Public Health England

Protecting and improving the nation's health

Public Health England
National Infection Service

Genomic Services and Development Unit
User Manual

About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-leading science, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and a distinct delivery organisation with operational autonomy. We provide government, local government, the NHS, Parliament, industry and the public with evidence-based professional, scientific and delivery expertise and support.

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Contents

Introduction

The Genomic Services and Development Unit (GSDU) provides generic nucleic acid sequencing and fragment sizing services. The unit receives nucleic acids, plasmids and PCR products from customers across Public Health England (PHE) Laboratories and other PHE-affiliated laboratories. Samples are processed based on specific protocols and service type selected by customers from a list in both the User Manual and a Memorandum of Understanding (MOU) provided to each customer. Sequence and fragment sizing data are checked by GSDU against internal standards and quality scores before electronically notifying and reporting the results to allow customers to analyse their own data.

The unit has a strong research and development commitment to improve methods for rapid and high throughput genomics. Established approaches are published and shared with microbiology specialists through training, shared protocols and workshops.

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Amendment history

Version No.	Date	Sections affected	Pages affected
10	January 2016	Change of unit name and reformatted to improve layout and access of information	All
11	April 2017	NGS run-only MiSeq service, NGS TAT, NGS repeat policy, Quality assurance section, NGS concentrations, NGS sample numbers	14 to 18
12	November 2017	Content page, NGS recharging, Sanger service codes	3, 9, 16
13	April 2019	Unit Head changed, updated link to Sanger LIMS, NGS TAT increased to 80%	6, 14

Contact details

Name	Designation	Email	Telephone
Dr Derren Ready	Head of GSDU	Derren.Ready@phe.gov.uk	020 8327 6068
Dr Meeta Desai	Complaints Manager	Meeta.Desai@phe.gov.uk	020 8327 6469

Sanger Sequencing / Fragment Analysis enquiries	afgu@phe.gov.uk	020 8327 7772
NGS enquiries	NGS.service@phe.gov.uk	020 8327 7898
GSDU Sanger Sequencing and Fragment analysis sample submission	http://158.119.147.153/gsle/mainPage	
GSDU NGS submission (Please paste the following in browser)	http://158.119.147.110/gsle/mainPage	
GSDU (outside working hours)		07766 421765
PHE Colindale switchboard		020 8200 4400

Postal address: GSDU/Central Stores National Infection Service Public Health England 61 Colindale Avenue London NW9 5EQ	DX address: GSDU PHE Colindale DX 6530002 Colindale NW

GSDU Services

GSDU offers various levels of DNA sequencing of extracted DNA, PCR fragments and plasmids for laboratories of the National Infection Service within PHE and to PHE affiliated laboratories (by prior arrangement).

Sanger sequencing:

Of short DNA fragments on Applied Biosystems capillary platforms. Up to 850 bases read length is generated

Fragment analysis:

using Applied Biosystems capillary platforms to size fluorescently-labelled PCR products for variable number tandem repeat (VNTR) analysis, microsatellites, ribotyping and CRISPRS (Clustered Regularly Interspaced Short Palindromic Repeat).

Next generation sequencing:

using Illumina sequencing technology to sequence genomic DNA or PCR amplicons. At least 150 Megabases of high quality trimmed sequence is provided (20Mb for MiSeq runs), as measured by internal positive control.

Technical details of services provided can be obtained from the relevant customer MOU. These can be obtained by contacting a member of GSDU and should be signed by an appropriate member of staff.

Hours of Service

For all services, normal service will be provided between 09.00 to 17.30 hours, Monday to Friday (excluding bank holidays). An out of hours emergency support will be available for Sanger sequencing and fragment analysis.

Emergency out of hours service

GSDU will provide emergency out of hours Sanger sequencing and fragment analysis for samples submitted by the customer. A request for this service should be placed by communicating with the on-call GSDU staff. Contact is by telephone on 07766 421765 at least 2 hours prior to sample submission. An early indication of when the samples are expected for processing will allow better co-ordination and a faster response by GSDU.

Order Submission

For Sanger Sequencing and Fragment Analysis:

Customers must create a GeneSifter account via the PHE Genomic services link:

http://158.119.147.153/gsle/mainPage

Sequencing orders should include the cost centre or project code to which the work will be charged. Please Note: Do not use these characters in sample or primer names in the order form – for instance, spaces \ /: *? “ < > |)

For Next Generation Sequencing:

Samples are received for processing Monday to Friday but orders submitted after 9.30 am will be processed the following day or on Monday for Friday submissions.
Customers must create a Genesifter account via the link:
http://158.119.147.110/gsle/mainPage

Delivery of Samples

For customers within Colindale site: Deliver the samples to level 2 Zone D and place inside the refrigerator marked ‘GSDU Customer fridge’. The GSDU customer sheet (BW0158) kept next to the fridge must be completed to ensure samples are processed with the priority of when they were submitted.

For PHE Customers external to the Colindale site, send the samples via Hays DX to: PHE Colindale, (GSDU), Bacteriology, DX 6530002, Colindale NW or via Royal Mail to GSDU, Central Stores, National Infection Service, PHE Colindale, 61 Colindale Avenue, London, NW9 5EQ.

Sample Rejection Criteria for NGS service

Samples will be rejected if:

- not submitted in the designated 96-well plate
- its volume is different from that specified for each service
- its concentration is not within the specified range
- the online submission form has not been completed
- plate layout does not match online submission form
- a unique purchase order number has not been provided in the order submission (non-PHE customer orders only)

Sanger Sequencing Service

GSDU provides single read sequencing of PCR products or plasmid DNA based on dideoxy Sanger sequencing using ABI Genetic Analyser 3730XL capillary platforms at PHE National Infection Service, Colindale.

Table 1: Sanger DNA Sequencing Services

Service Code	Description of Service	Turnaround time*	Read length
S1	Reaction-ready samples (DNA & primer)	36 hours	Long (850bp)
S4A	PCR products for clean-up and sequencing	48 hours	Long (850bp)
S5	PCR product clean-up	24 hours	
S5B	Urgent PCR product clean-up	3 hours	
S6A	Urgent Sequencing	8 hours	Long or short (850bp or 550bp)
S6B	Urgent PCR product clean-up and sequencing	10 hours	Long or short (850bp or 550bp)
S7A*	Emergency out-of-hours sequencing	8 hours	Long or short (850bp or 550bp)
S7B*	Emergency out-of-hours PCR product clean-up and sequencing	10 hours	Long or short (850bp or 550bp)
S8A	Reaction-ready samples (DNA & primer)	36 hours	Short (550bp)
S8C	PCR products for clean-up and sequencing	48 hours	Short (550bp)
S9	Reaction-ready samples (DNA & primer)	10 working days	Long or short (850bp or 550bp)
R0-1	Run only service	36 hours	Long or short
CE-S1*	Chain of evidence reaction ready long-read sequencing	36 hours	Long (850bp)
CE-S8A*	Chain of evidence reaction ready short-read sequencing	36 hours	Short (550bp)

*Turnaround times will be met >90% of the time

*All out of hours emergency requests/chain of evidence requests will be charged at a minimum of 10 samples.

Please Note:

Orders submitted after 4.00pm for services S1, S5 & S8A will be considered as following day orders and the turnaround time will start from 9.00am on the following day

Orders submitted after 3.00pm for PCR purification and Sequencing services – such as S4A & S8C – will be considered as following day orders and the turnaround time will start from 9.00am on the following day

Turnaround time maybe affected due to a surge in urgent/high priority samples received for sequencing from an outbreak or, due to an unavoidable interruption, to GSDU facilities. Specified turnaround times are for PHE customers only. Customers will be informed of major delays to turnaround time

Sanger guidelines

1. Samples must be provided in 0.2ml MicroAmp Optical 96-well semi-skirted reaction plates.
2. Wells G12 and H12 should be left empty for GSDU controls, unless otherwise specified.
3. For sequencing only services DNA must be provided using a method compatible with dideoxy sequencing.
4. For sequencing, only services a volume of 6µl must be provided which comprises cleaned-up DNA (see table 2) and 5 picomole of primer (reactions should be prepared in molecular biology grade water and not EDTA).
5. For PCR clean-up services a minimum of 10µl and maximum of 50µl unpurified DNA should be supplied and all wells should contain a uniform volume.
6. Where primers are to be added by GSDU then a minimum of 200µl of each primer at 5 pmol/µl should be provided (maximum 2 primers per plate).
7. For run-only service 25µl of purified post-cycled DNA products resuspended in nuclease-free water should be provided.
8. For long-read services GSDU aim to obtain a minimum read length of 850bp (Phred20 quality) from an internal control.
9. For short-read services GSDU aim to obtain a minimum read length of 550bp (Phred20 quality) from an internal control.
10. For chain of evidence services (medicolegal) a registered clinical or biomedical scientist must be provided to observe GSDU processes and countersign worksheets.

Table 2: Recommended concentrations of DNA for Sanger Sequencing

Template	Quantity of DNA
PCR product 100-200 bp	1-3ng
PCR product 200-500 bp	3-10ng
PCR product 500-1000 bp	5-20ng
PCR product 1000-2000 bp	10-40 ng
PCR product >2000 bp	40-100 ng
Single stranded DNA	50-100 ng
Double stranded DNA	200-500 ng
Plasmid <10Kb	100-300 ng
Plasmid >10 Kb	100-300 ng + 20 ng for each additional 1Kb
Large DNA (BACs, PACs, YACs, phage, cosmids and fosmids)	0.5-1.0 µg

DNA-based fragment sizing and analysis service

GSDU provides a DNA-based fragment analysis services using the ABI Genetic Analyser capillary platform (3730xl). The ABI 3730xl instruments are spectrally calibrated to run filter set G5 and detect five fluorescent dyes (DS-33 set). The five dyes are 6-FAM (blue), VIC (green), NED (yellow), PET (red) and LIZ (orange: size standard).

Table 3: Fragment Analysis Services

Service Code	Description of Service	Turnaround time*	Data Provided
F4	Ready to run polymorphic size based DNA fragment separation (for example VNTR, MLVA, ribotyping, CRISPRs)	36 hours	*.fsa files for customer analysis
F5	PCR reactions for electrophoresis separation requiring pre-processing	36 hours	*.fsa files for customer analysis
F6A	Urgent F4 service	6 hours	*.fsa files for customer analysis
F6B	Urgent F5 service	7 hours	*.fsa files for customer analysis
F7A†	Out-of hours F4 & F5 service	7 hours	*.fsa files for customer analysis

***Turnaround times will be met >90% of the time
† All out-of hours emergency requests for F4 & F5 will be charged at a minimum of 20 urgent samples (such as 20 X Urgent Sample Cost).

Please Note:

1. Orders submitted after 4.30pm for services F4 & F5 will be considered as following day orders and the turnaround time will start from 9.00am on the following day
2. Turnaround time maybe affected due to a surge in urgent/high priority samples received for sequencing from an outbreak or, due to an unavoidable interruption to GSDU facility. Customers will be informed off major delays to turnaround time
3. Customers must ensure that the sample position in the 96-well plate exactly matches the layout in the order submission form. Any mismatches will create incorrect data files, loss of data and processing delay by GSDU

Fragment analysis submission guidelines

Samples should be submitted in 0.2mL MicroAmp optical 96-well semi-skirted reaction plates.

A maximum of 94 samples can be submitted and wells G12 and H12 should be left empty for GSDU use.

For ready-to-run samples a total volume of 11.5µl comprising 1.0µl diluted FAFLP PCR reaction, 0.5µl LIZ-labelled size standard and 10µl of HiDi formamide should be submitted.

The LIZ-labelled size standard should be specified on the request form.

For PCR reactions requiring pre-processing then a minimum volume of 10µl PCR reaction should be provided and the dilution required for electrophoresis should be specified.

Next Generation Sequencing service

GSDU offers various levels of Next Generation Sequencing (NGS) of genomic DNA (usually bacterial DNA extracts) and PCR amplicons (usually generated from viral samples) for laboratories of the National Infection Service, Colindale, other centres within Public Health England and other PHE affiliated laboratories. NGS is performed using Illumina technology and aims to provide a minimum of 150 Mb (HiSeq) or 20 Mb (MiSeq) of high quality sequence (Q30 and above; Appendix 1), >95% of the time dependent on the quality of the DNA template supplied.

Quality control of the run and sequencing data will be by processing sequencing control DNA on each plate that reaches the yield target of 150 Megabases (HiSeq) and 20 Megabases (MiSeq) of data.

Table 4: NGS Services

Service Code	Description of Service	Turnaround time*	DNA Concentration	Other Information
N1	Standard genomic NGS	5 working days	6-100ng/µl	Usually bacterial
N2	Standard amplicon-based NGS	5 working days	2-10ng/µl	Usually viral
Other (T1, T2, T3, T4)	Research	On request	N/a	Individually agreed project arrangements
T5	Run only (Miseq) – customer supplied cartridge	5 working days from sample receipt	N/a	
T6	Run only (MiSeq)-GSDU supplied cartridge	5 working days from sample receipt		

*Turnaround time will be met >80% of the time

Please Note:

1. Orders submitted after 9.30am on way weekday will be processed on the following day or on Monday for Friday submissions.
2. Turnaround time (calculated in working days from LIMS receipt by GSDU to data delivery to customer) may be affected due to:

a. too few samples being submitted (fewer than 70 submissions in total, not per individual submission) because of cost effectiveness.
b. a surge in urgent/high priority samples received from an outbreak.
c. an unavoidable interruption to the GSDU facility.

Customers will be informed of major delays to turnaround time.

NGS Submission Guidelines

Samples must be provided in a clear 4titude PCR full skirted plate with unique GSDU barcode on the left plate edge A1-H1 side. Catalogue number SP-0238 (4titude, UK).

Up to 94 samples can be submitted per plate and wells G12 and H12 should be left empty for GSDU controls.

Concentration (See table 4) should be confirmed using the Quant-iT dsDNA BR Assay Kit (broad range) Cat No. Q-33130 (Life Technologies, UK), Quant-iT DNA High Sensitivity Assay Kit (Q33120) or a fluorescence based quantification method.

Provide a minimum of 60µl high quality DNA.

Complete online sample sheet and include a unique SampleID. If a MOLIS number is being used as the SampleID then also include it in the MOLIS ID column.

Quality control from GSDU will be provided using a negative control (molecular biology grade water) and positive control E. coli K12 for each 96-well plate processed. A minimum of 150 Megabases of quality-trimmed data >Q30 (HiSeq) and 20 Megabases (MiSeq) for the positive control will be obtained which is equivalent to 30 fold coverage for a 5Mbp genome on a HiSeq run.

For run-only services:

Contact NGS.Service@phe.gov.uk for availability of MiSeq instrument and agreed arrangements for sample handover. Please do not prepare the library without having confirmation of availability from a member of the team.

A clean, pooled, amplified indexed library should be supplied in a LoBind Eppendorf tube, labelled with a GSDU barcode available on request from GSDU.

Customers are advised to include a run control such as a phiX library spike. Data quality or quantity cannot be guaranteed without this.

Concentration should be confirmed using the KAPA SYBR FAST Universal qPCR kit

A minimum volume of 600µl should be provided.

Online sample sheet should be completed.

Policy on repeat reactions

If the run and control metrics pass the standard quality checks and the customer samples fail yield and quality metrics they will be repeated on request and the repeat service not charged for if they, and the run controls, subsequently pass yield and quality metrics. If they fail the run sample will be charged for twice.

Specialist and Technical Support

GSDU will provide support in designing upstream workflow to help ensure compatibility with sequencing reagents.

GSDU will provide support in sequencing/fragment analysis related troubleshooting and help with interpretation of results.

GSDU will continue to maintain its CPA or UKAS ISO15189 accreditation and will run an auditable quality system. The Unit will run an annual workshop to provide feedback, update customers on the range of services provided and give three months' notice before changes are made to the price of services where possible.

Financial Arrangements

For PHE partners please contact GSDU to sign up to a MOU, which will include current pricing information. Then samples sent in the prescribed GSDU format will have cost recovered via internal recharging to the partner's project or cost centre on a monthly basis. There will be an annual price review and this will be discussed with the partner at the same time as a review of the MOU. All repeats will be done free of charge if the error is deemed to be due to GSDU.

Please note that for non-PHE partners a valid Purchase Order Number must accompany each order submission. For any other queries from non-PHE partners, please contact GSDU.

Quality Assurance in GSDU

An overview of quality standards at microbiology standards, Colindale, can be found at:

https://www.gov.uk/government/publications/quality-standards-microbiology-services-colindale

Internal quality assurance (IQA) is carried out by replicate testing of 'blinded' Salmonella samples (DNA) submitted by the Gastrointestinal Bacteria Reference Unit. Following processing the results are unblinded and are assessed against the original result. Any discrepancies are investigated as to their root cause before any remedial action is implemented. Results of IQA and EQA (when available) are discussed at Unit and Management Review meetings as appropriate.

Complaints procedure

If there is a problem, or you are not satisfied with the GSDU service you have received:

In the first instance contact the Unit Head or Unit Complaints Manager. Contact details are given on page 5. We endeavour to be responsive to the changing needs of all users of our services. We welcome comments on how we can improve the provision of these services. Please contact the Unit if you have any queries.

Appendix: Definition of Q30 scores

Quality scores measure the probability that a base is called incorrectly. With SBS technology, each base in a read is assigned a quality score by a phred-like algorithm1,2, similar to that originally developed for Sanger sequencing experiments. The quality score of a given base, Q, is defined by the equation Q = -10log10(e) where e is the estimated probability of the base call being wrong. Thus, a higher quality score indicates a smaller probability of error. In the table below, a quality score of 20 represents an error rate of 1 in 100, with a corresponding call accuracy of 99%.

Table 5. The relationship between quality score and base call accuracy

Quality Score	Probability of Incorrect Base Call	Inferred Base Call Accuracy
10 (Q10)	1 in 10	90%
20 (Q20)	1 in 100	99%
30 (Q30)	1 in 1000	99.9%

References

1. Ewing B, Hillier L, Wendl MC, Green P. (1998): Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8(3):175-185
2. Ewing B, Green P. (1998): Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8(3):186-194.