



Elucigene  
QST\**Rplusv2*

# Instructions for Use

# INDEX

Instructions for Use	2
Elucigene QST®R	3
Intended Use	3-5
Principle of the Procedure	6
Warnings and Precautions	6
Symbols used on Labels	7
Materials Provided	8
Materials Required but not Provided	9
Additional Elucigene QST®R Documentation	10
Sample Collection and Storage	10
DNA Extraction	10-11
DNA Concentration	11-12
PCR Set Up	12-13
Capillary Electrophoresis	13-15
Interpretation of Results	16-19
Performance Characteristics	19
Appendix 1	
-examples	21-33
Appendix 2	
-tables of markers	34-37
Limitations of the Procedure	38
Disclaimer	38
Technical Support	39
Bibliography	39

## INSTRUCTIONS FOR USE

ELUCIGENE is a trademark of Gen-Probe Life Sciences Ltd.

QST<sup>®</sup>R is a trademark of Gen-Probe Life Sciences Ltd.

Elucigene kits are developed and manufactured by Gen-Probe Life Sciences Ltd. within quality systems accredited to ISO9001:2008 and ISO13485:2003

Elucigene QST<sup>®</sup>R kits are developed in collaboration with Guys and St Thomas' NHS Foundation Trust, London, UK

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GeneMarker<sup>®</sup> is a registered trademark of SoftGenetics LLC

InstaGene<sup>™</sup> is a trademark of Bio-Rad Laboratories Inc.

### Note to purchaser: Limited License

Polynucleotides labelled with VIC<sup>®</sup>, NED<sup>™</sup> and PET<sup>®</sup> dyes and/or their use may be covered by one or more patents owned by Applied Biosystems, LLC. The purchase price of this product includes limited, nontransferable rights under certain claims of certain patents owned by Applied Biosystems, LLC to use only this amount of the product solely for activities of the purchaser in detection of Target(s) within the field of human diagnostics. No other rights are conveyed. Further information on purchasing licenses relating to the dyes mentioned above may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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## Elucigene QST\*R

The Elucigene QST\*R range of products are DNA based multiplexed assays for the rapid prenatal determination of aneuploidy status for the three most common viable autosomal trisomies and the sex chromosomes X and Y. Elucigene QST\*R kits are available in the formats listed below. For more information on the kits in the QST\*R range please visit [www.gen-probe.com/products/](http://www.gen-probe.com/products/)

Product	Size	Product Code
Elucigene QST*R <i>plusv2</i>	50 tests	ANOPLB2
Elucigene QST*R	50 tests	AN003B2
Elucigene QST*R-XYv2	50 tests	ANOXYB2
	10 tests	ANOXYBX
Elucigene QST*R-13	10 tests	AN013BX
Elucigene QST*R-18	10 tests	AN018BX
Elucigene QST*R-21	10 tests	AN021BX

### INTENDED USE

#### QST\*R*plusv2*

For the routine *in vitro* quantitative diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). 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*plusv2* kit is the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. The devices are intended to be used on DNA extracted from either amniotic fluid or chorionic villus samples (CVS) taken during amniocentesis. The intended target population is pregnant women that have been assessed as being at 'high risk' of carrying an affected foetus by either biochemical or ultrasound diagnostic procedures or assessed to be 'at risk' due to either previous family history or maternal age. The device 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.

**QST®R**

For the routine *in vitro* quantitative diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). The method employed by the Elucigene QST®R kit is the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. The devices are intended to be used on DNA extracted from either amniotic fluid or chorionic villus samples (CVS) taken during amniocentesis. The intended target population is pregnant women that have been assessed as being at 'high risk' of carrying an affected foetus by either biochemical or ultrasound diagnostic procedures or assessed to be 'at risk' due to either previous family history or maternal age. The device 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.

**QST®R-XYv2**

For the routine *in vitro* quantitative diagnosis of the for the analysis of sex chromosome status including the common aneuploidies Klinefelter syndrome and Turner syndrome. The method employed by the Elucigene QST®R-XYv2 kit is the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. The devices are intended to be used on DNA extracted from either amniotic fluid or chorionic villus samples (CVS) taken during amniocentesis. The intended target population is pregnant women that have been assessed as being at 'high risk' of carrying an affected foetus by either biochemical or ultrasound diagnostic procedures or assessed to be 'at risk' due to either previous family history or maternal age. The device 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.

**QST<sup>®</sup>R-13, QST<sup>®</sup>R-18, QST<sup>®</sup>R-21**

Supplemental kits containing additional autosomal markers, to be used in conjunction with QST<sup>®</sup>R or QST<sup>®</sup>R*plusv2*, for the routine quantitative *in vitro* diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome) respectively.

These kits are available for extended chromosome testing where necessary or for confirmation of positive results.

The method employed by these kits is the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. The devices are intended to be used on DNA extracted from either amniotic fluid or chorionic villus samples (CVS) taken during amniocentesis.

The intended target population is pregnant women that have been assessed as being at 'high risk' of carrying an affected foetus by either biochemical or ultrasound diagnostic procedures or assessed to be 'at risk' due to either previous family history or maternal age. The device 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.

## **PRINCIPLE OF THE PROCEDURE**

The method employed by Elucigene QST®R kits uses the QF-PCR (Quantitative Fluorescence-Polymerase Chain Reaction) technique. 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. For professional *in vitro* diagnostic use only.
2. 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.
3. 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.
4. 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.
5. Store all components below -20°C.
6. 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.

## SYMBOLS USED ON LABELS

The symbols used on all labels and packaging conform to the harmonised standard EN980



Manufacturer



Number of tests



See Instructions for Use



Store below temperature shown



Use before date shown



Catalogue code



Lot or batch number

## MATERIALS PROVIDED

Each kit contains:

2 x 250µl (50 tests) or 1 x 100µl (10 tests) of QST<sup>®</sup>R Reaction Mix, containing primers to amplify a number of STR (short tandem repeat) markers. See appendix 2 for details of markers in each kit. The QST<sup>®</sup>R reaction mix also contains DNA polymerase and deoxynucleotide triphosphates in buffer.

Kit	0.2ml PCR Vials	Component Part No.
Elucigene QST <sup>®</sup> R <i>plusv2</i>	Clear	ANOPLTA
Elucigene QST <sup>®</sup> R	Orange	AN003TA
Elucigene QST <sup>®</sup> R-XYv2	Pink	ANOXYTA
Elucigene QST <sup>®</sup> R-13	Green	AN013TA
Elucigene QST <sup>®</sup> R-18	Purple	AN018TA
Elucigene QST <sup>®</sup> R-21	Yellow	AN021TA

1 x 50µl vial DNA Control, diploid for the markers detected by Elucigene QST<sup>®</sup>R:

Kit	Component Part No.
Elucigene QST <sup>®</sup> R <i>plusv2</i>	CR001TX
Elucigene QST <sup>®</sup> R	CR001TX
Elucigene QST <sup>®</sup> R-XYv2	CR001TX
Elucigene QST <sup>®</sup> R-13	CR001TX
Elucigene QST <sup>®</sup> R-18	CR001TX
Elucigene QST <sup>®</sup> R-21	CR001TX

50 (10) x 0.2ml PCR vials, colour coded as detailed above.

## KIT PREPARATION AND STORAGE

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

## ***MATERIALS REQUIRED BUT NOT PROVIDED***

### ***GENERAL***

Laboratory consumables – gloves; 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.

### ***DNA EXTRACTION***

DNA Preparation – InstaGene Matrix (Bio-Rad Laboratories, Cat No 732-6030), sterile de-ionised water.

### ***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) or GeneScan 600 LIZ (ABI Cat No 4366589), DS-33 (dye set G5) matrix standard (ABI Cat No 4345833), POP-6 Polymer (ABI Cat No 4316357) or POP-7 Polymer (ABI Cat No 4352759), 10x Genetic Analyzer Buffer (ABI Cat No 402824) and Hi-Di Formamide (ABI Cat No 4311320).

Applied Biosystems ABI 3\*\*\* series Genetic Analyzers (with GeneMapper software), 36cm capillary array, 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 (SoftGenetics LLC).

## ***ADDITIONAL ELUCIGENE QST\*R DOCUMENTATION***

These Instructions for Use include a basic section on interpretation of the results obtained. A supplemental **Guide to Interpretation of Results** with examples and glossary and a **Guide to Analysis Software** are available from the Gen-Probe website:

[www.gen-probe.com/products/](http://www.gen-probe.com/products/)

## ***SAMPLE COLLECTION AND STORAGE***

Chorionic Villus (CV) or Amniotic Fluid (AF) samples should be used. Sample collection devices have on occasion been reported to be detrimental to the integrity of certain analytes and could interfere with some method technologies. It is recommended that each user ensure that the chosen device is used according to the manufacturer's instructions and both sample collection devices and DNA preparation methods are compatible with this test.

## ***DNA EXTRACTION***

Elucigene QST\*R kits are validated on the InstaGene matrix method of DNA extraction and can be performed in a single tube, eliminating the necessity for tube-to-tube transfers. Other extraction methods have been shown to provide equally reliable results eg. Qiagen QIAamp® kits.

The InstaGene method of DNA extraction is described below.

### ***INSTAGENE EXTRACTION METHOD***

#### ***Amniotic Fluid (AF)***

Approximately 1-2ml of amniotic fluid should be used.

#### ***Chorionic Villus (CV)***

CV samples should be carefully cleaned to remove any adhering maternal decidua. It is important that cells from more than one region of the sample are tested and that cells from the mesenchyme core are represented. A small aliquot of the cell suspension, as prepared for conventional cell culture set-up, is recommended for QST\*R analysis. This ensures that the QST\*R result is obtained from the same population of cells used for karyotype analysis.

1. Resuspend the InstaGene matrix on the magnetic stirrer and set at a medium speed for at least 5 minutes.
2. Centrifuge the sample (AF or CV) at 12,000g for 1 minute in order to pellet the cells.
3. Remove the samples from the centrifuge and visually check the pellet for blood-staining. Make a note of the percentage of blood-staining, if any.
4. Carefully remove and discard the supernatant from the pellet, ensuring that the pellet is undisturbed. Leave approximately 10-20µl of supernatant behind to re-suspend the pellet.
5. Thoroughly mix the sample by vortexing.
6. If greater than 50% bloodstaining is observed proceed to Step 7. If less than 50% blood-staining is observed proceed to Step 8.
7. Add 200µl of sterile deionised water to the cell pellet. Thoroughly mix by vortexing. Centrifuge at 12,000g for 1 minute, remove the supernatant leaving 10-20µl of supernatant behind to resuspend the pellet.  
**Note:** this additional washing step helps to lyse the red blood cells and remove haem that could inhibit PCR.
8. Add 200µl of InstaGene matrix from step 1 to the samples using a pipette tip with a large bore, such as 1,000µl.  
**Note:** to optimise the extraction protocol the added volume of InstaGene matrix (Chelex-100 resin) can be varied using 100µl of InstaGene matrix for small AF cell pellets (barely visible), or 300µl for large pellets (covering the base of the tube), CV and tissue samples. Record the amount of InstaGene matrix added to each sample.
9. Thoroughly mix the samples by vortexing and incubate at 100°C for 8 minutes in a hot block or water bath.
10. Thoroughly mix again by vortexing at high speed for 10 seconds.
11. Centrifuge the samples at 12,000g for 3 minutes. The supernatant contains the extracted DNA.
12. Proceed to PCR set up or store the extracted DNA at -20°C until required.

### ***DNA CONCENTRATION***

It is recommended that alternative DNA extraction methods and sample types are thoroughly evaluated with the Elucigen QST<sup>®</sup>R test prior to the results being used for diagnostic use.

Under optimal PCR conditions and using the recommended sample injection settings\* stated in the capillary column run module (page 13), acceptable results are consistently obtained with input DNA amounts of 1.25ng to 10ng.

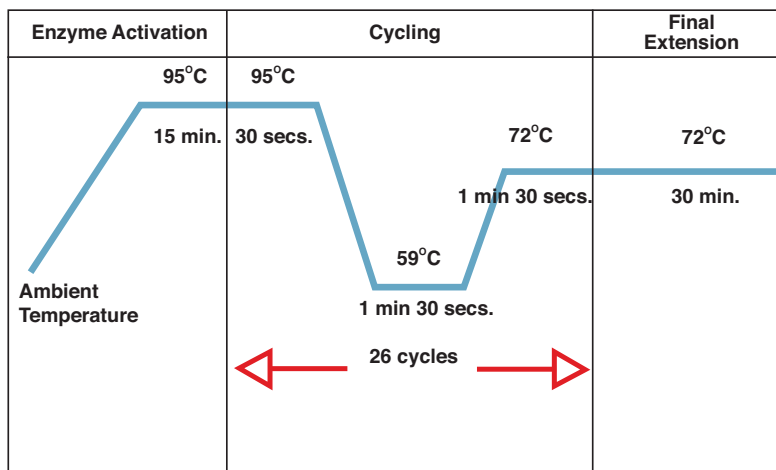
\***Note:** sample injection settings can be modified to suit the amount of amplicon produced during the PCR reaction which can vary due to amount of input genomic DNA added. Less amplicon can be applied to the column for analysis by reducing time of injection. Conversely, more amplicon can be applied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

### PCR SET UP

Where samples have been extracted using the Instagene method, it is recommended to use undiluted supernatant directly in the PCR reaction.

**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, eg. positive normal (DNA control supplied) and positive trisomy control (DNA not supplied).

3. Thaw sufficient vials of pre-aliquoted QST<sup>®</sup>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<sup>®</sup>R reaction mix and mix by pipetting up and down. Do this for all samples to be tested.

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

**Note:** care must be taken not to contaminate the PCR reaction with any InstaGene resin.

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

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

7. 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 ABI 3100 or ABI 3130 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.

**ABI 3100 GENETIC ANALYZER:**

Create a sample sheet using the 3100 data collection software with the following settings:

- Sample Name: this must be the sample specific name or number.
- Size Standard colour: ensure the orange coloured box is marked with a diamond so that the size standard is recognised.
- Colour Info: copy and paste sample name column data into this column.
- Colour Comment: copy and paste sample name column data into this column.
- Dye Set: G5
- Run Module: 36cm capillary run modules see below\*.

**\*Note:** Required 'run time' will vary dependent on the ambient temperature of the location in which the Genetic Analyzer has been installed. For more information on creating run modules please refer to your instrument user manual.

**ABI 3130 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.

**3100 RUN MODULES****FOR POP6 POLYMER****36cm Capillary Module: QSTR**

#	Parameter Name	Value	Range
1	Run Temperature	60	int 18...65 Deg.C
2	Cap Fill Volume	184	int 1...200 steps
3	Current Tolerance	100	int 1...100 uAmps
4	Run Current	100	int 1...200 uAmps
5	Voltage Tolerance	0.6	float 0.25...2.0 kVolts
6	Pre Run Voltage	15	float 0...15 kVolts
7	Pre Run Time	180	int 1...1000 sec.
8	Injection Voltage	3	float 1...15 kVolts
9	Injection Time	15	int 1...600 sec.
10	Run Voltage	15	float 0...15 kVolts
11	Number of Steps	10	int 1...100 nk
12	Voltage Step Interval	20	int 1...60 sec.
13	Data Delay Time	1	int 1...3600 sec.
14	Run Time	2900	int 300...14000 sec.

**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...3600sec.
11	Run_Voltage	15.0	0...15 kvolts
12	Run_Time	1200	300...14000 sec.

## 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 Clinical Molecular Genetics Society and Association of Clinical Cytogeneticists and are available for reference at:

<http://www.cmgs.org.uk>

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.

Software analysis guides for GeneMarker and GeneMapper are available from the Gen-Probe website:

[www.gen-probe.com/products/](http://www.gen-probe.com/products/)

The GeneMapper analysis guide gives details of software settings and instructions for importing analysed data into an Excel report template. GeneMarker has a trisomy analysis application that is compatible with Elucigene QST<sup>®</sup>R.

**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 for advice.

### General analysis guidelines for all QST<sup>®</sup>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 3100 and 3130 Genetic Analyzers is between 50 and 6000 relative fluorescent units (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 diagnostic 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 of Results** 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 chromosomes 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.

#### **Specific to QST<sup>®</sup>R-XYv2 and the Sex Chromosome Markers in QST<sup>®</sup>R $\plus$ usv2**

1. The AMEL marker amplifies non-polymorphic sequences on the X (104 bp) and Y (110 bp) 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 diagnosis of sex chromosome aneuploidy, for example Turner syndrome. In a normal female the markers should fall within a ratio window of 0.8 to 1.4. In a normal male or monosomy X the markers will give a ratio  $\geq 1.8$ . Further details on the interpretation of the TAF9L marker can be found in the Guide to Interpretation.

3. The DXYS267 and DXYS218 polymorphic STR markers are present on both the X and Y chromosomes and represents the total number of sex chromosomes. For informative male results it is not possible to determine which allele represents the X or Y chromosome.

4. Informative X-specific markers DXS981, DXS1187, XHPRT, DXS6807, DXS7423, DXS6803 and DXS6809 represent the number of X chromosomes.

5. The Y-specific marker, SRY, will give a single peak in normal males and will not amplify in normal females.
6. The Y-specific marker, DYS448, in most cases will give a single peak in normal males and will not amplify in normal females. It has been noted that on rare occasions, this marker can demonstrate a heritable di-allelic pattern (sub-microscopic duplication followed by replication slippage) or show no amplification (null allele).
7. A result exhibiting no amplification for Y specific markers and homozygous for all other markers is not necessarily diagnostic of Turner syndrome. Based on published data approximately 1 in 170,000 females will be homozygous for all 7 X specific polymorphic markers. This gives a Bayesian probability of approximately 1 in 1400 that a profile homozygous for all X specific markers represents a true monosomy X genotype rather than a normal homozygous female.

## ***PERFORMANCE CHARACTERISTICS***

### ***INTERNAL VALIDATION***

#### ***QST<sup>®</sup>R<sub>plusv2</sub>***

98 samples were tested blind using Elucigene QST<sup>®</sup>R<sub>plusv2</sub>. Of these, 22 were normal/XY, 17 were normal/XX, 12 were Trisomy 21/XY, 7 were Trisomy 21/XX, 9 were Trisomy 18/XY, 8 were Trisomy 18/XX, 2 were Trisomy 13/XY, 4 were Trisomy 13/XX, 8 were normal/X0, 1 was normal/XYY and 1 was triploid for all chromosomes tested.

One sample gave an uninformative result. Six samples failed to give analysable results due to poor sample quality. All analysable results showed 100% specificity and sensitivity with results previously obtained by an alternative established method.

#### ***QST<sup>®</sup>R***

312 samples were tested blind using Elucigene QST<sup>®</sup>R. Of these, 286 were normal, 2 showed trisomy 13, 7 showed trisomy 18, 13 showed trisomy 21 and 2 were triploid for all 3 chromosomes tested.

One sample exhibited maternal cell contamination preventing analysis and one sample failed to give an interpretable result despite repeat amplification. In total, 310 samples gave analysable results. All analysable samples showed 100% specificity and sensitivity with results previously obtained by karyotyping.

**QST®R-XYv2**

321 samples were tested blind using Elucigene QST®R-XYv2. Of these, 160 were normal male, 147 were normal female, 3 showed monosomy X, 2 showed XXY, 2 showed XYY and 1 showed XXX.

Six samples failed to give an interpretable result despite repeat amplification. In total, 315 samples gave analysable results. All analysable samples showed 100% specificity and sensitivity with results previously obtained by karyotyping.

**QST®R-13**

152 samples were tested blind using Elucigene QST®R-13. Of these, 144 were normal and 2 showed trisomy 13.

Six samples failed to give an interpretable result despite repeat amplification. All analysable samples showed 100% specificity and sensitivity with results previously obtained by karyotyping.

**QST®R-18**

152 samples were tested blind using Elucigene QST®R-18. Of these, 143 were normal and 4 showed trisomy 18.

Five samples failed to give an interpretable result despite repeat amplification. All analysable samples showed 100% specificity and sensitivity with results previously obtained by karyotyping.

**QST®R-21**

152 samples were tested blind using Elucigene QST®R-21. Of these, 148 were normal and 2 showed trisomy 21.

Two samples failed to give an interpretable result despite repeat amplification. All analysable samples showed 100% specificity and sensitivity with results previously obtained by karyotyping.

## APPENDIX 1: EXAMPLES

**ELUCIGENE QST<sup>®</sup>RPLUS<sub>v</sub>2**

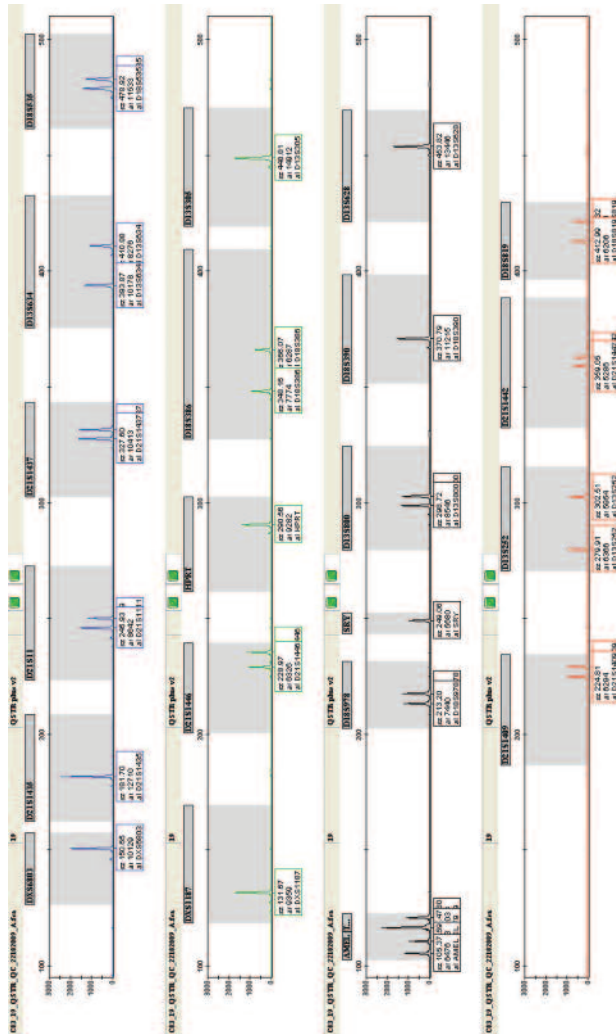
The markers are labelled as follows: -

6-FAM	VIC	NED	PET
DXS6803	DXS1187	AMEL	D21S1409
D21S1435	D21S1446	TAF9L	D13S252
D21S11	XHPRT	D18S978	D21S1442
D21S1437	D18S386	SRY	D18S819
D13S634	D13S305	D13S800	
D18S535		D18S390	
		D13S628	

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

## ELUCIGENE QST<sup>+</sup>RPLUSv2 - GENEMAPPER

An example of a normal male QST<sup>+</sup>Rplusv2 profile showing the relative position of the markers detected.



**ELUCIGENE QST®R**

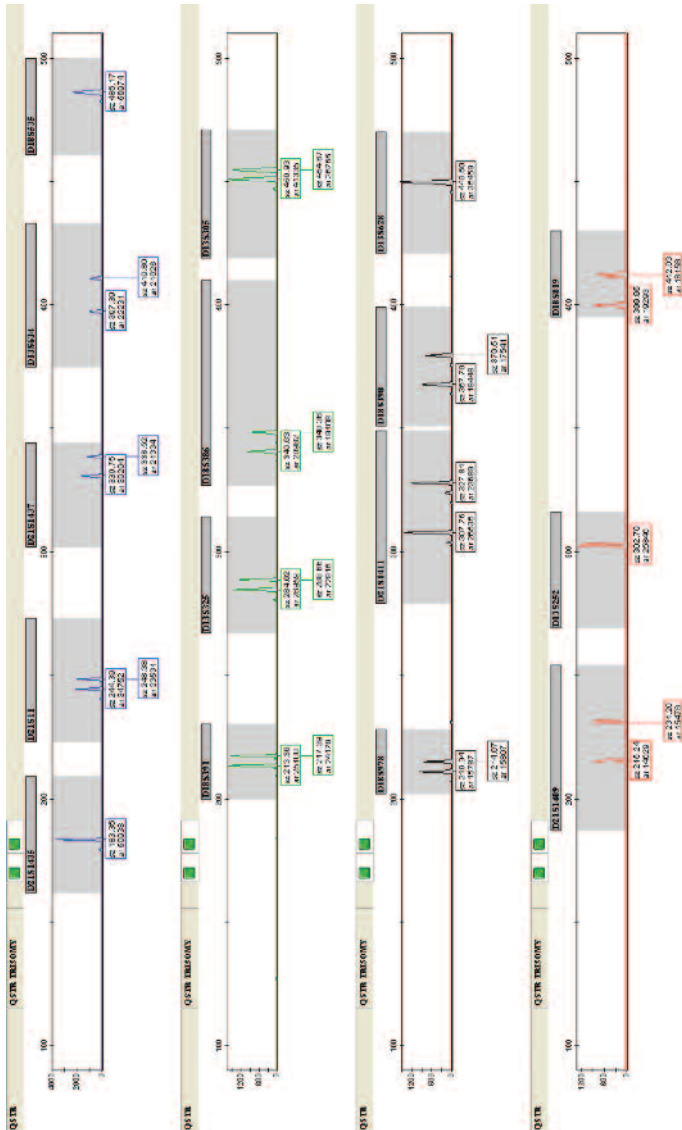
The markers are labelled as follows: -

6-FAM	VIC	NED	PET
D21S1435	D18S391	D18S978	D21S1409
D21S11	D13S325	D21S1411	D13S252
D21S1437	D18S386	D18S390	D18S819
D13S634	D13S305	D13S628	
D18S535			

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

## ELUCIGENE QST\*R - GENEMAPPER

An example of a normal QST\*R profile showing the relative position of the markers detected.



**ELUCIGENE QST\*R-XY2**

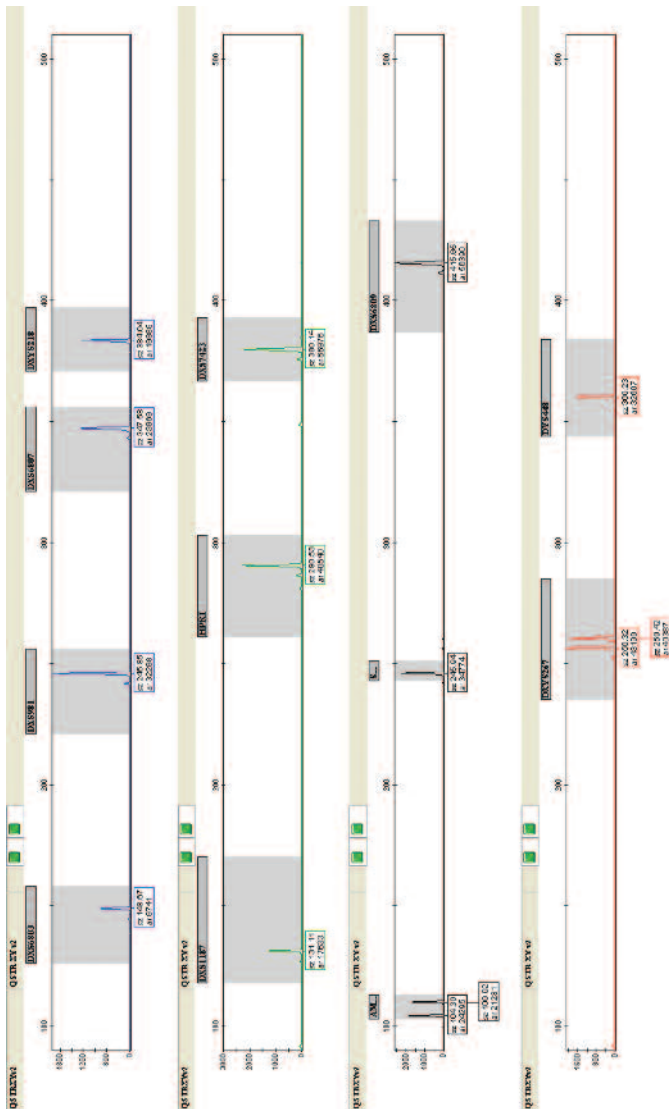
The markers are labeled as follows: -

6-FAM	VIC	NED	PET
DXS6803	DXS1187	AMEL	DXYS267
DXS981	XHPRT	SRY	DYS448
DXS6807	DXS7423	DXS6809	
DXYS218			

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

## ELUCIGENE QST<sup>®</sup>R-XYv2 - GENEMAPPER

An example of a normal male QST<sup>®</sup>R-XYv2 profile showing the relative position of the markers detected.



**ELUCIGENE QST\*R-21**

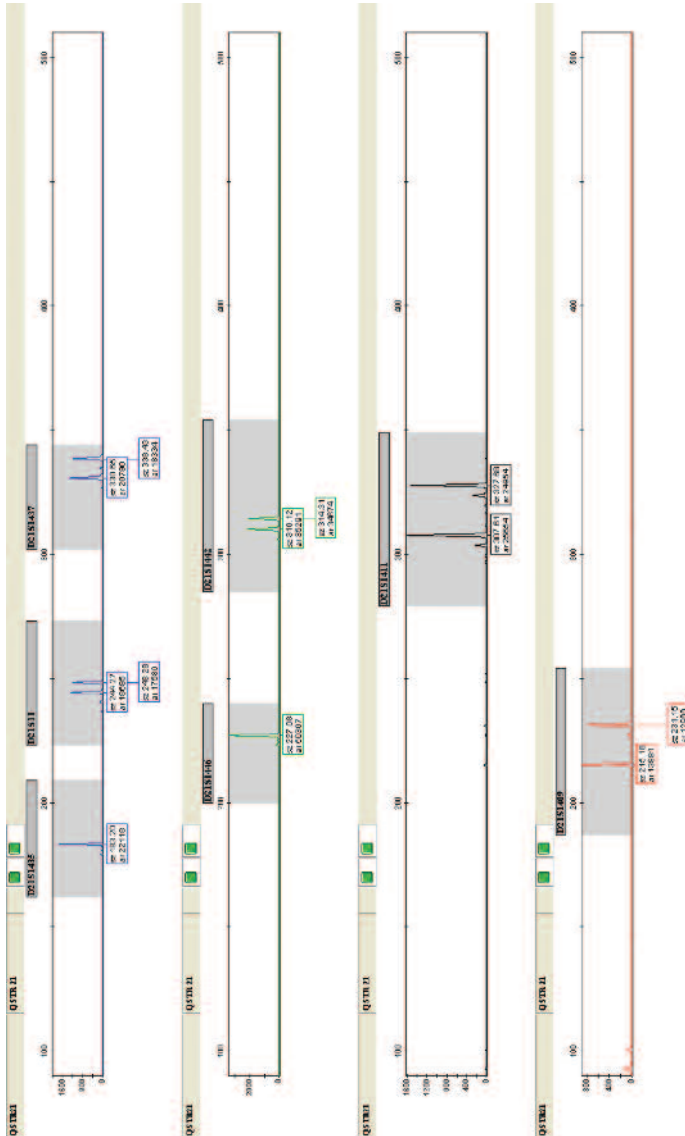
The markers are labelled as follows: -

6-FAM	VIC	NED	PET
D21S1435	D21S1446	D21S1411	D21S1409
D21S11	D21S1442		
D21S1437			

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

## ELUCIGENE QST®R-21 - GENEMAPPER

An example of a normal QST®R-21 profile showing the relative position of the markers detected.



**ELUCIGENE QST®R-18**

The markers are labelled as follows: -

6-FAM	VIC	NED	PET
D18S847	D18S391	D18S978	D18S977
D18S1002	D18S386	D18S390	D18S9819
D18S535			

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



**ELUCIGENE QST\*R-13**

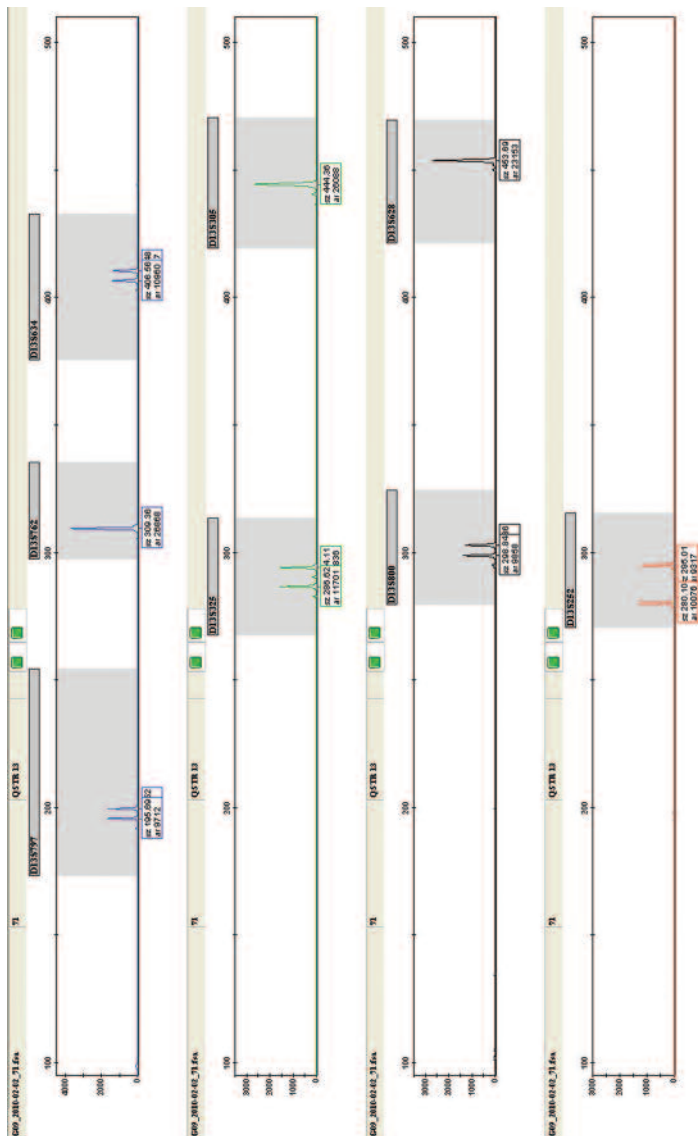
The markers are labelled as follows: -

6-FAM	VIC	NED	PET
D13S797	D13S325	D13S800	D13S252
D13S762	D13S305	D13S628	
D13S634			

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

## ELUCIGENE QST®R-13 - GENEMAPPER

An example of a normal QST®R-13 profile showing the relative position of the markers detected.



## APPENDIX 2

### TABLES OF MARKERS USED

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

\*Observed heterozygosities are based on number of alleles observed with Gen-Probe's validation panel. These figures may therefore differ from published data and may also vary according to the population being tested.

Table 1. Markers in Elucigene QST\**Rplus2*

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D13S252	13q12.2	0.74	274-311	red
D13S305	13q13.3	0.79	424-466	green
D13S634	13q21.33	0.84	380-428	blue
D13S800	13q22.1	0.72	284-320	yellow
D13S628	13q31.1	0.75	426-465	yellow
D18S819	18q11.2	0.73	400-425	red
D18S535	18q12.3	0.77	466-498	blue
D18S978	18q12.3	0.71	207-223	yellow
D18S386	18q22.1	0.92	332-405	green
D18S390	18q22.3	0.69	356-394	yellow
D21S11	21q21.1	0.82	228-279	blue
D21S1437	21q21.1	0.76	307-347	blue
D21S1409	21q21.2	0.74	191-239	red
D21S1442	21q21.3	0.85	332-389	red
D21S1435	21q21.3	0.74	167-204	blue
D21S1446	21q22.3	0.76	205-235	green
AMEL	Xp22.22/Yp11.2	n/a	104/110	yellow
TAF9L	Xq21.1/3p24.2	n/a	116/121	yellow
DXS6803	Xq21.31	0.86	131-153	blue
XHPRT	Xq26.2	0.72	266-298	green
DXS1187	Xq26.2	0.73	123-165	green
SRY	Yp11.31	n/a	248	yellow

Table 2. Markers in Elucigene QST®R

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D13S252	13q12.2	0.74	274-311	red
D13S305	13q13.3	0.79	424-466	green
D13S325	13q14.11	0.80	272-309	green
D13S634	13q21.33	0.84	380-428	blue
D13S628	13q31.1	0.75	426-465	yellow
D18S391	18p11.31	0.70	205-225	green
D18S819	18q11.2	0.73	400-425	red
D18S535	18q12.3	0.77	466-498	blue
D18S978	18q12.3	0.71	207-223	yellow
D18S386	18q22.1	0.92	332-405	green
D18S390	18q22.3	0.69	356-394	yellow
D21S11	21q21.1	0.82	228-279	blue
D21S1437	21q21.1	0.76	307-347	blue
D21S1409	21q21.2	0.74	191-239	red
D21S1435	21q21.3	0.74	167-204	blue
D21S1411	21q22.3	0.83	283-344	yellow

Table 3. Markers in Elucigene QST®R-XYv2

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
DXYS218	Xp22.32/Yp11.3	0.74	376-392	blue
AMEL	Xp22.22/Yp11.2	n/a	104-110	yellow
DXYS267	Xq21.31/Yp11.31	0.75	240-280	red
DXS6807	Xp22.3	0.66	326-351	blue
DXS981	Xq13.1	0.73	226-260	blue
DXS6803	Xq21.31	0.86	131-153	blue
DXS6809	Xq21.33	0.78	392-436	yellow
DXS1187	Xq26.2	0.73	123-165	green
XHPRT	Xq26.2	0.72	266-298	green
DXS7423	Xq28	0.67	360-388	green
SRY	Yp11.31	n/a	248	yellow
DYS448	Yq11.223	n/a	349-372	red

Table 4. Markers in Elucigene QST®R-21

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D21S11	21q21.1	0.82	228-279	blue
D21S1437	21q21.1	0.76	307-347	blue
D21S1409	21q21.2	0.74	191-239	red
D21S1442	21q21.3	0.85	290-349	green
D21S1435	21q21.3	0.74	167-204	blue
D21S1411	21q22.3	0.83	283-344	yellow
D21S1446	21q22.3	0.76	205-235	green

Table 5. Markers in Elucigene QST\*R-18

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D18S391	18p11.31	0.70	205-225	green
D18S1002	18q11.2	0.76	337-365	blue
D18S819	18q11.2	0.73	400-425	red
D18S847	18q12.1	0.71	204-232	blue
D18S535	18q12.3	0.77	466-498	blue
D18S978	18q12.3	0.71	207-223	yellow
D18S977	18q21.31	0.70	248-285	red
D18S386	18q22.1	0.92	332-405	green
D18S390	18q22.3	0.69	356-394	yellow

Table 6. Markers in Elucigene QST\*R-13

Marker	Location	Observed Heterozygosity*	Allele Size Range (bp)	Marker Dye Colour
D13S252	13q12.2	0.74	274-311	red
D13S305	13q13.3	0.79	424-466	green
D13S325	13q14.11	0.80	272-309	green
D13S634	13q21.33	0.84	380-428	blue
D13S800	13q22.1	0.72	284-320	yellow
D13S628	13q31.1	0.75	426-465	yellow
D13S762	13q31.3	0.75	302-331	blue
D13S797	13q33.2	0.77	178-250	blue

## LIMITATIONS OF 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<sup>®</sup>R 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<sup>®</sup>R 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

The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. Gen-Probe Life Sciences Ltd. cannot accept responsibility for any clinical decisions that are taken.

These Elucigene reagents are supplied for professional *in vitro* diagnostic testing. This product does not provide a licence to perform PCR under patents owned by any third party including F. Hoffman-La Roche (F. Hoffmann-La Roche Ltd, Diagnostics, CH-4070 Basel, Switzerland) and Roche Molecular Systems, Inc (Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501).

Further details of Elucigene QST<sup>®</sup>R products are available at: [www.gen-probe.com/products/](http://www.gen-probe.com/products/)

## ***TECHNICAL SUPPORT***

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