Technical description of the service	The accuracy of the raw data has to be considered as follows: the obtained sequences are generally reliable in about the first 600 bases. The useful reading you get from about 50 bases from the primer 3' end. Cycle Sequencing is realized by ABI (Life Technologies) Big Dye Terminator version 3.1. It is generally achieved the 98.5% of accuracy. The amount of ambiguity, often to be interpreted by electropherogram analysis, is generally attributable to the preparation of the template, which is prepared by the applicant, by the complexity of the sequence, for example, high G and C content, repeated sequences etc., and from the choiche of the oligo. Each request for sequencing is for the single strand. In the case of DNA fragments that require more reactions to be completely sequenced, the choice of new primers to proceed in the sequence must be done by the applicant. Delivery of the results is guaranteed no later than the next four working days at the receipt of samples. The results, .ab1 format, will be sent to the email address provided by the customer. It should be noted that the sequence "failed" will be considered in any case fulfilled. For Bac ends, lambda-phage and other special cases, please ask for advice by contacting the staff of the Service, by telephone at 0815833269 or e-mail: sbmseq@szn.it. **PURIFICATION AND DELIVERY OF THE SAMPLES:** **PLASMIDIC DNA** The quality of the plasmid DNA from bacterial cultures that have reached the stationary phase is not optimal. For cultures grown at 37°C is recommended to block bacterial growth to an O.D.600 not greater than 1.5. A simple alternative is to incubate inocula O.N. at 30°C. The preparation of the plasmid DNA must be done through the use of ion exchange columns that provide the best degree of purification. We recommend the use of kits from companies: Qiagen, Sigma, Roche, Macherey Nagel, etc. It is recommended to use no more of the half of the inoculum volume recommended by the owner's manual of the columns. The sample must be quantized by spectrophotometry and the 260/280 ratio should be greater than or equal to 1.8. It is required photographic documentation of the sample with a mass DNA marker (we recommend 1 µl of lambda HindIII [500 ng/µl]). It is required a mix: plasmid DNA [25 fmol/ul] in H_2O + primers [1.25 pmol / ul] in H_2O, in a total volume of 10 µl for each sequence into 1.5 mL Eppendorf tube. We recommend starting with a concentration of the sample and the primer respectively [100-500 ng/ul] and [5 pmol/ul]. Please clearly label on the test tube the sample name and the primer (for a maximum of seven digits). P.S.: to calculate the volume (µl) of the plasmid DNA to be used in the mix (10 uL), for convenience, you can use the following formula: µl = 0.165 x (bp) / [ng / ul]. **PCR PRODUCTS** The purification of the PCR products must be done through the use of columns or by gel. We recommend the use of kits from the companies: Qiagen, Sigma, Roche, Macherey Nagel, etc. The sample must be quantized by spectrophotometry and the 260/280 ratio should be greater than or equal to 1.8. It is required photographic documentation of the sample with a mass DNA marker (we recommend 1 µl of lambda HindIII [500 ng/µl]). For PCR fragments greater than 1,500 bp, please contact the Service.

It is required a mix: DNA fragment [15 nmol/ul] in H_2O + primer [4.5 pmol/ul] in H_2O, in a total volume of 10 µl per each sequence, in 1.5 mL Eppendorf tube. We recommend starting with a concentration of the sample and the primer, respectively, of [10-25 ng/ul] and [20 pmol/ul].
For the PCR products the use of internal primers is preferred.
Please clearly label on the test tube the sample name and the primer (for a maximum of seven digits).

P.S.: to calculate the volume (µl) of the DNA fragment to be used in the mix (10 uL), for convenience, you can use the following formula:
µl = 0.1 x (bp)/[ng/ul].

PRIMERS
It is preferable that:
-the oligo's TM is greater than 50°C;
-the sequence does not contain more than four successive equal bases, especially if G or C;
-the 3' is stable and does not possess complementarity zones;
-the content of G/C is about 50% and that the length is between 19 and 26 mers.

The following primers are available from the service:
T3 (19 mer):
5'-ATT AAC CAC TAA CCT AGG G -3'

T7 (19 mer):
5'- AAT ACG ACT CAC TAT AGG G -3'

Sp6 (19mer):
5'-GAT TTA GGT GAC ACT ATA G -3'

M13 fw (21 mer):
5'- TGT CGT AAA ACG ACG GCC AGT -3'

M13 RV (24-mer):
5'-TTT CAC ACA GGA AAC AGC TAT GAC -3'

Please check that the oligo sequence coincides perfectly with that of the plasmid.

1. **Request form for Sanger Sequencing.**

2. **Download, fill and send to:**

Sequencing and genotyping: sbmseq@szn.it

Multiple sample (30) sequencing/genotyping request form
SBM_seq_geno_request_form multiple sampl[...]

3. Request (sequencing)

4. Internal users

Online ordering forms