



DNA Sequencing Area
Tel.: +34 91 837 31 12 ext.4449
Fax: +34 91 804 72 93
www.secugen.es
Madrid - España

SERVICE REQUEST OF DNA SEQUENCING

To submit samples for DNA sequencing please read carefully the following instructions (Operational Procedure) where the steps to follow are detailed.

***Important Note:** Secugen assumes that when a customer request a service from Secugen, he/she has previously **read the operational procedure and agrees with the terms and conditions of the service** that are detailed in our website. If you have any question, please do not hesitate to contact us, we will be pleased to attend you.

OPERATIONAL PROCEDURE

In order to simplify and speed up the DNA sequencing service offered by **Secugen**, there is an application available to all customers in our website that facilitates the traceability of samples and allows the on-line access to the results.

Next, there are the steps our customers should follow to use the on-line application correctly. It is important to read all the instructions that the application is showing, because they will guide you to use the web application properly.

1. [Customer registration](#)
2. [Centers registration](#)
3. [Material request](#)
4. [Service request](#)
5. [Samples shipment](#)
6. [Results delivery](#)
7. [Sequence file visualization](#)

1. CUSTOMER REGISTRATION

The first step to get into the web application is to register as a new client. To do so, you click in this [link](#).

Then, you must complete the registration form following the instructions.

Once all the information has been introduced, you can access the system through the **Customer Area** panel typing your **Email** and **Password**.

***Important Note:** Remember to write down your email and password because you will need them every time you want to enter our system to request a service or consult any information of your orderings.



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2. CENTERS REGISTRATION

The next step will be to register your list of centers.

To work with us it is necessary you supply a **Work Center**, a **Material Shipment Center** and at least one **Billing Center**.

The Billing Centers are denominated '**Accounts**' and you can create all billing centers that you need it.

These Accounts can represent projects, personal, laboratories, etc.

For each Account generated you have associated a Results Folder, we will send to these folders the results requested by each Account.

3. MATERIAL REQUEST

The samples must be submitted to **Secugen** using special tubes and sticky labels with personalized barcodes that **Secugen** will previously provide to the customer by standard mail.

These personalized barcodes identify the customer samples and allow their track through the entire sequencing process.

By clicking on the option **Material Orders**, you can order the materials you may need and those will immediately be sent to your postal address.

Samples sent to **Secugen** using other tubes or labels will not be accepted, because the tube colors and labels with barcodes are necessary to identify the customers and samples through the sequencing process.

Available material:

Colored Eppendorf type tubes

96-well plates (Recommended for 48 or 96 samples):

3.1 TUBES

White Eppendorf type Tubes: These are used for sequencing reactions that use primers from the list provided by **Secugen**.

Purple Eppendorf type Tubes: These are used for sequencing reactions that use primers provided by the customer.

Every package of materials (tubes) includes 50 Eppendorf type tubes and 50 triplicate bar-coded labels.

3.2 PLATES

If the number of samples to be submitted to **Secugen** is large, we recommend to use a 96-well plate format that will also be identified with bar code labels (see figure).

Every package of material (plates) includes 10 (ten) 96-well plates, 10 sealing sheets and 10 triplicated plate barcoded labels.

***Important Note:** Plates should have 48 or 96 samples, if there were more than 48 or less that 96, the samples that exceeded the 48 samples must be submitted in tubes. If less than 48 samples are submitted in half plate they will be charged as 48 samples. (See paragraph 5.1.1. "use of Eppendorf type tubes or plates")

4. SERVICE REQUEST

Once you have received the material provided by **Secugen**, you should fill in the service request form. Clicking on **Request Form** you will be able to select a service request form that corresponds with the service you are requesting.

You must download, print and fill in the service request form to submit samples to **Secugen** because the **Request Form** contains a barcode that identify the customer, billing data and the characteristics of the samples.

If you are submitting an analysis that need special conditions, it is **very important** that you clearly state that in the **Request Form**, because it is the only way we can attend your specific request.

The analysis of samples that are incorrectly processed due to lack of specific instructions in the request form, will be charged to the customer. Please, be sure that the **Request Form** you are sending contains all the necessary information to process your samples correctly.

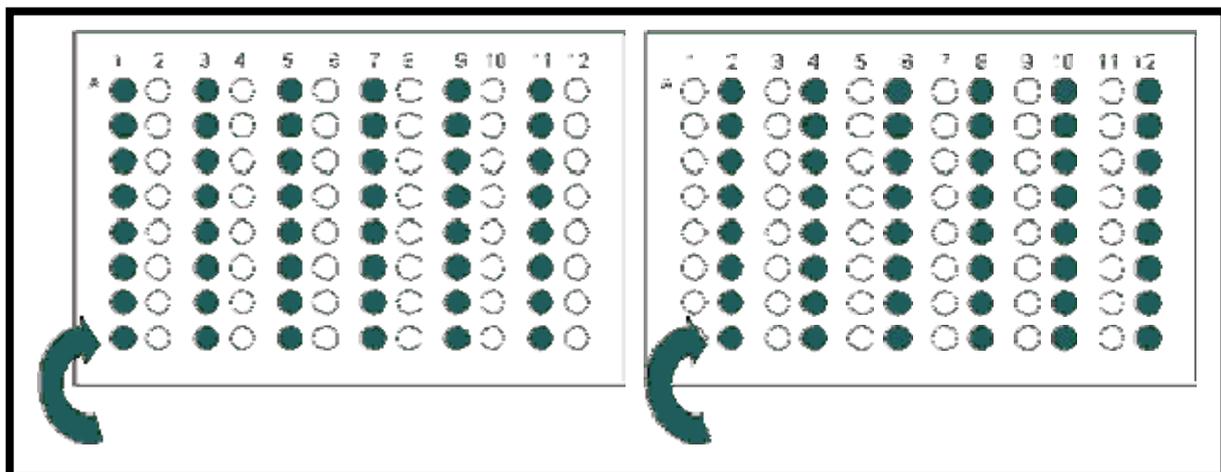
5. SAMPLES SHIPMENT

5.1. SAMPLES PREPARATION

5.1.1. Use of Eppendorf tubes or plates

As it was mentioned in the paragraph **3. Material Request**, for sample submission to **Secugen** you can send your sequencing samples to **Secugen** in Eppendorf type tubes or 96-well plates, previously supplied by **Secugen**.

96 Well plates should be used to send 48 or 96 samples (half plate or whole plate). In case of sending only half plate (48 samples), these should be plated only in even or odd columns, as it is shown in the following picture.



We cannot process in a half plate a number of samples below 48. Therefore, if you have less than 48 samples and you do not want to be charged for a completed half plate (48 samples), please sent the samples of the incomplete plate in Eppendorf® tubes. If fewer than 48 samples are sent in a half plate, 48 samples will be charged. The same applies in the case of 96 samples.

5.1.2. DNA concentrations and volumes required for a correct sequencing

For correct sequencing, it is required than samples have a suitable DNA volume and minimum concentration prepared in bidistilled water (see attached tables).

| DNA type | Concentration |
|-----------------------------------|-----------------|
| Plasmids (3-20 kb) | 100 ng/ μ l |
| Plasmids (>20 kb) and cosmids | 200 ng/ μ l |
| PCR products up to 500 pb | 10 ng/ μ l |
| PCR products between 500-1000 pb | 20 ng/ μ l |
| PCR products between 1000-1500 pb | 30 ng/ μ l |
| PCR products between 1500-2500 pb | 40 ng/ μ l |
| PCR products between 2500-4000 pb | 50 ng/ μ l |
| PCR products > 4000 pb | 100 ng/ μ l |

* For different DNA type, please contact us.

| Type of samples | Final Volume | |
|---|--------------|-------------------|
| | Tube | Plate |
| Plasmids and PCR Fragments | 15 μ l | 5-10 μ L/well |
| Phages | 20 μ l | |
| Cosmids | 15 μ l | |
| BACs | 20 μ l | |
| Direct sequencing of plasmids from colonies | 15 μ l | 15 μ L/well |
| Loads | 20 μ l | 20 μ L/well |
| Fragments | 15 μ l | 15 μ L/well |

***Important Note:** If this is the first time you are sending samples in 96-well plates or samples that require to be processed under special conditions (BACs, fragments phages...), please, contact our lab sequencing staff for technical support.

5.1.3. Type of primer

Preparation of samples to be sequenced using Secugen's primers.

The DNA sample at the suitable concentration will be dispensed in a white Eppendorf® tube or 96-well plate, marking the code (table below) of the primer needed for sequencing in the **Request Form**:



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| Code | Primers availables in Secugen service |
|-----------|---|
| F17 | M13 forward (-20): 5´d(GTAAAACGACGGCCAGT)3´ 17mer |
| R19 | M13 reverse (-24): 5´d(GGAAACAGCTATGACCATG)3´ 19mer |
| F24 | M13 forward (-47): 5´d(CGCCAGGGTTTTCCAGTCACGAC)3´ 24mer |
| R24 | M13 reverse (-48): 5´d(AGCGGATAACAATTTACACAGGA)3´ 24mer |
| T3 | T3 promoter (-20): 5´d(ATTAACCCTCACTAAAGGGA)3´ 20mer |
| T7p | T7 promoter: 5´d(TAATACGACTCACTATAGGG)3´ 20mer |
| T7pCl-neo | T7 pCl-neo: 5´d(TAATACGACTCACTATAGG)3´ 19 mer |
| T7t | T7 terminator: 5´d(GCTAGTTATTGCTCAGCGG)3´ 19 mer |
| SP6 | SP6 promoter: 5´d(ATTTAGGTGACACTATAG)3´ 18mer |
| Seq T | SeqT: 5´d(TTTTTTTTTTTTTTTTTTTTTT)3´ 25 mer |

***Important Note:** The Promega pCl-neo plasmid cannot be sequenced with the T7 primer due to a base pair of difference at the 3'-end. For sequencing this vector, primer T7 (pCl-neo) should be used.

Preparation of samples to be sequenced using primers provided by the customer.

In a single purple tube or 96-well plate, you must dispense both the DNA at the concentration indicated above and your own specific primer at 5 µM, both in bidistilled water as follows.

Required volumes:

Samples in tube: 15 ul of DNA + 1,5 ul primer at 5 µM.

Samples in plate: 5 ul of DNA + 0,5 ul primer at 5 µM.

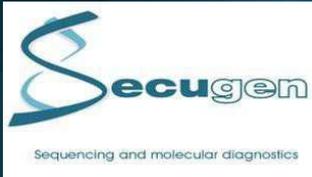
5.1.4. Preparation of special samples

If you expect any difficulties in sequencing your samples, it is important that you contact us in advance. Our laboratory staff will give you advice and technical support to solve these difficulties.

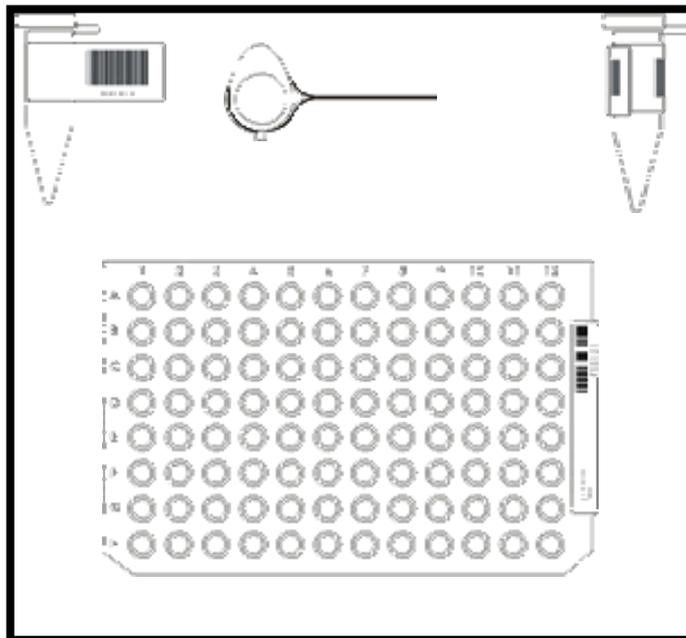
5.2. USING THE LABELS WITH BARCODES

Together with the tubes (white or purple) or the plates that you request prior to sequence with us, you will receive a set of barcoded labels. For each sample you will get a set of three identical labels.

1. Attach the first label to the sample tube as indicated below picture.
2. Place a second label in the space available for it in the **Request Form**. At the same time please indicate the annealing temperature of the primer and any other remarks about your sample, using the blank space available at the label side.
3. The third label is for the customer. We recommend that you stick it to your lab notebook or registration book together with a description of your sample. You will need this label to later on be able to identify the DNA sequence corresponding to that sample.



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5.3. SAMPLES SUBMISSION TO SECUGEN

Samples (tubes or plates), together with the **Request Forms** should be sent to **Secugen** by standard mail or courier at room temperature.

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6. RESULTS DELIVERY

Secugen will inform you by e-mail when your results are ready and will upload the sequences in your **Personal Folder for each Account** (Billing Center). You can access to this folder via internet, entering **Secugen** web page with your user and password. A **Statistical Report** for number of sample analyzed is also available and can be accessed by clicking on Statistics.

Upon receiving your sample at **Secugen**:

1. An e-mail will immediately be sent to you indicating the beginning of the sequencing process.
2. Once sequencing is finished, you will receive a new e-mail indicating that the analysis has been completed and the name of the Folder deposited in your **Personal Folder** that contains your results in a ZIP format. This folder should be copy and saved in your own computer. This process takes two and a half days to be finished after the sample reception.



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***Important Note:** The longest sequence may be obtained up to 1000 nucleotides.

***Important Note:** The personal folders should not be used to store the sequences. We recommend you delete the sequence files when these samples have been downloaded to free up storage in your folders.

With time the customer will generate a history profile with **Secugen**. Clicking on the button **Statistics**, you will have access to your sequencing statistics.

7. SEQUENCE FILE VISUALIZATION

Sequence files can be visualized using different software depending on your computer. Some freeware programs can be found in the following links:

1. **Chromas** (Free to PCs)
2. **BioEdit** (Free to PCs)
3. **4peaks** (Free to MacOS)
4. **FinchTV** (Free to Linux, PCs, yMACs)
5. **Sequence Scanner Software** (Free to PCs)

PRICES

Registration as a **Secugen** customer is free and will allow you to request a package of tubes and labels for the sequencing of three samples without any charge. You will be able to verify the simplicity and quality of our DNA sequence services and test our procedures for future orders.

- Price of sequencing a plasmid or PCR product: 6,5 €.
- Price of running a ready-to-load samples and fragments: 2,25 € (minimum samples required: 48)

*For other samples, special samples or sequencing difficulties must first consult with Technical Services to consult its price.

- Price of package of tubes: 50 tubes + 50 triplicated barcoded labels: 3,50 €.
- Price of package of plates: 10 96-well plates + 10 triplicated barcoded labels + 10 sealing sheets: 25 €

*Prices do not include VAT.

Secugen offers special prices, prior consulting, for large numbers of samples or when contracting multiple sequencing analyses on account.

***Important Note:** Samples that failed to be sequenced for reasons beyond our control will be charged in the invoice. For this reason, if the customer anticipates sequencing difficulties of any sample we recommend contacting in advance our Customer Service to receive technical support.



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CUSTOMER SUPPORT

Customer satisfaction is the priority. Contact us for technical support, quotation or any other information that you need. We have a long and solid experience sequencing difficult samples. Our staff will help you to find the best strategy to fulfill your particular needs.

In addition, feel free to contact our Customer Service for any question related to sequencing troubleshooting. If you are not fully satisfied with your sequencing results, please contact the Laboratory Manager, **in a period of 10 days**, to evaluate whether the process could be repeated without any additional charge.

Secugen guarantees the confidentiality of customer information and data. Upon request **Secugen** can provide a confidentiality agreement where it will be detailed that none of the information concerning your DNA samples will reach third parties.