

USER GUIDE

Sanger Sequencing Solutions One-Shot and Optimized protocols

A - GENERAL INFORMATION

Technical steps description:

- Samples reception and quality controls (tubes, non-completed plates and/or completed plates).
- PCR products purification.
- Quantification of all your samples.
- DNA Sequencing on a 3730XL sequencer.
- Reading of the sequences (up to 1000bp).
- Sequencing process validation by an analysis of the quality control results (C+/C-).

Optimized option specificities:

- *Analysis of each sequence profile by GenoScreen's Sanger department.*
- *Free reprocessing of failed sequences under certain conditions.*

You obtain:

- Chromatograms (.abi files) and sequences in fasta format.
- The detailed results by email or/and by transfer to a secured FTP server by a login and password.

Optimized option specificities:

- *Specific report containing a detailed commentary of each sequence profile.*
- *Personalized monitoring of your project.*

Delivery time:

- Delivery time of 24h to 48h up to 8 plates received in one batch.

Included services:

- Confidentiality of received information and sent results.
- Direct access to our platform's engineers and experts from Monday to Friday from 9:00 a.m. to 17:30 p.m.
- Free hotline.
- Storage of samples and specific primers for 15 working days (more on client's request).
- GenoScreen provides free universal primers.
- Free shipment of samples under certain conditions (see section D – Samples shipping)

Possible conditioning:

- Samples received in tubes.
- Samples received in non-completed 96 wells plates:
 - Provide from 24 to 94 samples per plate,
 - 4 primers per plate maximum, to be provided in tubes,
 - For more than 4 primers, client should provide a mirror plate containing the respective primers.
- Samples received in completed 96 wells plates:
 - Provide 94 samples per plate,
 - Only 1 primer per plate is allowed, to be provided in a tube.

Samples received in microtubes fixed on an annotated support will be considered as sendings in plates starting from 24 samples.

B - DNA SEQUENCING REQUEST FORM

1/ Fill in the electronic version of the sequencing request form

Since the Sanger service is partly automated, it's asked to clients to pay special attention when filling the sequencing application form:

- Please complete all columns; each has its importance for the management of your sequencing request,
- Please match each lane of the table with a single sequencing reaction.

Note: Some cells are limited in number of characters (10 to 15 characters maximum).

For samples provided in tubes:

Example of a form for a sample (named A) to be sequenced with two 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												
<input checked="" type="radio"/> Tube(s) <input type="radio"/> 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	Genoscreen	-	A	PCR	900 to 1000	40	20		1	5	10	
2	Genoscreen	-	A	PCR	900 to 1000	40	20		2	5	10	
3		-										
4		-										
5		-										
6		-										
7		-										
8		-										
9		-										
10		-										
11		-										
12		-										
13		-										
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17		-										
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19		-										
20		-										
21		-										
22		-										
23		-										
24		-										
25		-										
26		-										
27		-										

For samples provided in plates:

In case of a plate with « n » quantity of primers to use for the same sample, «n» forms should to be filled in (one form per primer) while keeping the same plate name (in order to keep the same wells references).

Also, please prefer an organization in columns instead of lanes and avoid empty wells between samples.

Example of a form for the B sample to be sequenced with the 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												
<input type="radio"/> Tube(s) <input checked="" type="radio"/> Plate Name of plate: <u>Genoscreen-Plate1</u>												
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)		
Genoscreen	A1	B	PCR	900 to 1000	40	20		3	5	10		
	B1											
	C1											
	D1											
	E1											
	F1											
	G1											
	H1											
	A2											
	B2											
	C2											
	D2											
	E2											
	F2											
	G2											
	H2											
	A3											
	B3											
	C3											
	D3											
	E3											
	F3											
	G3											
	H3											
	A4											
	B4											
	C4											

Example of a form for the B sample to be sequenced with the primer 4

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												
<input type="radio"/> Tube(s) <input checked="" type="radio"/> Plate Name of plate: <u>Genoscreen-Plate1</u>												
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)		
Genoscreen	A1	B	PCR	900 to 1000	40	20		4	5	10		
	B1											
	C1											
	D1											
	E1											
	F1											
	G1											
	H1											
	A2											
	B2											
	C2											
	D2											
	E2											
	F2											
	G2											
	H2											
	A3											
	B3											
	C3											
	D3											
	E3											
	F3											
	G3											
	H3											
	A4											
	B4											
	C4											

2/ Send back your sequencing form by e-mail the day of your samples shipment to the address below:

seq1@genoscreen.fr

The subject of your e-mail should be in the following format: **[Sanger - Applicant name]**.
The applicant name in your e-mail should correspond to the name appearing in your sequencing form.

Example:

Applicant Name: Mr Dupont

ADDRESS FORM

Laboratory / Unit : _____
Address : _____
Post code : _____
Town : _____
Country : _____
Phone number : _____
Fax number : _____
Name : Dupont

Rédaction : [sanger-Dupont]

Expéditeur : Dupont@viu.fr

Pour : seq1@genoscreen.fr

Sujet : [Sanger-Dupont]

3/ The sequencing request form should be printed and sent along with the corresponding samples

The sequencing service will start as soon as the completed sequencing request form is received. GenoScreen reserves the right to hold any sequencing project whose form does not comply with one of the points outlined above.

C – SAMPLES CONDITIONING

1/ Conditioning in plates and/or tubes:

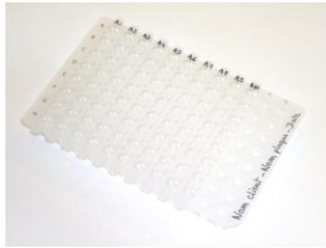
GenoScreen recommends the following references:

- **96 wells plate:** Sorenson Bioscience, Inc
0.2mL Rigid PCR Ultra Plate 96 Well
Reference 26190
- **Sealable adhesive film:** 4titude
Adhesive film PCR Cseal
Reference 016028F
- **Sealable film:** 4titude
Sealable film Peel Seal
Reference 016279F
- **Caps strips:** Thermo Scientific
Domed Caps Strip
Reference AB-0265
- **Tubes 1,7mL:** Sorenson Bioscience, Inc
Tubes 1,7mL Multi
Reference 017040A

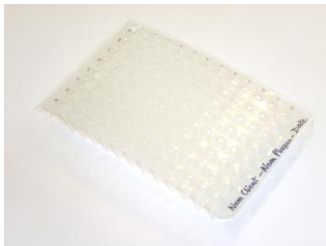
Technical notes for samples conditioning:

- For 96 wells plates samples shipping, please leave 2 empty wells for GenoScreen (for the internal quality controls).
- Please dilute your samples and primers in water (neither EDTA nor TE).
- Please annotate tubes and plates with an indelible marker to avoid any erasing (adhesive strips are not allowed on tubes).
- Your samples can be sent at room temperature in suitable packaging, such as bubble envelopes.
- For any plate's shipment: we advise the insertion of a rigid separation (cardboard type) between 2 plates in order to avoid any risk of drilling.

GENOSCREEN ACCEPT YOUR SAMPLES IN THESE CASES:



Annotated plates sealed with caps strip



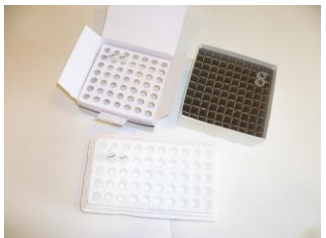
Annotated plates with adhesive PCR/heat-sealed films (see Annex 1)



1.5mL or 2mL reaction annotated tubes (Eppendorf type or flat cap/screw cap tubes)

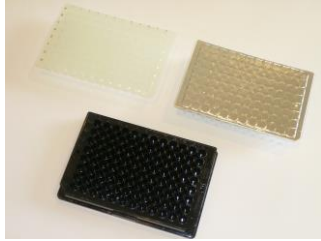


Annotated microtubes fixed on an annotated support



Annotated thermotubes arranged in order on a support or in a box (Genobox type)

GENOSCREEN DOESN'T ACCEPT YOUR SAMPLES IN THE FOLLOWING CASES:



Round/flat bottom PCR plates, use of simple adhesive, aluminum films or not-heat-sealed films



Loose thermotubes strips and non fixed on support



Parafilm, badly closed tubes, bulk tubes, tubes lower than 1.5 mL (ex: microtubes), large tubes (culture type)



Loose tubes

- BE CAREFUL, GENOSCREEN DOESN'T ACCEPT NON ANNOTATED SAMPLES -

2/ Quantities to provide for sequencing:

	PLASMID	PCR PRODUCT	COSMID	BAC
QUANTITY TO PROVIDE	500ng	PCR < 500bp: 50ng 500 < PCR < 1000bp: 100ng PCR > 1000bp: 200ng	2µg	2µg
CONCENTRATION	50 at 200ng/µL	5ng/µL/100bp	200 at 300ng/µL	200 at 300ng/µL
<u>MINIMAL VOLUME</u>	10µL	20µL	10µL	10µL

The indicated quantities are those required to perform one sequence reaction.

For any sample to be sequenced with more than one primer, please multiply the indicated amounts by the number of sequencings you would like to perform (taking into account possible reprocessings).

3/ Primers preparation:

If you want us to perform the sequencing reactions with your primers, please notice the following considerations:

- Volume and concentration: 10µL of primer minimum, at 2.5pmol/µL or 5pmol/µL,
- No secondary hybridation sites,
- No mismatches,
- Length of 18-25 bases,
- GC percentage comprised between 40% and 60%,
- T_m (melting temperature) between 50°C and 60°C,
- No hairpins > 3bp,
- No salts, EDTA or other contaminants.

**Minimal volume:
1µL/reaction**

D - SAMPLES SHIPPING

Transportation envelopes (provided by Genoscreen's approved carrier) and Genobox boxes are available upon client's request to the Sanger technical hotline (under certain quantity conditions).

1/ Sample shipment services by GenoScreen:

GenoScreen takes in charge your samples transportation for free as long as it respects the following conditions:

- The shipment should contain a minimum of 20 sequences for customers based in continental France*.
- A minimum of 48 to 96 sequences per sending for customers in other countries** and in the DOM-TOM**.

** If the package does not contain the minimum number of sequences indicated in the quote, transportation costs will be invoiced to the customer (for your information and except particular terms, a shipment from continental France will be invoiced 15.00€ without VAT)*

*** Please contact the Sales department to enquire about the minimum quantity shipment conditions for your country or DOM-TOM (Shipments that will not comply with these conditions will be invoiced 25.00€ without VAT for other countries, and 45.00€ without VAT from the DOM-TOM).*

To benefit from this service from continental France:

1 / GenoScreen will provide you with pre-filled and pre-paid transportation envelopes and Genobox boxes for your shipments, upon email or telephone request to the Sanger technical hotline.

2 / The client sends the package and we will receive it within 24 hours.

To benefit from this service from other countries or the DOM-TOM:

1 / Customer should inform us of the samples shipment by e-mail (seq2@genoscreen.com) providing us with the exact collection address.

2 / GenoScreen will contact the carrier for the collection scheduling.

3/ The client receives an email from the carrier to fill in an online form.

4/ We will receive your shipment within 24 hours after collection.

2/ Mailing by the customer:

Please send your package to the following address:

GENOSCREEN/Service SANGER
1 rue du professeur Calmette
59000 LILLE- France

Please make sure to write, visibly, the corresponding quote number on the package and join the printed sequencing request form to it. The contact(s) indicated on the sequencing form will receive an acknowledgment email from the Sanger department.

3/ GenoScreen lab samples drop-off:

In order to maintain quality services and insure short delivery schedules, **your samples must be dropped off before 12:00 a.m.**

Samples received after 12:00 a.m. will be considered as received the following day.

We store your samples for 15 working days.
This period can be extended upon client's request (by telephone or email).

E – ADMINISTRATIVE MODALITIES

The sequencing will start at samples/data, sequencing form and purchase order reception.

The following information must be mentioned in the purchase order:

- Quote number,
- Contact details of the invoice receiver's administration (address, phone number and e-mail, contact, VAT number),
- Laboratory contact details for results delivery (name, address, phone number and e-mail).

The invoicing terms are the following:

- Unique invoicing: if the whole analysis is for less than 1 month (duration in the quote), Genoscreen will send only one invoice related to performed and delivered results.
- Graduated invoicing: if the whole analysis is for more than 1 month (duration in the quote), Genoscreen will send graduated invoices, each one related to the performed and delivered results, in accordance with the steps mentioned in the quote.

The payment delay for invoices is of 30 days.

30.00€ without VAT is the administrative fee that will be invoiced for any purchase order lower than 250.00€ without VAT.

ANNEX 1: HEAT-SEALING PROTOCOL

In order to heat-seal your plates, 2 solutions exist:

- Heat-sealing with a heat-sealer: 3 sec at 180°C with an adapted film (see section C – Samples conditioning)
- Heat-sealing with a thermocycler: 5 min at 80-100°C with an adapted film (see section C – Samples conditioning) and only heat the cover of the device.