

How to send your samples for “One Shot” DNA sequencing

For each reaction, we need **5 µL** of sample containing:

- **100 femtomols template DNA**

- > **250-500 ng** of plasmid DNA
- > 1 µg for large plasmids (cosmids or BACs)
- > **a visible band** of PCR product or 10-200 ng (approx. 2 ng per 100 bp)

Difficult templates: special protocols are not available for “One Shot” sequencing.

As the DNA template is one of the main factors influencing sequence quality, it should be purified using commercial kits.

It should be provided in distilled water (or Tris), free of EDTA and other salts, proteins, RNA, or genomic DNA.

PCR products have to be purified from reaction buffer, primers and nucleotides. They must appear as single band in an agarose gel.

- **5 picomols primer**

If your primer is at **10 µM**, add **0.5 µL** per reaction.

If at least 5-8 reactions are to be performed with the same primer, we can add it to the sequencing mix, *i.e.* primer should be supplied at 10 µM, except for [standard primers](#).

- **Sending the samples**

Samples should be provided in 48 or 96-wells plates (or at least strips of 8 x 0.2 mL tubes). We recommend the following plates from AB-GENE:

- ThermoFast 48, Cat. No AB-0648
(these plates can be split in groups of 3 x 8-wells strips)
- ThermoFast 96 non-skirted, Cat. No AB-0600
- Flat Cap Strip, Cat. No AB-0784

For plates, samples must be placed in **columns**, *i.e.* A1, B1, C1...H1, A2, B2,...

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2										
B	B1	B2										
C	C1	C2										
D	D1	↓										
E	E1											
F	F1											
G	G1											
H	H1											