



**Summary on how to prepare samples for sequencing at GATC and how to evaluate sequences from GATC – please check for updates online:**

**1. Sample requirements - options:**

- a) PCR products (concentration must be known, measure e.g. with nanoDrop).
- b) Plasmid DNA (concentration must be known, measure e.g. with nanoDrop).
- c) Clones on Master plate.
  - Concentration of PCR-Products: 10-50 ng/μl
  - Concentration of Plasmid-DNA: 30-100 ng/μl
  - Concentration of Primer: 10 pmol/μl
  - Volume requirements (for ~ 8 sequencing reactions): 20μl in microplates; 30 μl for single tubes.

**2. Other requirements:**

- GATC sample table (excel file can be downloaded from the lab website or from GATC).
- For PCR products: sterile 1,5 ml screwcap tubes or empty sterile 96 microwell plates and appropriate labels that close tightly.
- For clones: 96 microwell plates with LB agar and antibiotics (can be ordered for free from GATC). **Note, these plates have a limited shelf life.** Be sure to prepare a copy of your master plate when inoculating the 96 microwell plate for GATC.
- Pipettes/sterile pipette tips/sterile tooth sticks.
- GATC boxes for shipping (can be ordered for free from GATC).
- Consider appropriate primers for sequencing (can be selected from the GATC website) or provided in tubes for GATC if special primers would be needed).
- Bar codes for sequencing and for primers, respectively – note, they come in different colors). Must be ordered from GATC (may take a few days). Ask your supervisor for barcodes.

### 3. Procedure:

#### 1) Select how to prepare your samples:

- **If less than 96 samples (only for PCR products or plasmid DNA):** Pipette your sample (amplificate or plasmid DNA) into 1,5 ml screwcap tubes. Label the tubes with bar codes provided by GATC (via your supervisor). Do not add tape on the tubes. Make sure that you know which sample will be labeled with which barcode. Use the GATC sample excel table provided by GATC.
- **If 96 samples and PCR product/plasmid DNA:** Pipette your samples into the wells in a 96 microwell plate. Be aware of how you pipette your samples so that you know which well contains which sample. Use the GATC sample excel table provided by GATC. Label the plate with the ordering number provided by GATC when the order and the corresponding excel sample table was posted on their website.
- **If 96 samples and clones:** Inoculate clones from a master plate into 96 microwell plates with LB agar and antibiotics. Be aware of how you pipette your samples so that you know which well contains which sample. Use the GATC sample excel table provided by GATC. Close lid tightly and incubate for a couple of hours/over night at 37 °C. Label the plate with the ordering number provided by GATC when the order and the corresponding excel sample table was posted on their website.

2) Storage: All samples can be stored at RT or at +4°C for a couple of hours prior to shipping. For longer storage periods, PCR products and plasmid DNA should be stored at -20°C.

3) Shipping: On the day you wish to send away the tube(s) or the plate(s) for sequencing, log in at the GATC website <http://www.gatc-biotech.com/en/>, (info about the User, eMail address and customer number can be provided from the supervisor) and provide the following info:

- Info about the contents of the tubes/microtiterplate. Use the GATC sample excel table.
- Which primer should be applied (GATC has a list on their website of a number of routinely used primers. If other primers should be desired, these should then be provided as a solution in a tube labeled with a special GATC bar code for primers).
- The box with the tube(s) or the microtiterplate(s) can be deposited at the Mensa building. Watch out for the GATC post box at the front door, just next to the beverage machine, close to the cafeteria. If you post it before 16:30, you may get your sequences on the next day already (however usually it takes 2 days).



**4. Format of results:** Data are provided in three different files (e.g. FASTA file and ABI Chromatogram). You need a special software to open the chromatograms - these can be downloaded for free from the GATC website - e.g. GATC viewer or chroma light.

#### 5) How to evaluate sequence data:

- a) Check the size of the sequence (amount of nucleotides).
- b) Check the quality of the peaks in the chromatogram. Discard or correct sequence information.
- c) Copy the sequence from the text file and submit to online data bases for further identification of the sequence, e.g.:
  - **For general search:** NCBI blast <http://blast.ncbi.nlm.nih.gov/Blast.cgi> (select appropriate blast database, e.g. nucleotides if you sequence genes). Note, this website is updated daily.
  - **For 16S rRNA genes:** RDP sequence match [http://rdp.cme.msu.edu/seqmatch/seqmatch\\_intro.jsp](http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) Note, this website is updated every couple of months.
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  - **For ribosomal genes:** Silva alignment <http://www.arb-silva.de/aligner/> Note, this website is updated every 6 months or longer.
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- d) Record results with regard to e.g.:
  - Affiliation to next closest sequence(s) – distinguish between parameters such as culturable/unculturable; eco system type, etc.
  - Degree of similarity(ies) in %.
  - Record the next relatives and where appropriate download those sequences (check first that they are not already available in former databases) for further comparison with own sequences in e.g. the bioinformatic software package arb [www.arb-home.de/](http://www.arb-home.de/)
- e) **Download all files from the GATC website and keep them in your record (together with the key of your sample entries). These are regarded as raw data and must not be lost.**