cogentech

DNA Sanger Sequencing

REV. 01/24

Samples Submission Guidelines

1. DNA Sanger Sequencing

1a. General information

If you want to access our DNA Sanger sequencing service, you are kindly asked to:

- **fill out** the on-line request form @www.cogentech.it after registration and **print** it (see "How to fill out the request form" document);
- **prepare** the sample. Please refer to the tables below;
- **deliver** us the samples together with the printed form

1b. Samples' formats

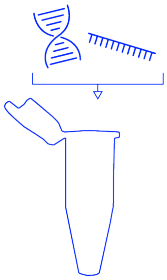
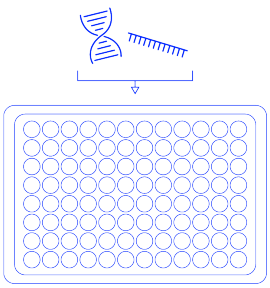
You can choose between two different formats:

- Single 1.5 ml tubes: select the "Single tube Format" option in the request form (maximum 29 samples for each request)
- 96 well plate: select the "96 Well Format" option in the request form (maximum 96 samples for each request, automatically named as plate's coordinates). Please note that the cost of this format is optimized for 96 samples (full plate) that must contain **the same primers and template's type**.

For each format there are two options:

- Ready-To-Go: primers and template are premixed together (not applicable to PCR products which have not yet been purified)
- Standard: primers and template are in separate 1.5 ml tubes.

Ready-to-go

TEMPLATE				PRIMER		TEMPLATE + PRIMER	
SUBMITTED IN		DNA TYPE	CONCENTRATION	VOLUME	CONCENTRATION	VOLUME	PRE-MIXED TOTAL VOLUME
	1.5mL reaction tube	Plasmids purified	50-100 ng/µl	12 µl	5 pmol/ µl (µM)	2 µl	14 µl
		PCR fragments purified	20-50 ng/µl				
	96-well plate	Plasmids purified	50-100 ng/µl	1 2 µ l / well	5 pmol/ µl (µM)	2 µl/well	14 µl/well
		PCR fragments purified	20-50 ng/µl				

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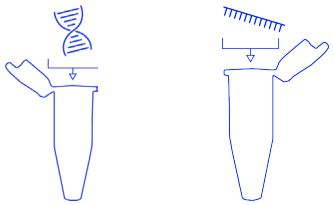
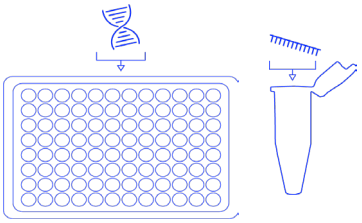
Standard

TEMPLATE					PRIMER		
SUBMITTED IN		DNA TYPE	CONCENTRATION	VOLUME	SUBMITTED IN	CONCENTRATION	VOLUME
	1.5mL reaction tube	Plasmids purified	50-100 ng/µl	15 µl	1.5mL reaction tube	5 pmol/ µl (µM)	5 µl
		PCR fragments purified/ unpurified	20-50 ng/µl				
	96-well plate	Plasmids purified	50-100 ng/µl	1 5 µ / welll	1.5mL reaction tube	5 pmol/ µl (µM)	250 µl/ platel
		PCR fragments purified/ unpurified	20-50 ng/µl				

1c. How to submit your samples

Plasmids

Plasmid DNA prepared by commercially available column-based kits usually have sufficiently good quality for routine sequencing. Difficult templates might better be prepared by high performing kits such as the QIAprep Spin Miniprep from Qiagen or the Wizard® Plus SV Miniprep from Promega.

PCR

Sequencing of PCR products will only be successful if a single band has been amplified. The Sequencing facility will purify the PCR reaction for you, unless otherwise indicated in the request form, with enzymatic method: column-based purification protocols often allow to lose material and concentrate salts that inhibit sequencing reactions.

If the PCR reaction does not generate a strong single band, we warmly suggest to optimize the PCR protocol, because sequencing reaction of gel purified PCR usually either fail or are of poor quality.

ATTENTION: colored PCR buffers and some Taq Polymerase kits might result in poor quality sequencing reads. In particular we have noticed that PCR reactions carried out with the **Q5 Hot Start High-Fidelity Master 2x mix (NEB cod#M0494S)** give poor results (using our standard PCR purification protocol).

Concentration & Quality

After template preparation, dilute your samples in H2O (Plasmids or PCR fragments) following the conditions in the table above: the A260/280 should be >= 1.8 (free of protein contamination and organic compounds).

To quantify the concentration of your DNA sample you may use:

- Qubit or spectrophotometric analysis (for plasmids or purified PCR fragments)
- agarose gel analysis: for single band unpurified PCR, please refer to the specific handout in our web page

During the filling of the request form you are asked to upload the picture of the agarose gel and to provide some information. The agarose gel analysis helps us to evaluate the quality of samples provided.

2. Other services

For Cell ID full Service, fragment analysis or DNA sanger sequencing specific projects, please contact us at service-sequencing@cogentech.it

3. Data Delivery time and Storage

The results are usually delivered in 1-2 working days. You will receive an email when the results are ready.

For special projects or DNA Sanger sequencing of more that 2 plates at ones, please contact us at service-sequencing@cogentech.it

We guarantee data storage for at least 1 month and sample/primer storage for 1 week.

4. Shipping procedure

We accept samples from Monday to Friday, until 9.00 a.m. Samples that are delivered after 9:00 a.m. will be processed the next working day.

Sample delivery is not possible over the weekend. If you send samples on Friday, please make sure they are delivered by 4:00 p.m.

External Users can ship the samples at controlled temperature, using either a cooling pad or shipped in dry ice, to the following address:

DNA Sanger Sequencing Service

c/o Cogentech Via Adamello 16, 20139, Milano

Internal Users can bring the samples any time to Sequencing Facility at Building 11, second floor, and place them in the dedicated fridge, leaving the printed request form in the box close to the fridge.