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

Instructions for Use

Cat Code: AN6XYR1 – **25 tests**

Research Use Only

The performance characteristics of this product have not been established and must not be used for diagnostic purposes.

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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

The QST*R-PL assay can be used for the detection 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 assay can be used on DNA extracted from either fetal material obtained post-miscarriage or whole blood (of fetal origin. The 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.

This Product is for Research Use Only (RUO).

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).

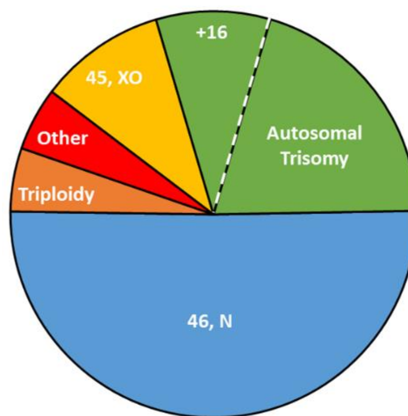


Figure 1: Showing the chromosomal findings in products of conception with 46N representing normal results.

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.

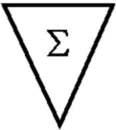
4. In line with current good laboratory practice, laboratories should process their own internal QC samples of known type in each assay, so that the validity of the procedure can be assessed.

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

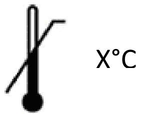
The symbols used on all labels and packaging conform to the harmonised standard ISO 15223

Manufacturer



Number of tests

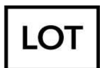
See Instructions for Use



Store below temperature shown

Use before date shown

Catalogue code



Lot or batch number

Materials Provided

Store all components below -20°C

The Elucigene QST*R-PL RUO kit contains:

450048, 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.

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 GeneMapper 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 3.7 (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. A supplemental Guide to Interpretation with examples and glossary and a Guide to Analysis Software 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/µl.

DNA Concentration

Using the recommended PCR conditions and sample injection settings* stated in the capillary column run module (pages 10 and 11), acceptable results are consistently obtained with input DNA amounts of 1.25ng to 10ng.

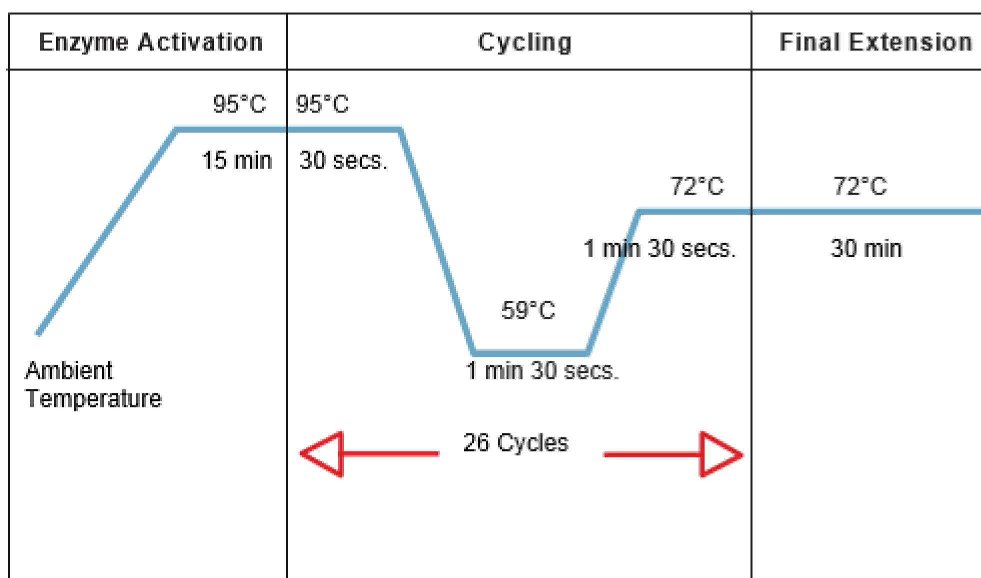
***Note:** sample injection settings can be modified to suit the amount of amplicon produced during the PCR reaction which can vary due to amount of input genomic DNA added. Less amplicon can be applied to the column for analysis by reducing time of injection. Conversely, more amplicon can be applied 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 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 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.

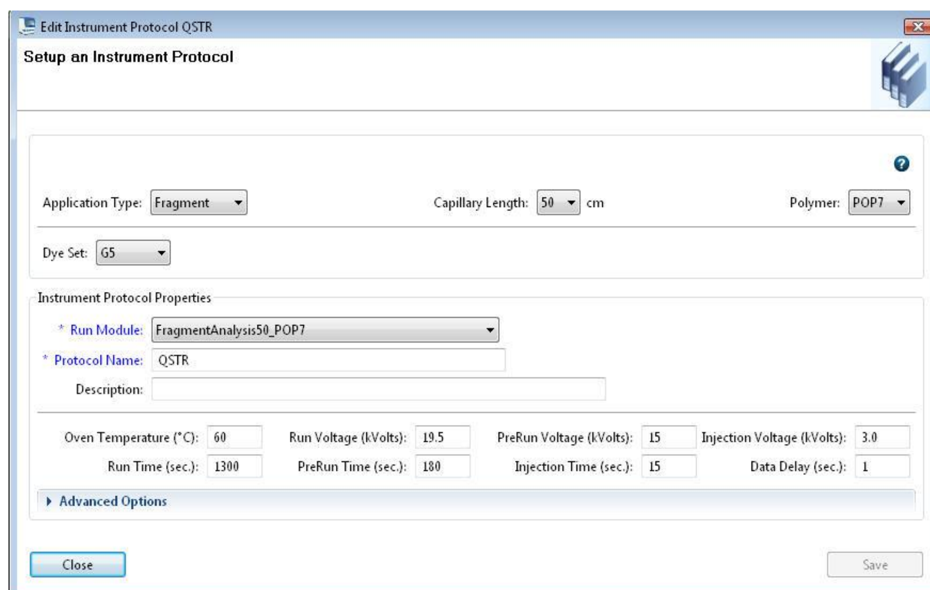
ABI3500 GENETIC ANALYZER

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 of Results

General Interpretation of Results

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 Guide to Analysis Software for GeneMarker and GeneMapper is available from the Elucigene website: www.elucigene.com. Please note that IVD labelling on these QST*R guides does not apply to QST*R-PL.

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 all QST*R kits

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.

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	

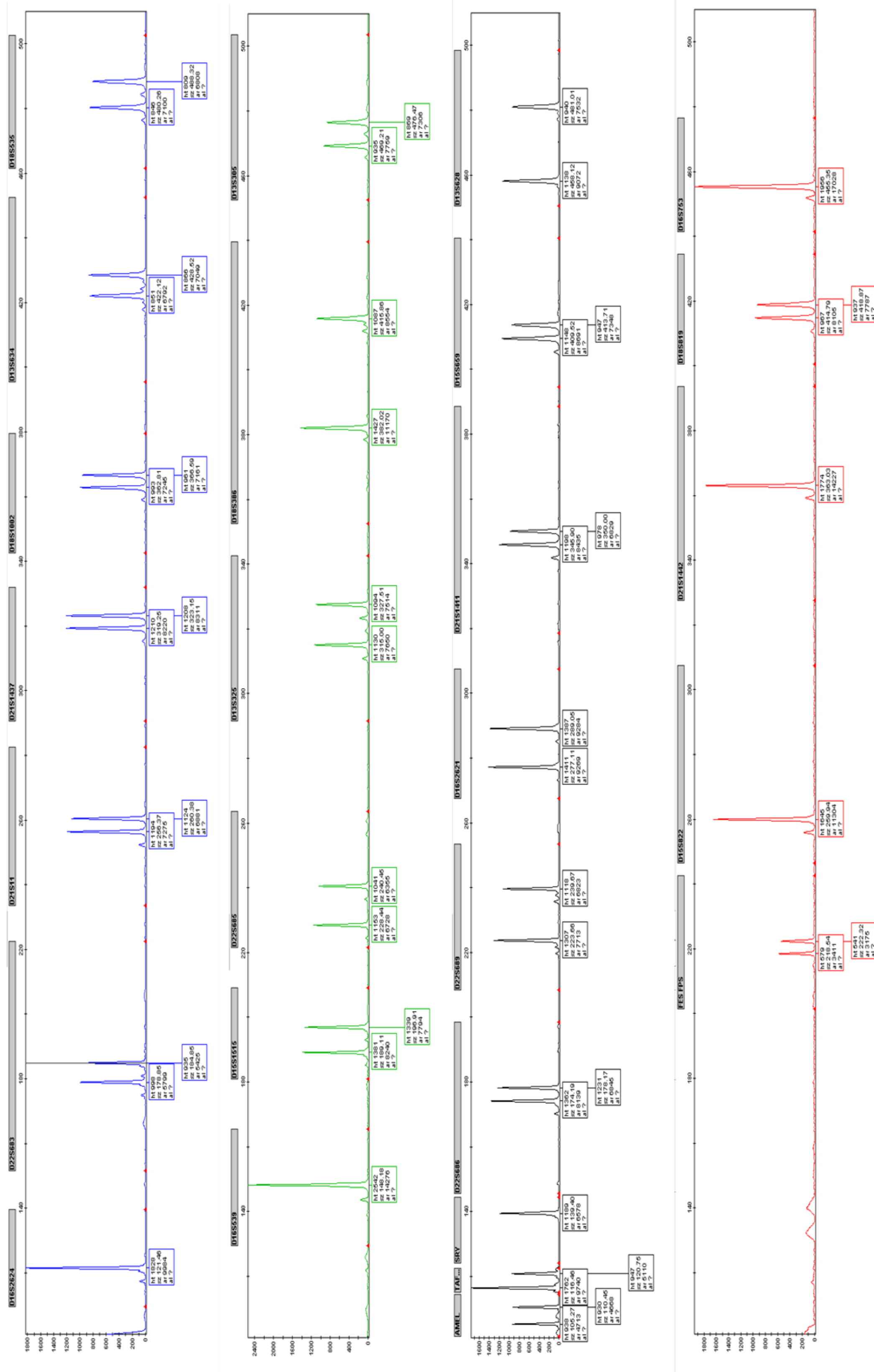
APPENDIX 2: - Table of Maker location, Observed Heterozygosity, allele size range

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

For **Research use only (RUO)**, the performance characteristics of this assay have not been established

Not for In Vitro Diagnostic Use.

Further details of Elucigene QST*R products are available at:

www.elucigene.com

References

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