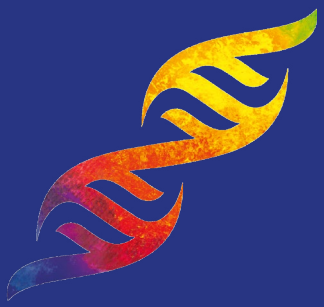
GenoScreen



USER MANUAL

Sanger Sequencing

One-Shot / One-Shot Plus / Optimized

Mix & Run / Run Only

Crédit Impôt Recherche CIR

Entreprise membre de bpifrance EXCELLENCE 2019

Agrément MOT ansm

ISO 9001 ISO 13485 BUREAU VERITAS Certification

Illumina Propel Certified™ 2019-20 MiSeq®

SUMMARY

A – GENERAL INFORMATION

Technical specifications for
One-Shot / One-Shot Plus / Optimized offers

- One-Shot offer
  - Sample reception
  - Purification of PCR products (free option)
  - Fluorimetric assay
  - Sequencing reaction
  - Purification on G50 resin
  - Migration on ABI 3730XL capillary sequencer - Reading up to 1200 bp
  - Validation of the sequencing process by analysis of our internal controls
- One-Shot Plus offer
  - Automated enhancement and correction of raw sequences (IUPAC codes)
- Optimized offer
  - Manual analysis and correction of each sequence profile by an operator (IUPAC codes)
  - QC report with reading comments for each sequence
  - Free reprocessing of sequences with optimized protocol

Technical specifications for the Mix & Run offer

- Samples reception with your own DNA and primer mix
- Sequencing reaction
- Purification on G50 resin
- Migration on ABI 3730XL capillary sequencer - Reading up to 1200 bp
- Validation of the sequencing process by analysis of our internal controls

Technical specifications for the Run Only offer

- Samples reception with sequencing reactions, already done and purified by you
- Migration on ABI 3730XL capillary sequencer
- Validation of the sequencing process by analysis of our internal controls

Deliverables

- Chromatograms and raw or corrected* sequences in .ab1/.seq format
  *One-Shot Plus and Optimized offers
- QC report with reading comments for each sequence (Optimized offer)

Results are sent by email and/or on a secure server.

Indicative turn-around time

- Results within 1 to 2 working days, upon computerized form and samples reception
- 24h for Mix & Run and Run Only
- 72h for 8 or more sequencing plates to be processed simultaneously*.

* *for 8 or more plates, results can be sent gradually.*

Included services

- Free shipping of samples under certain conditions (see part C)
- Confidentiality of all received information and obtained results
- Direct access to our technical team by phone, from Monday to Friday, 9am to 12pm and 2pm to 5.30pm
- Use of universal primers (see list in Appendix 3)
- Specific protocols for AT/GC-rich sequences on request
- Conservation of your samples and/or primers for 1 month (or longer on request)

Furthermore, we offer optional services to complete the sequencing of your samples:

- Purification of PCR products by gel cutting
- Primer synthesis
- Verification of PCR products on QIAxcel before sequencing

If you have any technical questions before sending your samples, please contact our team at seq1@genoscreen.fr

If you have any questions about your results, reprocessing requests or logistics (Genobox, consumables/Chronopost collections), please contact our team at seq2@genoscreen.fr

	One-Shot	One-Shot Plus	Optimized
Results within 24 to 48 hours after receipt	✔	✔	✔
Technical support by email and/or phone	✔	✔	✔
Accepted sample formats : - Tubes - Plates (complete or not)	✔	✔	✔
Sample purification free of charge (on request)	✔	✔	✔
Reading up to 1200 pb	✔	✔	✔
Sequence review and corrections with IUPAC annotations		Semi-automated	Manual by operator
Deliverables	.seq/.ab1	.seq/.ab1	.seq/.ab1 QC (Quality Control) report with comments for for each sequence
Reprocessing with optimized protocol (on request)			✔
Monthly activity report (including remaining credits)	✔	✔	✔

B – ADMINISTRATIVE PROCEDURES

Our sales team is available to help you with any requests for quotes or annualized pricing conditions.

Contact our sales department by e-mail: commercial.dept@genoscreen.fr

Before sending any sample, please ensure that you send us an order form referring to the current quotation or price conditions.

The service will be initiated once samples and the computerized sequencing form have been received.

The order form must include the following information:

- Up-to-date quotation number or pricing conditions referring to the service,
- Details of the organization to which the invoice is addressed (address, telephone and email numbers, contact, VAT number),
- Details of the laboratory delivering the results (address, telephone number and email address, contact details).

Your services will be invoiced according to the following conditions:

- One-off invoice: if the project takes less than a month to be completed (as specified in the quotation), GenoScreen will send the customer a one-off invoice for the work carried out and delivered.
- Staggered invoicing: if the project lasts more than one month, particularly under annualized pricing conditions, GenoScreen can send the customer staggered invoices, based on the services actually carried out during the period in question.

Our invoices are to be paid within 30 days net.

In compliance with our general terms and conditions of sale, any order for an amount below €250 will be increased by €30 excl. VAT for administration fees, if not stated otherwise in the quotation.

C – SAMPLES DISPATCH

You can send us your samples by Chronopost, tracked letter, simple letter, TNT or other carriers. We also accept drop-off at our facilities, in particular for users of the Pasteur Institute Campus in Lille.

In all cases, please ensure that you enclose the completed and printed Sanger form with your parcel. Contacts listed on the form will receive an email acknowledging receipt.

1. Dispatch solutions

GenoScreen offers free sample dispatch for orders placed under these conditions:

- A minimum of 5 to 19 sequences per shipment by tracked letter for customers based in Metropolitan France*.
- A minimum of 20 sequences per Chronopost shipment for customers based in Metropolitan France**.
- A minimum of 48 sequences per shipment for customers from other countries*** and 95 sequences from DOM-TOM***.

** If the parcel does not contain the minimum number of sequences indicated, the cost of transport will be invoiced to the customer (for information only, and except for special conditions, a shipment from Metropolitan France will be invoiced at €5.00 excl. VAT).*

*** If the parcel does not contain the minimum number of sequences indicated in the quotation, the cost of transport will be invoiced to the customer (for information only, and unless special conditions apply, a shipment from Metropolitan France will be invoiced at €17.00 excl. VAT).*

**** Please contact our sales department to find out about minimum shipping conditions from your country or French overseas departments and territories (as a guide, if free shipping quotas are not reached, shipping from another country will be invoiced at €65.00 plus VAT, and shipping from French overseas departments and territories will be invoiced at €95.00 to €115.00 plus VAT).*

From France

On request to the Sanger team at the following e-mail address seq2@genoscreen.fr, GenoScreen will provide you with pre-filled waybills, envelopes and Genoboxes (for tubes and/or plates) to send your samples.

The package can be sent by post for delivery within 24 hours.

If you don't have a courier team, GenoScreen can arrange for Chronopost collection on request by email (seq2@genoscreen.fr) no later than the day before the desired pick-up date, specifying the exact pick-up address, the desired date and time slot, and a telephone number.

From another country or from French overseas departments and territories

GenoScreen can arrange a TNT or Chronopost International pickup upon request by email to seq2@genoscreen.fr specifying the exact address for collection of the samples, a telephone number, the dimensions and weight of the parcel, and the desired date and time slot.

We will receive your shipment within 24/48 hours of collection.

2. Dispatch by customer

Your samples can also be sent by post or by your regular carrier.

Please send your parcels to the following address:

GenoScreen – Plateforme 3730
1 rue du professeur Calmette
59000 LILLE - France

3. Drop-off samples at our facilities

You can drop off your samples at our facilities from Monday to Friday, 9am to 12pm and 2pm to 5:30pm.

We are located on the 1st floor of the Emile Roux building on the Pasteur Institute Campus in Lille.

In order to maintain a high level of service and ensure short turnaround times, please hand in your samples before 12:00pm.

Samples dropped-off after 12:00pm will be considered as having been received the following day.

D – SAMPLES PACKAGING

1. Packaging options

- Samples in labeled tubes and primer(s) supplied in tube(s)
- Samples in labeled strips and primer(s) supplied in tube(s)
- Samples in «incompleted» plates:
  - 24 to 93 samples per plate to be sequenced with the same primer supplied in a tube.
  - 24 to 94 samples to be sequenced with a mix of 2 to 4 primers per plate (primers to be supplied in tubes) or more than 4 primers per plate (supply a primer mirror plate).

For the primer mirror plate, supply 5µL of primer per well in a plate following the plate map indicated on the form.

- Full plate samples (94 samples) to be sequenced with the same primer
  - 94 samples per plate with primer supplied in tube

NB: A plate of less than 24 samples will be priced as samples in tubes.

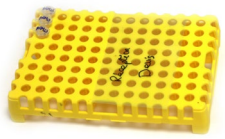
Samples in strips are charged as samples in plates from 24 or more samples.

A full plate is a plate of 94 samples sequenced with a single primer. It can be sequenced with several different primers if needed.

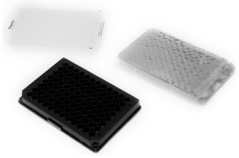
Technical recommendations for sample packaging

- The references of the consumables we recommend are given in Appendix 1.
- When sending samples in 96-well plates, please ensure that you leave 2 empty wells for our internal controls. Without these controls, we cannot guarantee the reliability of your results.
- To avoid any leakage during transport, we recommend the use of thick heat-sealable films or caps adapted to your plates.
- To avoid disrupting the sequencing reaction, please dilute your samples and primers preferably in water.
- In order to avoid any erasure during transport or handling of your samples, please mark your tubes and plates with an indelible marker.
- Samples can be sent at room temperature.
- For shipments of several plates, please ensure that the plates are protected from each other to avoid any damage during transport (crushing, punctured film, etc.). We also provide dedicated boxes for transporting plates.
- For Run Only, samples must be supplied in a half-skirted plate with a the upper right edge beveled, suitable for migration cassette on capillary sequencer.

Accepted packaging:

	Labeled plates heat-sealed or sealed with cap strips (see **Appendix 1**)
	Labeled 1.5 or 2.0mL Eppendorf-type tubes or screw caps
	Microtubes in labeled strips fixed on an annotated support
	Labeled tubes arranged in order on a rack or in a box (Genobox type)

Refused packaging:

	Round/flat-bottom plates, with adhesive film, with aluminium film, or non-heat-sealed
	Individual microtubes or unlabeled strips
	Parafilm, tubes smaller than 1.5mL or culture tubes
Unlabeled samples in tubes or plates	

2. Quantities to provide

The indicated quantities are optimum required to carry out a sequencing reaction. If the concentrations are lower, we cannot guarantee that usable sequences will be obtained.

For any sample to be sequenced with several primers, you can multiply the quantity indicated by the number of reactions to be carried out, taking into account any potential reprocessing.

It is therefore not necessary to supply an aliquot of sample for each reaction. The same sample can be used for all reactions if the quantity/concentration provided is sufficient.

3. Primers preparation

A list of our universal primers is available at Appendix 2.

Under conditions, we can also order the primer pairs of your choice.

If you wish to process sequencing reactions with your own primers, we recommend to respect the following guidelines:

- Volume and concentration: 10µL of primer minimum, between 2.5 and 5µM
- Absence of secondary hybridization sites,
- No mismatches,
- Length of 18 to 25 bases,
- GC percentage between 40 and 60%,
- Hybridization temperature between 50 and 60°C,
- Absence of secondary structures > 3bp,
- Avoid designing primers close to repeated sequences (poly-N or microsatellite).

NB: Please ensure that you supply a minimum of 1µL of primer per sequencing reaction, allowing for additionnal volume for pipetting (dead volume) and any potential reprocessing.

	PLASMID	PCR PRODUCT	COSMID/BAC
Recommended quantities per reaction	500ng	PCR < 500pb : 50ng 500pb < PCR < 1000pb : 100ng PCR > 1000pb : 200ng	2µg
Concentration	50 to 200ng/µL	5ng/µL for 100pb	200 to 300ng/µL
Minimum volume to provide	10µL	10µL	10µL

**We recommend a fluorimetric assay for estimating your DNA concentration, as optical method tends to often overestimate values.*

For PCR products longer than 150bp, optional purification step is performed using a membrane and eluted in 30µL. If you submit a volume below 30µL, please take into account the resulting dilution of your samples.

E – DNA SEQUENCING REQUEST FORM

1. Computerized sequencing form preparation

As the Sanger service is partly automated, please pay particular attention to fill-in the computerized form (Excel file).

Two forms are available, with or without purification of your PCR products (free option).

Please observe the following rules:

- Complete all the columns, each one being important for managing the sequencing request.
- Match each line of the table to a single sequencing reaction
- Some cells are limited in the number of characters (10 or 15 maximum)
- Special characters are not allowed

NB : The size of the PCR product is essential for processing your samples. For plasmid samples, please indicate their size in the comment column of the form without purification.

For Mix & Run and Run Only offers, please use the form without purification and specify the type of offer in the comment column.

The comment column of the form should be filled in with any information useful to our team: gel electrophoresis or migration on QIAxcel before sequencing, Mutation detection (for One-Shot Plus and Optimized offers), etc.

Header filling

Notice : This form is only valid for samples to be purified by Genoscreen before sequencing.

ADDRESS FORM		INVOICE ADDRESS	
Laboratory :		Laboratory / Unit :	
Address :		Address :	
Post code :		Post code :	
Town :		Town :	
Country :		Country :	
Phone number :			
Fax number :		Quote number :	
Name of contact :		Purchase order number :	
Team manager			

Email(s) for results (.seq et .ab1) : _____

Date of sending : _____

Please complete all administrative and billing information. The up-to-date quotation or pricing conditions number is required to process your samples.

The contacts whose emails are indicated on the form will be notified of the good reception of samples and will receive the results.

Please provide the shipment date for your samples in the designated section.

For samples supplied in tubes

Example form for sample A to be sequenced with two specific primers (1 and 2):

QUANTITY

Quantity for ONE sequencing reaction : PCR product : 50 to 200 ng - Plasmid : 500 ng - Cosmid or BAC : 2 µg - Primer : 2,5 to 5 µM

Please provide a suitable amount to the number of desired sequences as well as any reprocessings

PACKAGING

(•) Tube(s) ( ) Plate

	User name	SAMPLES						PRIMERS				COMMENTS
		Tube	Sample name	Sample type	Product size	Conc. (ng/µL)	Vol. (µL)	Universal primer	Specific primer	Conc. (µM)	Vol. (µL)	
1	USER NAME	-	A	PCR	500 to 600		20		1	5	10	
2	USER NAME	-	A	PCR	500 to 600		20		2	5	10	
3		-										
4		-										
5		-										
6		-										
7		-										
8		-										
9		-										
10		-										
11		-										

For samples supplied in plates

The form is designed to be filled in column by column. For easily completion, we recommend this layout.

In the case of a plate with «n» number of primers to be used for the same sample, «n» forms should be completed (one form per primer), keeping the same plate name, in order to keep the right reference. For example, for a plate to be processed in Forward and Reverse, 2 forms must be completed with the same plate name. Only the primer column changes.

Below: Example form for a plate of 8 samples to be sequenced with primer 3

QUANTITY

Quantity for ONE sequencing reaction : PCR product : 50 to 200 ng - Plasmid : 500 ng - Cosmid or BAC : 2 µg - Primer : 2,5 to 5 µM

Please provide a suitable amount to the number of desired sequences as well as any reprocessings

PACKAGING

( ) Tube(s) (•) Plate *Name of plate:*

	User name	SAMPLES						PRIMERS				COMMENTS
		Well	Sample name	Sample type	Product size	Conc. (ng/µL)	Vol. (µL)	Universal primer	Specific primer	Conc. (µM)	Vol. (µL)	
1	USER NAME	A1	1	PCR	500 to 600		20		3	5	10	
2	USER NAME	B1	2	PCR	500 to 600		20		3	5	10	
3	USER NAME	C1	3	PCR	500 to 600		20		3	5	10	
4	USER NAME	D1	4	PCR	500 to 600		20		3	5	10	
5	USER NAME	E1	5	PCR	500 to 600		20		3	5	10	
6	USER NAME	F1	6	PCR	500 to 600		20		3	5	10	
7	USER NAME	G1	7	PCR	500 to 600		20		3	5	10	
8	USER NAME	H1	8	PCR	500 to 600		20		3	5	10	
9		A2										
10		B2										

2. Form submission

Please return your completed forms on the day you send your samples to the following address seq1@genoscreen.fr, and enclose a printed copy with your parcel.

Sequencing services will only be provided on receipt of a duly completed sequencing request form.

GenoScreen reserves the right to put on hold any service for which the form does not comply with the points above.

APPENDIX 1: Consumables references recommended

GenoScreen recommends the following references:

- 96-well plates: 0.2mL Ultra Rigid 96-well PCR plate - Sorenson Bioscience, Imc - Ref. 26190
- Half-skirted 96-well plates (Run Only offer) : Half-skirted 96-well PCR plate - Mμlti - Ref. 035800/035900
- Heat-sealable adhesive films : Cseal PCR adhesive film - 4titude - Ref. 016028F
- Heat-sealable films: Peel Seal - 4titude - Ref. 016279F
- Cap strips : Domed PCR capsules, strips of 8 - Thermo Scientific - Ref. AB-0265
- Tubes 1.5mL : Multi Sorenson Bioscience, lmc - Ref. 017040A

APPENDIX 2: List of universal primers

3'AOX1	5' - GCAAATGGCATTCTGACATCC - 3'
40M13	5' - CGCCAGGGTTTTCCCAGTCACGAC - 3'
48REV	5' - AGCGGATAACAATTTCACACAGGA - 3'
5'AOX1	5' - GACTGGTTCCAATTGACAAGC - 3'
Alpha_factor	5' - TACTATTGCCAGCATTGCTGC - 3'
BGHREV	5' - TAGAAGGCACAGTCGAGG - 3'
CMVPRIMER	5' - CGCAAATGGGCGGTAGGCGTG - 3'
EBV-Rev	5' - GTGGTTTGTCCAAACTCATC - 3'
EGFP-C	5' - CATGGTCCTGCTGGAGTTCGTG - 3'
EGFP-N	5' - CGTCGCCGTCCAGCTCGACCAG - 3'
EYFP-617	5' - GCTCAAGCTTCGAATTCTGC - 3'
GFPForward	5' - CACAATCTGCCCTTTCGAAA - 3'
GLP2GEX	5' - CTTTATGTTTTTGGCGTCTTCCA - 3'
ITS1	5' - TCCGTAGGTGAACCTGCGG - 3'
ITS1F	5' - CTTGGTCATTTAGAGGAAGTAA - 3'
ITS4	5' - TCCTCCGCTTATTGATATGC - 3'
ITSL	5' - TCGTAACAAGGTTTCCGTAGGTG - 3'
KS	5' - CCTCGAGGTCGACGGTA - 3'
Lacop	5' - GGGAATTGTGAGCGGATAAC - 3'
lacZ-F	5' - GGGTGGGATCATCTCCAGTA - 3'
lacZ-R	5' - TGGTTTGTCCAAACTCATCAA - 3'
M13-20	5' - GTAAAACGACGGCCAG - 3'
M13-21	5' - TGTAAAACGACGGCCAGT - 3'
M13Fw-44	5' - CAGGGTTTTCCCAGTCAC - 3’
M13Fw-bis	5’ - GTTGTAAAACGACGGCCAG - 3’
M13rev	5' - CAGGAAACAGCTATGACC - 3'
M13rev-bis	5' - CACAGGAAACAGCTATGAC - 3'

PGEX3	5' - CCGGGAGCTGCATGTGTCAGAGG - 3'
PGEX5	5' - GGGCTGGCAAGCCACGTTTGGTG - 3'
PolyT	5’ - TTTTTTTTTTTTTTTTTTTTTTTVV - 3'
RBgus2	5' - TCACGGGTTGGGGTTTCTACAGGAC - 3'
RVP3	5' - CTAGCAAAATAGGCTGTCCC - 3'
SeqLA	5' - TCGCGTTAACGCTAGCATGGATCTC - 3'
SeqLB	5' - GTAACATCAGAGATTTTGAGACAC - 3'
SK	5' - CGCTCTAGAACTAGTGGATC - 3'
SP6	5' - GATTTAGGTGACACTATAG - 3'
SP6bis	5' - GATTTAGGTGACACTATA - 3'
Sp6prom	5' - ATTTAGGTGACACTATAG - 3'
SV40pA-Rv	5' - GAAATTTGTGATGCTATTGC - 3'
T3	5' - ATTAACCCTCACTAAAGGGA - 3'
T7PROM	5' - TAATACGACTCACTATAGG - 3'
T7TERM	5' - GCTAGTTATTGCTCAGCGG - 3 '
T7Term-bis	5' - GGTTATGCTAGTTATTGCTCAG - 3'
T7UP1	5' - CGGCGTAGAGGATCGAG - 3'
U19	5' - GTTTTCCCAGTCACGACGT - 3'
ATTB2	5' - CCACTTTGTACAAGAAAGCTGGGT - 3'
ATTB1	5' - GTTTGTACAAAAAAGCAGGC - 3'
HCO-2198	5' - TAAACTTCAGGGTGACCAAAAAATCA - 3'
LCO-1490	5' - GGTCAACAAATCATAAAGATATTGG - 3'
RVP4	5' - GACGATAGTCATGCCCCGCG - 3'
pDON-R	5' - GTAACATCAGAGATTTTGAGACAC - 3'
pBabeF	5' - AAGCCCTTTGTACACCCTAAGCCT - 3'
pBabeR	5' - GCGGGACTATGGTTGCTGACTAAT - 3'
XL39	5' - ATTAGGACAAGGCTGGTGGG - 3'

GenoScreen

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www.genoscreen.fr

SANGER TECHNICAL SUPPORT

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Phone : +33 (0)3 62 26 37 76

Crédit Impôt Recherche CIR
Entreprise membre de bpifrance EXCELLENCE
Agrément MOT ansm
ISO 9001 ISO 13485 BUREAU VERITAS Certification
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