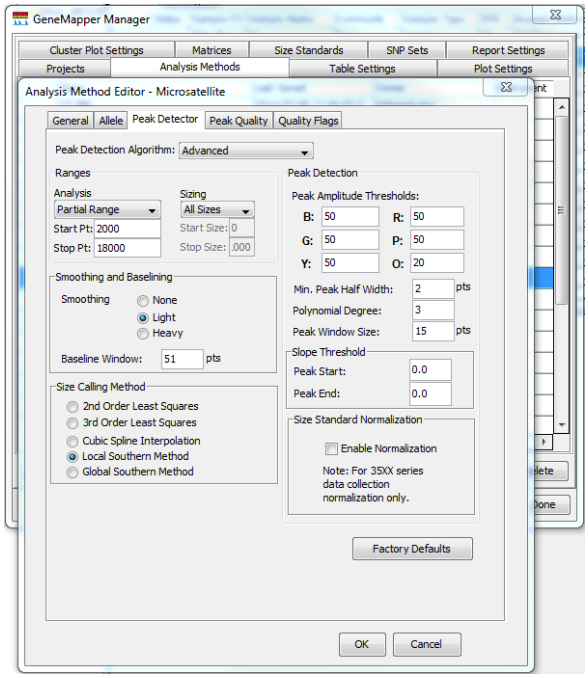
It may be necessary to modify the default '**Analysis Ranges**' in the QST*R analysis settings to account for local variance in run conditions. The minimum analysis range will depend on the capillary and polymer being used during data collection.

To view current analysis settings:

1. Open '**GeneMapper Manager**' by clicking on the  icon.
2. Select the '**Analysis Methods**' tab. The Imported QST*R file will be listed as '**QSTR Analysis v02**'.
3. Click on '**QSTR Analysis v02**'. The row will now appear highlighted.
4. Click the 'Open' button and select the 'Peak Detector' tab (Figure 5)

By default the analysis ranges are set to start at 2,000 and finish at 18,000.

Figure 5: Analysis Ranges.



To find the correct analysis range for your laboratory:

1. From the main GeneMapper window, double click on the imported Run Folder to view the list of .fsa files it contains.

2. Select an .fsa file.

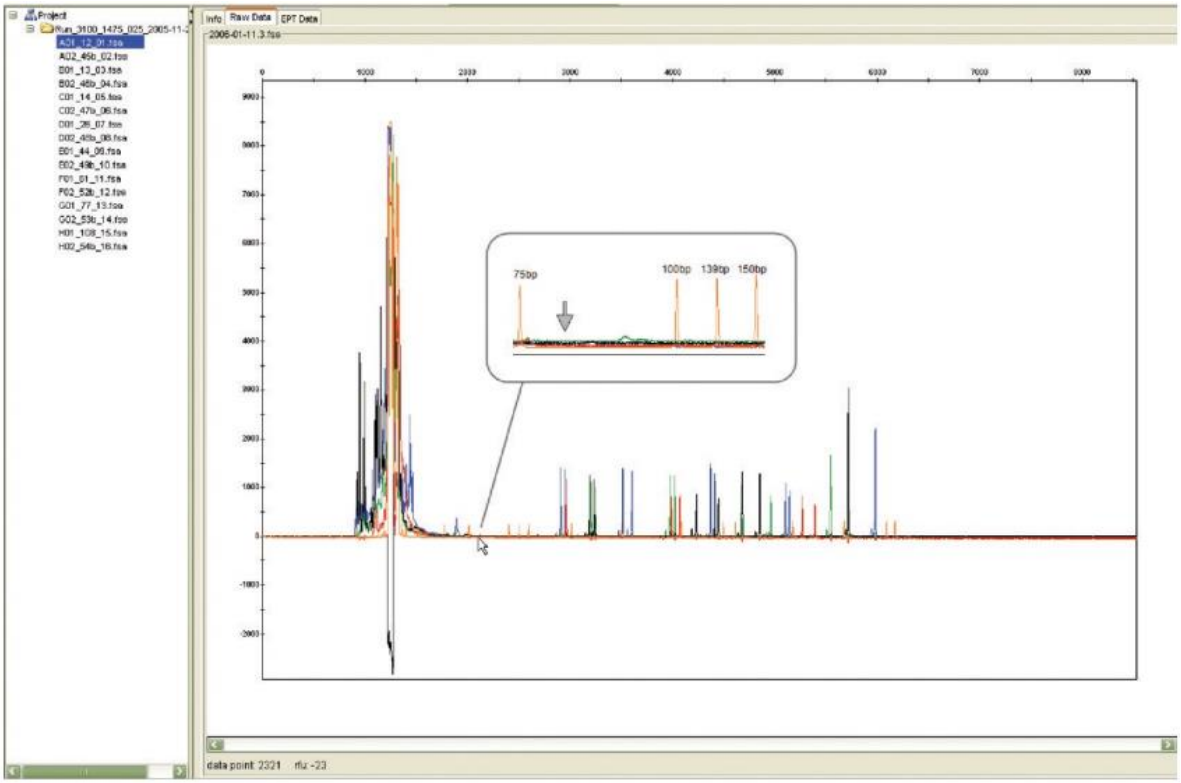
3. Clicking the 'Raw data' tab will display the electropherogram of the raw data.

4. Using the first peak of the size standard (e.g. 75bp of GS500LIZ) as a guide, select on a data point approximately 100 data points larger (Figure 6). This determines the lowest point in the analysable range.

5. Ensure the maximum analysis range encompasses the largest peak of the size standard (e.g. 500bp of GS500LIZ or 600bp of GS600LIZv2).

6. Input the new values into the QST*R Analysis file (accessing it as described above).

Figure 6: Finding the minimum range using sample raw data



Analysis of Imported QST*R-PL Files

1. In the main GeneMapper window select the '**QST*R Table Settings v02**' (Figure 7)

Figure 7: Setting QST*R table settings

2. Under '**Analysis Method**' select '**QSTR Analysis v02**'. Fill down each column by pressing '**Ctrl+D**'. Repeat this process selecting '**QSTR-PL**' for under the '**Panel**' heading and '**QSTR**' under the '**Size Standard**' heading. Each time remember to fill down by pressing '**Ctrl+D**' to ensure each setting is applied to the full list of samples.

3. Click  to initiate sample analysis. Assign a project name when prompted.

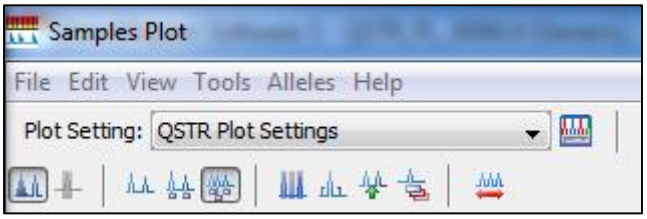
Reviewing QST*R Data

1. Select the sample for analysis (highlight sample row).

2. Click  to '**Display Plots**'.

3. Select the '**QST*R Plot settings**' (Figure 8).

Figure 8: QST*R Plot settings, drop down menu.

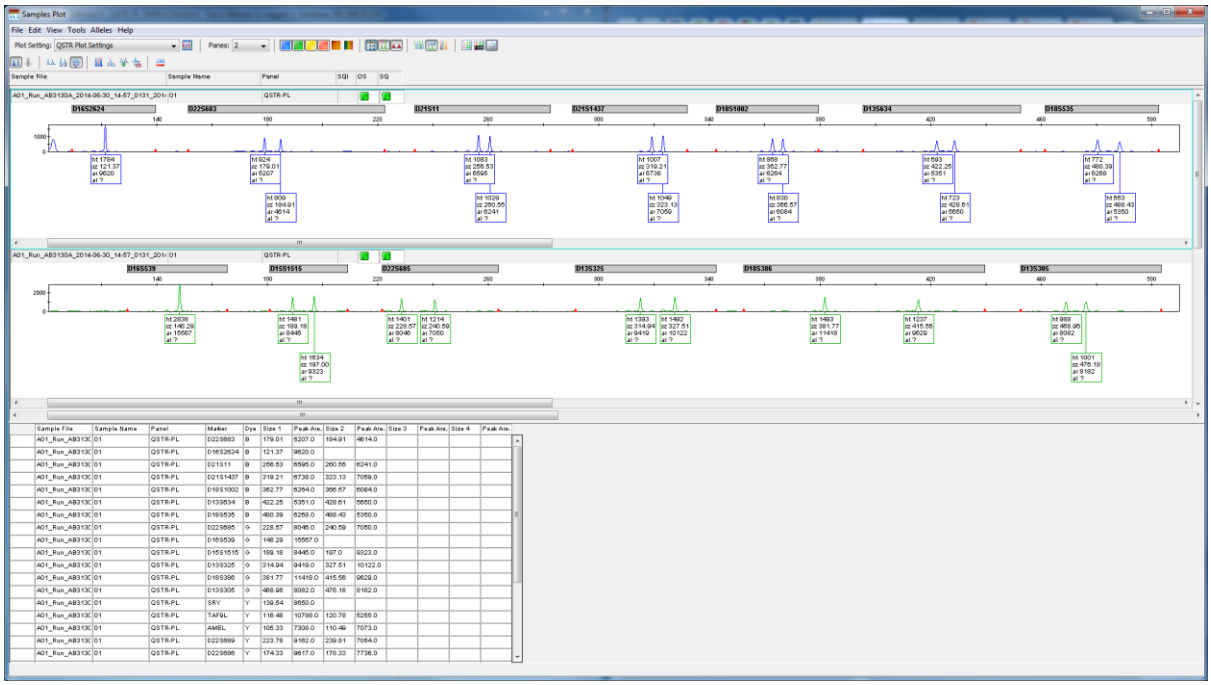


4. The plot window will display sample profile with the tabulated data (Figure 9).
A maximum of two peaks for each marker will be labelled automatically by GeneMapper. If three alleles are present for a marker, the third unlabelled peak should be manually labelled (see: Manual Editing of Profiles, below).

Note: *Allele size ranges for each marker are based on previously observed data. Rare alleles may fall outside the given marker size range and it may be necessary to adjust the bin set accordingly.*

5. It is recommended that the plot window has '**Single click editing**' activated. To do this select '**Alleles/set click editing**' and ensure that this option is checked.

Figure 9: Sample Plot window displaying labelled trace data and correlating genotype table



Manual Editing of Profiles

WARNING!

GeneMapper will only assign labels for up to 2 peaks per marker. Manual editing of profiles may therefore be required, i.e. when assigning labels to 3rd peaks (if present) or removing labels from stutter peaks.

To add a peak label; left click on the unlabelled peak. You will get the option to add allele comment. Click '**OK**'. The peak will now be labelled with its size in base pairs and its peak area. The table will automatically incorporate the newly labelled peak.

To remove a peak label; left click on the peak label. You will get the option to delete allele comment. Click '**OK**'. The peak label will now be removed. The table will automatically remove the deleted peak data.

Copying Table Data

1. Highlight all rows with the table at the bottom of the plot window.

2. Copy the selected rows by typing '**Ctrl+C**' keys.

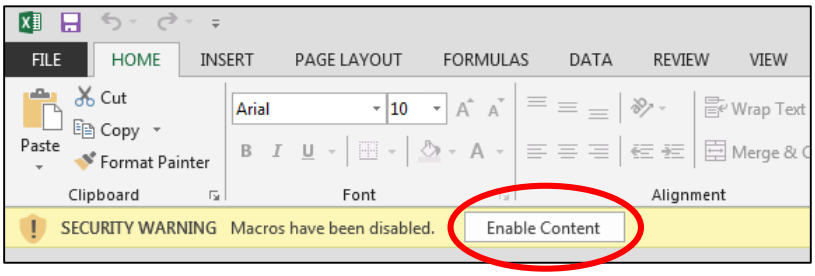
QST*R-PL Report Template

The associated QST*R-PL report template can be used to determine the peak ratios for a marker and assist in analysis of data. The QST*R-PL specific Report Template is available from the website: www.elucigene.com/product-category/rapid-aneuploidy-analysis/

1. Open the QST*R-PL Report Template file (QSTR-PL Report Template.xlsm).

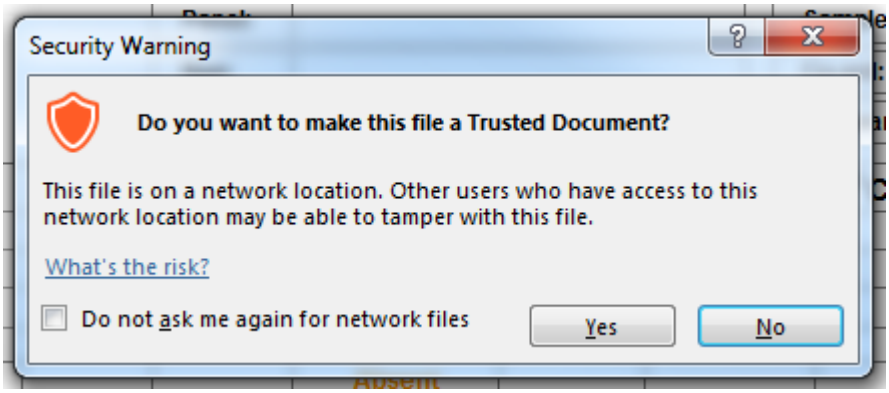
2. If the Report Template displays a warning indicating that macros have been disabled, click the '**Enable Content**' button to enable macros (Figure 10).

Figure 10: Enabling macro function in QST*R-PL Report Template



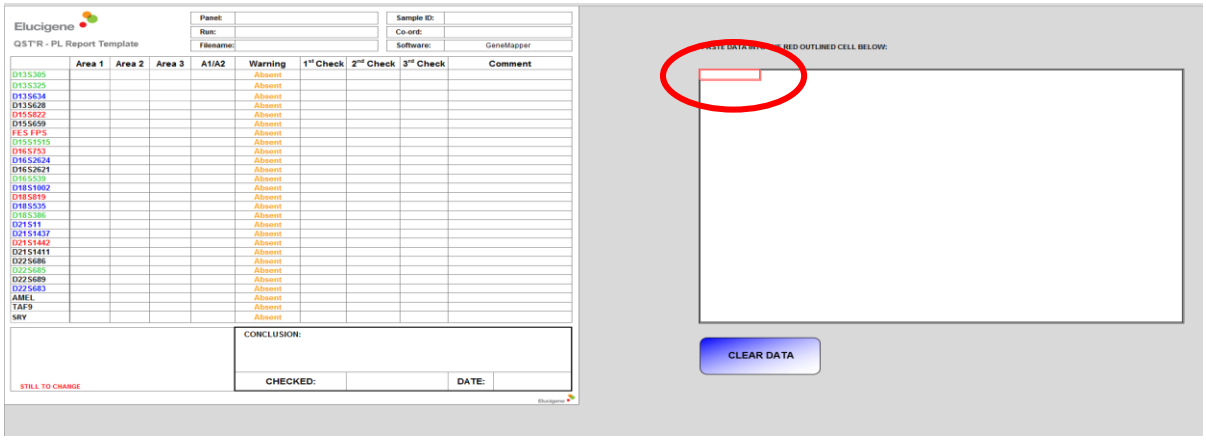
3. If a security warning is shown (as seen in Figure 11), click **Yes** in order to proceed.

Figure 11: Allowing security access



4. Paste the data copied from the GeneMapper data table (see above, '**Copying Data Table**') using '**Ctrl+V**' into the top left cell in the outlined area (See Figure 12).

Figure 12: Importing raw GeneMapper data into the QST*R-PL Report Template.



5. The calculated ratios for each marker will now be shown in the data table to the left. The data table can be printed or saved as a new file for each specific sample.

6. In order to process subsequent samples it is important that all data is fully cleared from the report template. In order to do this, click the '**CLEAR DATA**' button located underneath the raw data table within the QST*R-PL Report Template. New sample data can now be imported following the procedure outlined above.

Scoring the Report

1. Trisomy is determined by either:-

    a. Two peaks of uneven height due to one of the peaks representing two alleles which are common to both parents. In this case the ratio between the two peaks will be classed as 2:1 or 1:2. Where A1/A2 will give a result in the region of 1.8 to 2.4 when the peak representing the shorter length allele is greater in area than the peak representing the longer length allele, or where A1/A2 will give a result in the region of 0.45 to 0.65 when the peak representing the shorter length allele is smaller in area than the peak representing the longer length allele.

In both cases '**Ratio**' will appear in the '**Warning**' column.

    b. Three peaks of comparable height present. The ratio of the peaks will be classed as 1:1:1 and their values fall within the normal range of 0.8 – 1.4 (although for alleles separated by more than 24bp an allele ratio of up to 1.5 is acceptable).

In this case '**3 Alleles**' will appear in the '**Warning**' column.

2. To interpret a result as abnormal (i.e. trisomy present), at least two informative markers consistent with a tri-allelic genotype are required with all other markers being uninformative. It is not recommended to interpret a result as abnormal based on information from only one marker.

3. To interpret a result as normal, at least two informative markers consistent with a diallelic genotype are required with all other markers being uninformative. A normal result indicates the normal complement of two for the chromosomes tested.

4. Peak area ratios that fall between the normal and abnormal ranges are classed as inconclusive. Inconclusive results may be resolved by using the single chromosome kits.

In the absence of any peak data for a marker '**Absent**' will be displayed in the warning column. This warning will be routinely observed in the absence of Y chromosome markers.

GeneMarker Analysis Guide

Note: The following screen shots have been taken from GeneMarker v2.6.3.

Adding Sample Files to GeneMarker

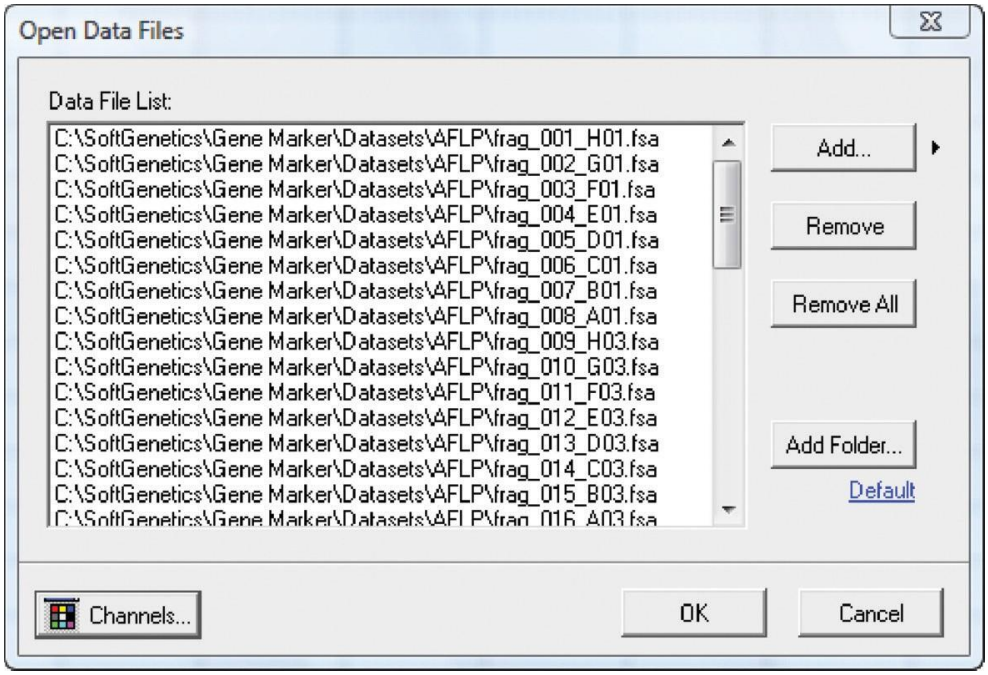
Open the GeneMarker program file and when prompted, select '**Open Data**'. The Open Data Files box will appear.

Click the '**Add'** button. The Open dialog will appear. Navigate to directory containing raw data files;

1. Select all files by CTRL+A or use CTRL and/or SHIFT keys to select individual samples.
2. Click '**Open'** button in the Open dialog

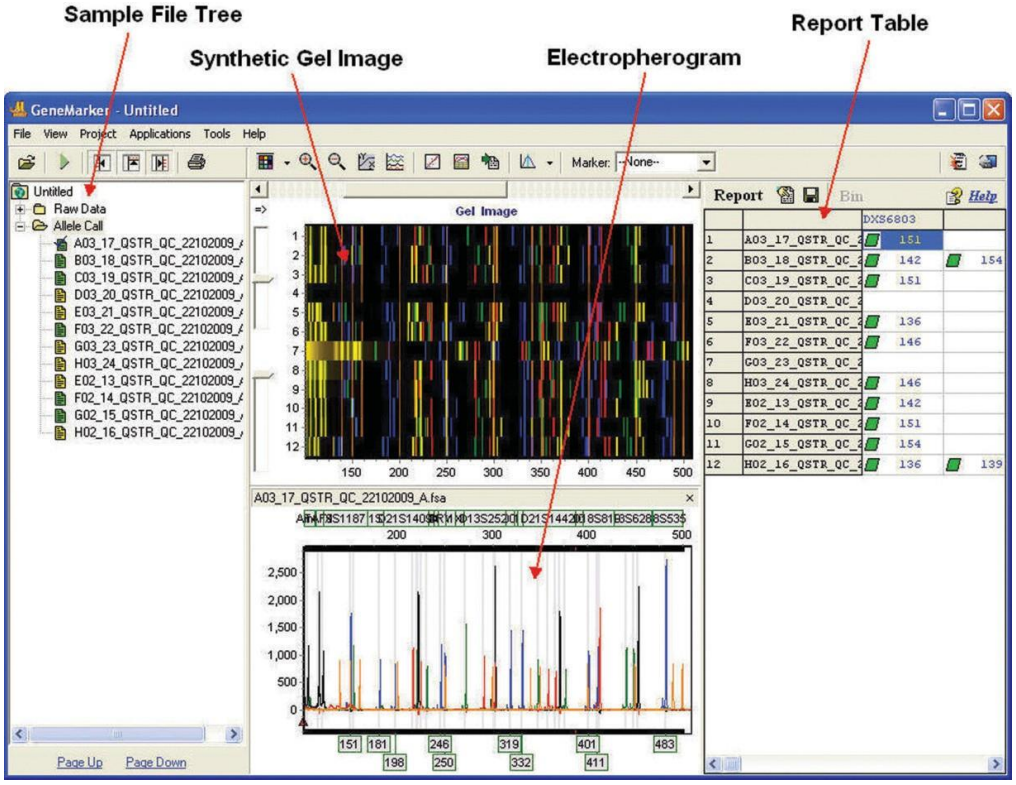
The files selected will appear in the Data File List field (Figure 13).

Figure 13: Samples added to the Data File list.



Click 'OK' button in the Open Data Files box and the samples will be uploaded to GeneMarker. The software will then automatically open the Raw Data Analysis window (Figure 14).

Figure 14: Raw Data Analysis Window



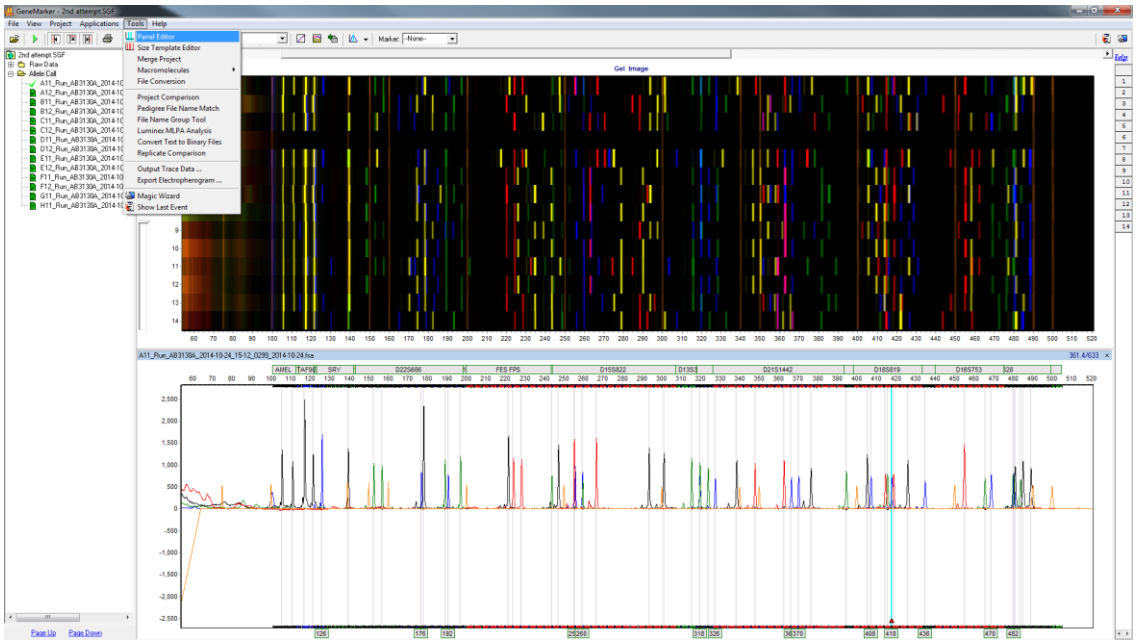
Importing QST*R-PL GeneMarker Panel Settings

It is necessary to import the QST*R panel settings for GeneMarker. This process is controlled through the 'Panel Editor' interface.

QST*R-PL GeneMarker panel settings are available from the Elucigene Website: www.elucigene.com/product-category/rapid-aneuploidy-analysis/

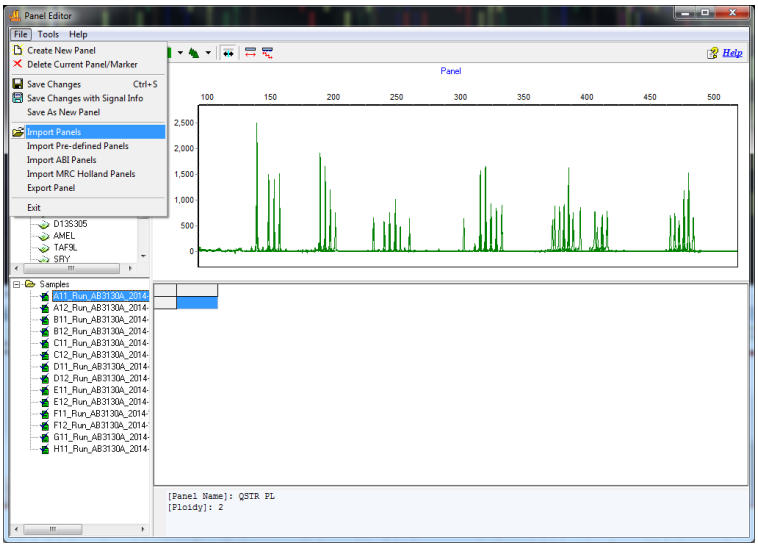
1. Open '**Panel Editor**' from the 'Tools' drop-down menu (Figure 15).

Figure 15: Selecting Panel Editor



2. Select 'Import Panels' from the 'File' drop-down menu (figure 16)

Figure 16: Importing Panels



3. Navigate to and import the panel QSTR-PL.xml

4. Repeat the process as required for other relevant panel files.

Processing Data

After the raw data files have been uploaded to GeneMarker, they are ready to be processed. The processing step includes application of a sizing standard, filtering of noisy peaks, and comparison to a known allelic panel if desired.

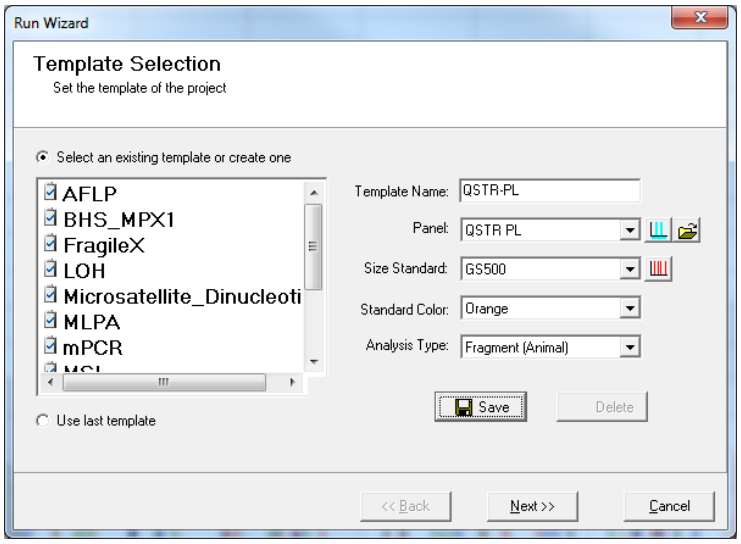
GeneMarker combines all these steps in one simple tool called the '**Run Wizard**' (Figure 17). To access the Run Wizard simply click the 'Run Project' icon in the main toolbar.

Run Wizard – creating a Run Template

It is necessary to create a run template the first time this software is used to analyse QST*R-PL data. This is done through the Run Wizard;

1. To access the Run Wizard simply click on the '**Run Project**' icon in the main toolbar.
2. Assign a Template Name e.g. **QSTR-PL.**
3. Select the Panel, Size Standard, Standard Colour and Analysis Type as shown in Figure 17 below.
4. Click '**Save**' to store the template for future analyses.
5. Click '**Next**' to continue.

Figure 17: Run Wizard – Template Selection Window



Run Wizard – Data Process

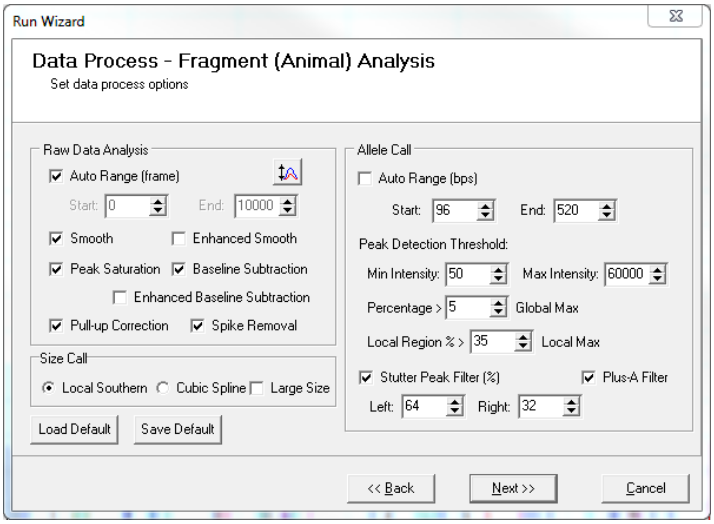
The Data Process window of Run Wizard allows the user to select the peak filtering parameters.

1. Select the appropriate analysis settings in the Data Process window as shown in the figure below (Figure 18).

2. Click '**Next**' to continue

Note: *The analysis range setting within the raw data analysis bow will vary depending on the Polymer used during data collection. The operator should select a start data point value that includes the 75bp size standard peak*.

Figure 18: Run Wizard – Data Process Window



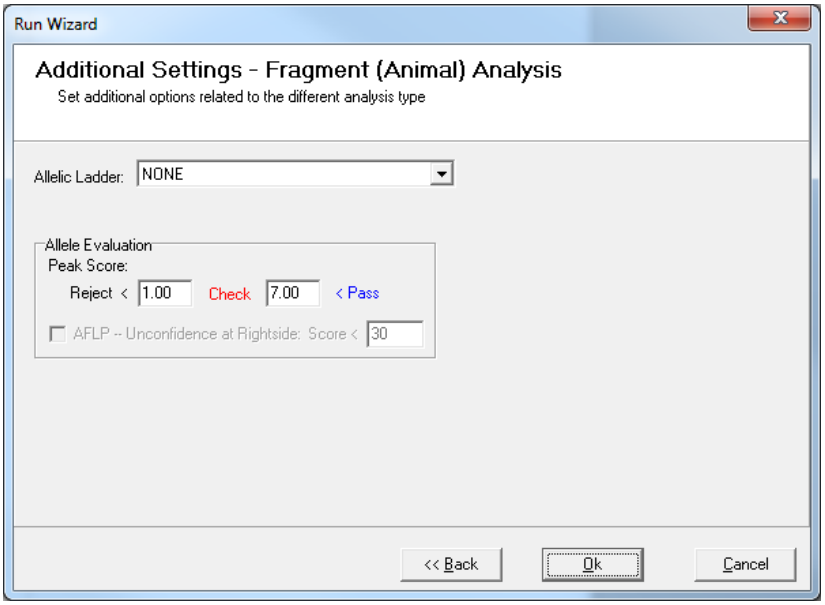
Note: *For 3500 data, increase the Minimum Intensity to 150*

Run Wizard – Additional Settings

There are no additional settings required when performing QST*R analysis (Figure 19).

1. Click '**OK**' to continue.

Figure 19: Run Wizard – Additional Settings

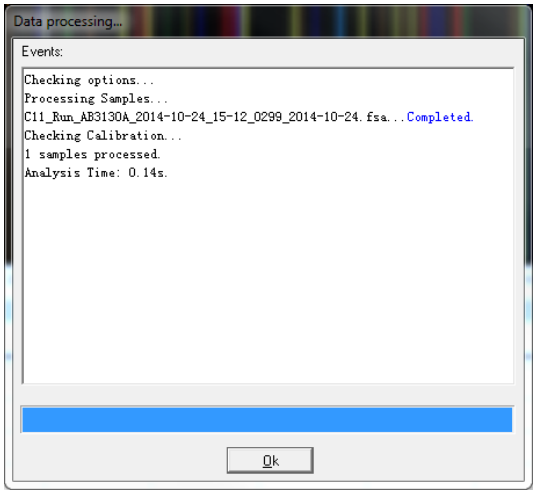


Data Processing Box

After clicking '**OK**' in the Run Wizard Additional Settings box, the Data Processing box appears (Figure 20). The raw data is processed and sized, then the filtering parameters and the selected QST*R Panel are applied.

1. Click '**OK**' in the Data Processing box when analysis is complete.

Figure 20: Data Processing Box



Main Analysis Window

The Main Analysis window (Figure 21) of GeneMarker has an easy to use layout. This layout includes:

- The sample files list - displayed on the left side of the window.
- The Synthetic Gel Image - displayed at the top of the window.
- Data Electropherograms - below the gel image.
- A Report Table - displayed on the right side of the window.

In this window it is important to check that all the appropriate peaks in each profile have been called correctly.

1. Double-click on each sample in turn in the sample file tree on the left-hand side of the screen. Right-click on any peaks in question and choose from the options in the dialogue box e.g. edit or delete allele, confirm or unconfirm as appropriate.

2. From the Main Analysis window select the '**Applications**' drop down menu option from the top of the screen. Select '**Trisomy Analysis**'. The Trisomy Analysis Settings box will then open (Figure 22).

Figure 21: Main Analysis Window

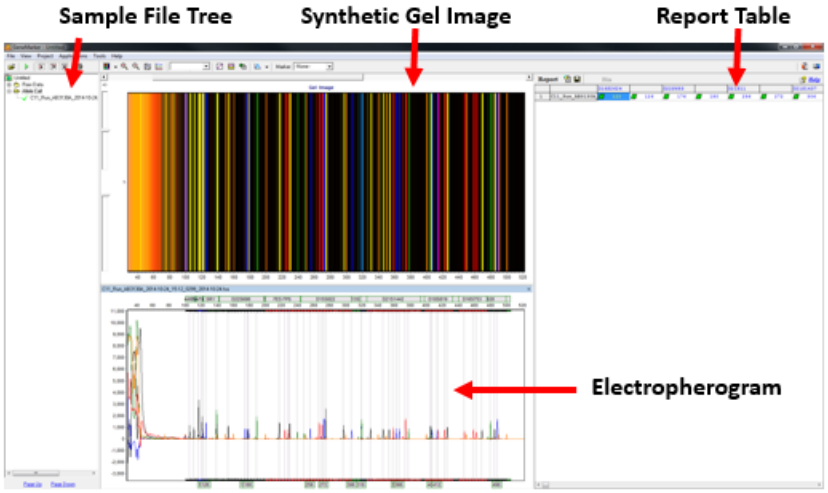
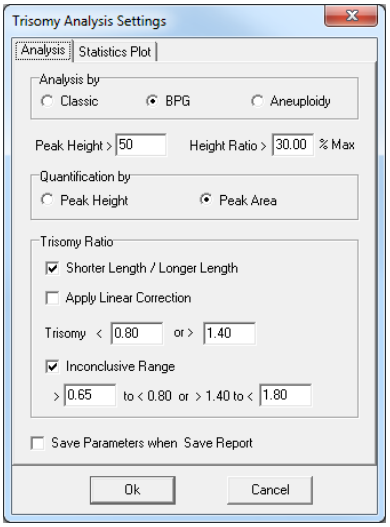


Figure 22: Trisomy Analysis Settings Box



Trisomy Analysis Settings

Within the Trisomy Analysis settings box two tabs are available:

1. Analysis tab.

2. Statistics Plot tab.

Analysis Tab

The Analysis tab provides threshold setting options for Trisomy analysis. Ensure that '**BPG**' is selected in the analysis box and the following settings displayed:

- Peak Height 50: Minimum height of 50 for peaks to be called. (150 if using 3500 data)
- Height Ratio 30%: Maximum percentage of the main peak the second peak must reach in order for two alleles to be identified.
- Quantification by Peak Area.
- Shorter Length/Longer Length selected.
- Trisomy Ratio thresholds are 0.80 – 1.40.
- Apply Linear Correction de-selected.
- Click '**OK**'
-

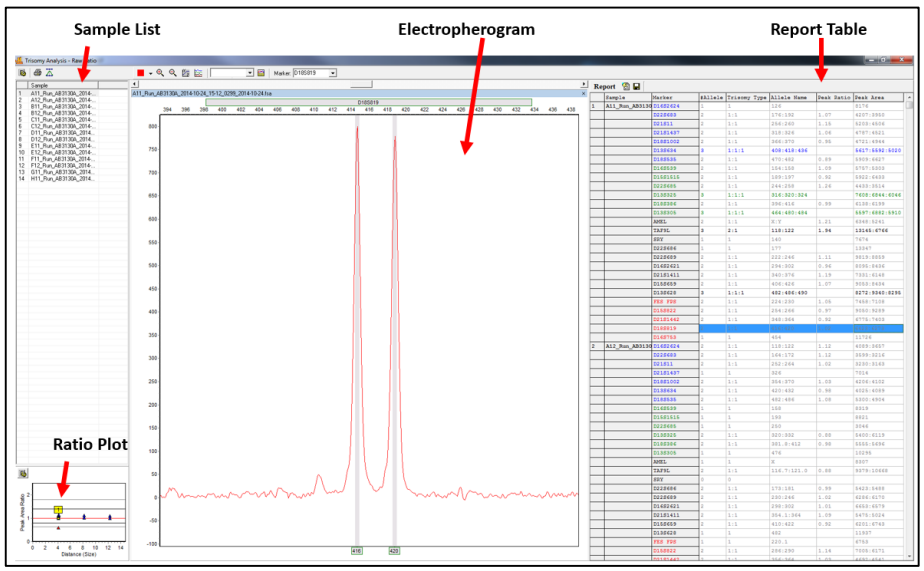
Trisomy Analysis Window

The Trisomy Analysis window (Figure 23) allows the operator to review QST*R sample data and display the ratio of peaks for each marker and access the GeneMarker report.

There are a number of displays which assist the operator in data analysis: They are:

- Sample List
- Electropherogram
- Ratio Plot
- Report Table

Figure 23: Trisomy Analysis Window



For more detailed information regarding Trisomy analysis features and their use, please refer to the GeneMarker Manual.

GeneMarker Report

GeneMarker includes a report template that is compatible with Elucigene QST*R kits. To access the report click the 'Print' icon located on the toolbar at the top left of the Trisomy Analysis Window.

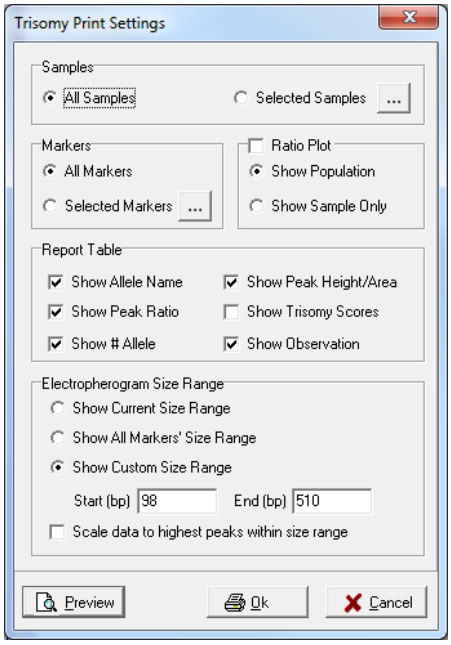
This will open the Trisomy Print Settings window (Figure 24).

Trisomy Print Settings Window

The Trisomy Print Settings detail the options for including and visualising sample data in the GeneMarker report.

Select the options as shown in Figure 24. Ensure that '**Custom Size Range**' is set to 98bp (Start) and 510bp (End).

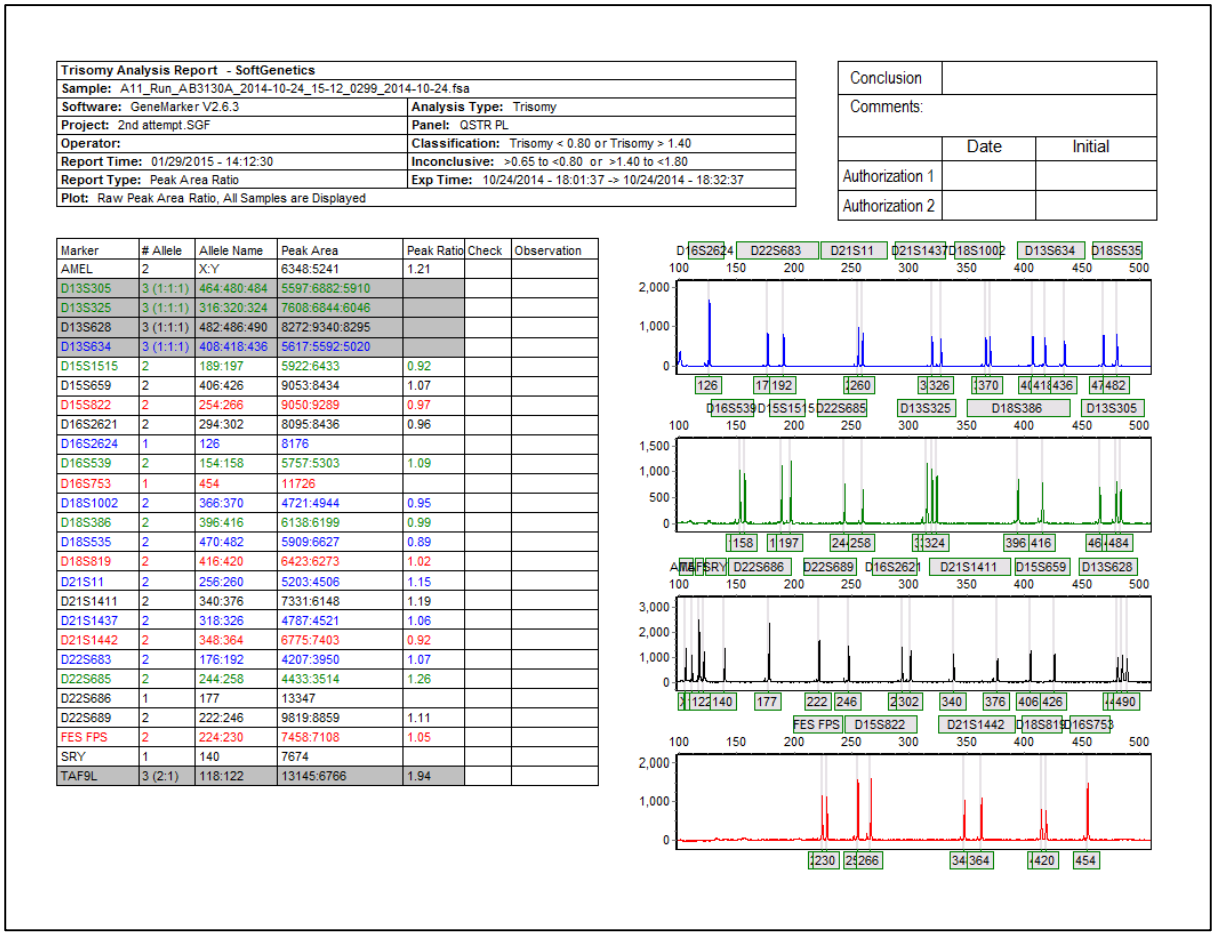
Figure 24: Trisomy Print Settings Window.



Previewing the GeneMarker Report

Click '**Preview**' to view the GeneMarker report (Figure 25). From this window the operator can review and print each sample's peak data from across all markers, providing a simple, one or two page sample report.

Figure 25: GeneMarker Report



Trisomy Analysis Report - SoftGenetics

Sample: A11_Run_AB3130A_2014-10-24_15-12_0299_2014-10-24.fsa	
Software: GeneMarker V2.6.3	**Analysis Type:** Trisomy
Project: 2nd attempt.SGF	**Panel:** QSTR PL
Operator:	**Classification:** Trisomy < 0.80 or Trisomy > 1.40
Report Time: 01/29/2015 - 14:12:30	**Inconclusive:** >0.65 to <0.80 or >1.40 to <1.80
Report Type: Peak Area Ratio	**Exp Time:** 10/24/2014 - 18:01:37 -> 10/24/2014 - 18:32:37
Plot: Raw Peak Area Ratio, All Samples are Displayed	

Conclusion		
Comments:		
	Date	Initial
Authorization 1		
Authorization 2		

Marker	# Allele	Allele Name	Peak Area	Peak Ratio	Check	Observation
AMEL	2	X:Y	6348:5241	1.21		
D13S305	3 (1:1:1)	464:480:484	5597:6882:5910			
D13S325	3 (1:1:1)	316:320:324	7608:6844:6046			
D13S628	3 (1:1:1)	482:486:490	8272:9340:8295			
D13S634	3 (1:1:1)	408:418:436	5617:5592:5020			
D15S1515	2	189:197	5922:6433	0.92		
D15S659	2	406:426	9053:8434	1.07		
D15S822	2	254:266	9050:9289	0.97		
D16S2621	2	294:302	8095:8436	0.96		
D16S2624	1	126	8176			
D16S539	2	154:158	5757:5303	1.09		
D16S753	1	454	11726			
D18S1002	2	366:370	4721:4944	0.95		
D18S386	2	396:416	6138:6199	0.99		
D18S535	2	470:482	5909:6627	0.89		
D18S819	2	416:420	6423:6273	1.02		
D21S11	2	256:260	5203:4506	1.15		
D21S1411	2	340:376	7331:6148	1.19		
D21S1437	2	318:326	4787:4521	1.06		
D21S1442	2	348:364	6775:7403	0.92		
D22S683	2	176:192	4207:3950	1.07		
D22S685	2	244:258	4433:3514	1.26		
D22S686	1	177	13347			
D22S689	2	222:246	9819:8859	1.11		
FES FPS	2	224:230	7458:7108	1.05		
SRY	1	140	7674			
TAF9L	3 (2:1)	118:122	13145:6766	1.94		

The GeneMarker report includes the following features:

- Report Header: Contains information about the analysis, project, sample and parameters.
- Signature Box: Date and initial space for report reviewers.
- Electropherogram: Similar to the Trisomy analysis window, displays all dye colours of the sample trace.
- Report Table: Displays selected peak and marker values for the current sample. Trisomy calls are highlighted grey. An additional check column is provided for reviewer initials.
- Corrected Ratio Plot: Contains the entire dataset's plot points for all markers in the dye colour. Symbol shapes represent different markers and can be deciphered from the 'Symbol' row in the 'Report Table'. Yellow filled symbols represent the current sample's data points. Red outlined symbols represent trisomy calls.

Note: *The Corrected Ratio Plot appears on a second page for each sample only when Ratio Plot is selected in the Trisomy Print Report Settings box.*

APPENDIX 1: Dye Labels

The markers are labeled as follows:

6-FAM	VIC	NED	PET
D16S2624	D16S539	AMEL	FES FPS
D22S683	D15S1515	TAF9L	D15S822
D21S11	D22S685	SRY	D21S1442
D21S1437	D13S325	D22S686	D18S819
D18S1002	D18S386	D22S689	D16S753
D13S634	D13S305	D16S2621	
D18S535		D21S1411	
		D15S659	
		D13S628	

See Appendix 2 for further details of the STR markers including size ranges.

APPENDIX 2: - Table of Maker location, Observed Heterozygosity, allele size range

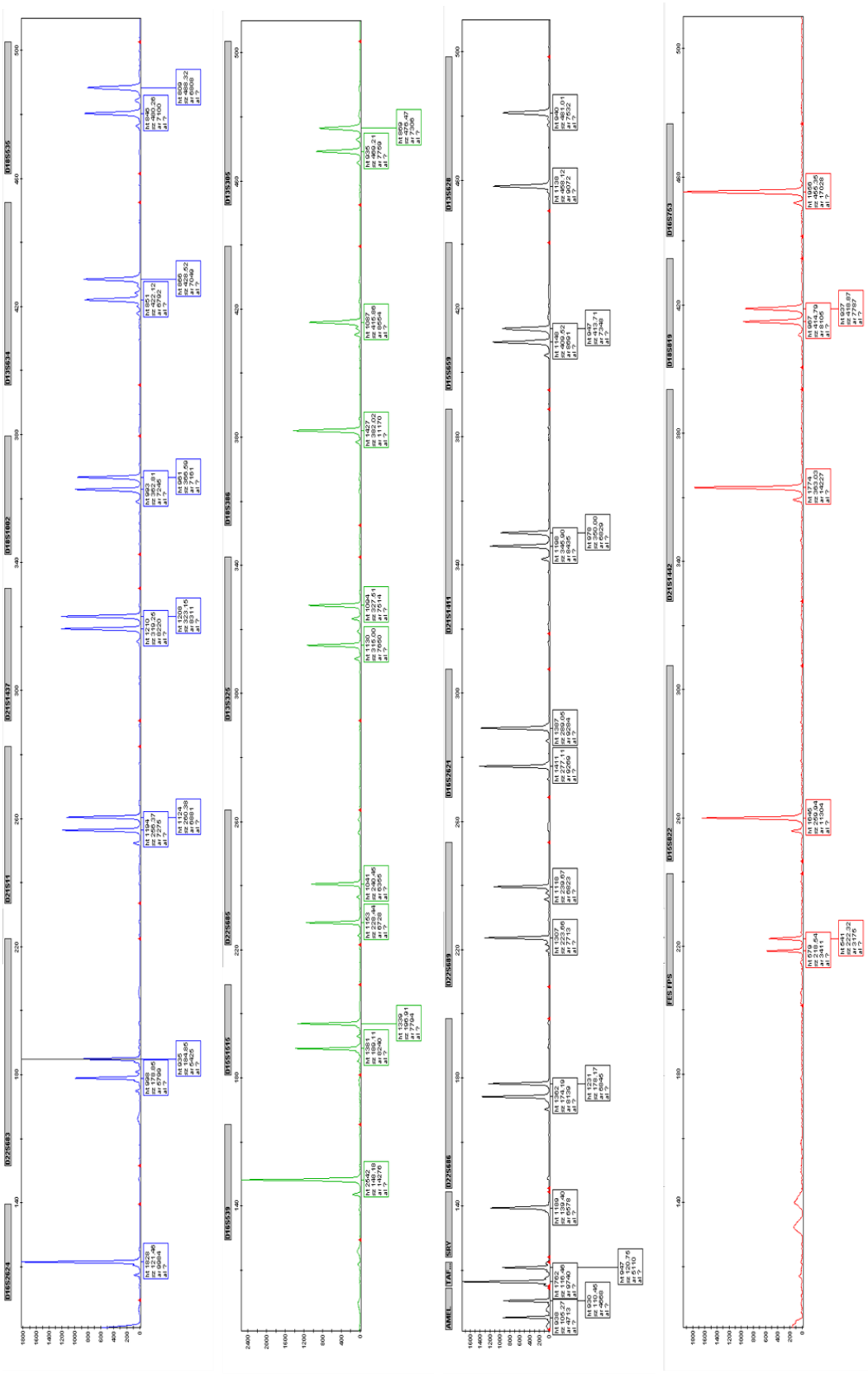
Note: *the NED dye used in the kits is identified spectrally as a yellow dye. It is conventionally displayed in black type for clarity.*

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D13S305	13q13.3	0.79	453-504	green
D13S325	13q14.11	0.80	292-343	green
D13S634	13q21.33	0.84	396-453	blue
D13S628	13q31.1	0.75	451-499	yellow
D15S822	15q12	0.86	247-308	red
D15S659	15q21.1	0.86	395-441	yellow
FESFPS	15q25.2	0.61	202-243	red
D15S1515	15q26.3	0.81	181-209	green
D16S753	16p11.2	0.77	442-477	red
D16S2624	16q22.3	0.71	110-140	blue
D16S2621	16q23.2-q24.2	0.79	268-308	yellow
D16S539	16q24.1	0.76	130-166	green
D18S1002	18q11.2	0.76	343-380	blue
D18S819	18q11.2	0.73	401-435	red
D18S535	18q12.3	0.77	462-503	blue
D18S386	18q22.1	0.92	353-440	green
D21S11	21q21.1	0.82	234-283	blue
D21S1437	21q21.1	0.76	291-332	blue
D21S1442	21q21.3	0.85	328-394	red
D21S1411	21q22.3	0.83	319-389	yellow
D22S686	22q11.2	0.69	146-199	yellow
D22S685	22q11.23	0.77	222-264	green
D22S689	22q12.1	0.74	209-254	yellow
D22S683	22q12.3	0.87	152-223	blue
AMEL	Xp22.22/Yp11.2	n/a	104/110	yellow
TAF9	3p24.2/Xq21.1	n/a	116/121	yellow
SRY	Yp11.31	n/a	124-145	yellow

***Observed heterozygosities are based on number of alleles observed with Elucigene Diagnostics testing panel. These figures may therefore differ from published data and may also vary according to the population being tested**

APPENDIX 3: GeneMapper Profile

GeneMapper normal male profile showing relative positions of the markers detected by QST*R-PL.



Limitations to the Procedure

This test is designed to detect specific chromosomal trisomies and sex chromosome aneuploidies as detailed in the Instructions for Use. It may not detect structural rearrangements involving the chromosomes tested and will not detect abnormalities in any other chromosomes. Mosaicism for the chromosomes tested may not be detected. A QST*R-PL result can only be directly applied to the tissue tested and may not represent the fetal karyotype. Maternal cell contamination (MCC) and confined placental mosaicism (CPM) may result in discrepancies between the QST*R-PL and karyotype results.

Note: *Heterozygosities of the markers used were derived from a random set of samples submitted for routine analysis from a predominantly Northern European Caucasian population. Any calculations using these heterozygosities strictly only apply to the population from which the samples were taken. A small study using locally derived samples may be carried out as part of a validation study to establish heterozygosities in the population to be tested. It is not expected that population variation will significantly alter the overall informativeness of the assay.*

Disclaimer

Results from this diagnostic assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

These Elucigene reagents are supplied for *In Vitro* diagnostic testing.

Further details of Elucigene QST*R products are available at:
www.elucigene.com.

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