**Sanger DNA sequence analysis at KIGene**

The ABI 3730 PRISM® DNA Analyzer enables high quality sequencing up to 1000 bases and high resolution (1 bp) fragment analysis. The ABI 3730 instruments are in the 96 or 384 well format using a 48 x 36 cm or 50 cm capillary array.

We have the appropriate matrices set up on the instruments for BigDyeTM terminators v.3.1 and 1.1 (Applied Biosystems).

## How we want your samples

We prefer to receive the samples placed in PCR plates or PCR strip-well tubes when sending more than 8 reactions/template DNA’s.

Only mark tubes with sample numbers and your initials. It is sufficient to add the complete sample names in the order form. When sending plates: add the sample names in the column order (A01-H01, A02-H02 etc) in the order form.

The order form is submitted within iLab to the core and a hard copy is placed together with the samples.

## pre-made Sanger sequence samples

After completion of the sequence reactions, each reaction is precipitated and dried before delivery to us.

* **full-service Sanger sequence samples**

In order to optimize the sequencing reaction we need information about template and primer concentrations, size of the template DNA and annealing temperature of the primer.

**Required volumes and concentrations of template-DNA and primers**

Purified PCR fragments: 2-50 ng depending on length (~2 ng/100 bp) in a volume of 6 µl or less

Plasmid DNA: 150 ng-200 ng in a volume of 6 µl or less

Sequence primers: Diluted to 5 pmol/µl. Provide 1 µl per reaction

**Note**: If you can spare, please provide more than the required volumes so that we can set up new reactions if needed.

**Universal primers stocked at the facility**

* **BGH-R** 5'-TAG AAG GCA CAG TCG AGG-3'
* **CMV-F** 5'-CGC AAA TGG GCG GTA GGC GTG-3'
* **EF-1a Forward** 5'-TCA AGC CTC AGA CAG TGG TTC-3'
* **EGFP-C** 5'-CAT GGT CCT GCT GGA GTT CGT G-3'
* **EGFP-N** 5'-CGT CGC CGT CCA GCT CGA CCA G-3'
* **Gal4-AD** 5'- AAT ACC ACT ACA ATG GAT-3'
* **Gal4 N-term** 5'- GAG TAG TAA CAA AGG TCA A-3'
* **GFP-F** 5'- GGT CCT TCT TGA GTT TGT AAC-3'
* **GFP-R** 5'- CCA TCT AAT TCA ACA AGA ATT GGG ACA AC-3'
* **IRES-F** 5'- TGG CTC TCC TCA AGC GTA TT-3'
* **IRES-R** 5'- CCT CAC ATT GCC AAA AGA CG-3'
* **LKO.1 5'** 5'- GAC TAT CAT ATG CTT ACC GT-3'
* **M13 F (-20)** 5'-GTA AAA CGA CGG CCA GTG-3'
* **M13-F (-40)** 5'-GTT TTC CCA GTC ACG AC-3'
* **M13 R (-27)** 5'-CAG GAA ACA GCT ATG AC-3'
* **M13 R (-48)** 5'-AGC GGA TAA CAA TTT CAC AC-3'
* **PGEX-3’** 5'-CCG GGA GCT GCA TGT GTC AGA GG-3'
* **PGEX-5’** 5'-GGG CTG GCA AGC CAC GTT TGG TG-3'
* **PolyA-B** 5'-AAA AAA AAA AAA AAA AAA AAA AAA B-3'
* **PolyT-V** 5'-TTT TTT TTT TTT TTT TTT TTT TTV-3'
* **RVprimer3** 5'- CTA GCA AAA TAG GCT GTC CC-3'
* **Sp6** 5'-ATT TAG GTG ACA CTA TAG-3'
* **SV40pA-R** 5'-GAA ATT TGT GAT GCT ATT GC-3'
* **SV40pro-F** 5'-TAT TTA TGC AGA GGC CGA GG-3'
* **T3 Promoter** 5'-ATT AAC CCT CAC TAA AGG-3'
* **T7-EEV** 5’-ATG TCG TAA TAA CCC CGC CCC G-3'
* **T7 Promoter** 5'-AAT ACG ACT CAC TAT AGG-3'
* **T7 Reverse** 5’-TAG TTA TTG CTC AGC GGT G-3'
* **T7 Terminator** 5'-GCT AGT TAT TGC TCA GCG G-3'
* U6 Primer 5'- GGG CAG GAA GAG GGC CTA T-3'

**Note**: the oligo sequences might differ between universal primers from different companies despite the same name. Remember to check that our universal primer can anneal to your target DNA.

## Sample delivery.

Put your samples in an envelope together with the appropriate order form. There is no need to add parafilm over the lids when placing them in our drop boxes.

***Internal Users***: Send samples by internal mail or place samples in any of our four drop boxes at Solna Campus area. After 10:00, note that your samples might be processed faster if you place them in our CMM drop box.

**CMM**

**Pick-up time:**

* 10:00 and 13:00 each business day

**Drop box location:**

* outside the Translational Psychiatry Unit, L8:00 (CMM entrance floor)

**BioClinicum**

**Pick-up time:**

* 10:00 each business day

**Drop box location:**

* Plan 5, close to the Skyway entrance

**BioMedicum**

**Pick-up time:**

* 10:00 each business day

**Drop box location**

* Plan 4, outside the lunch room located close to the Skyway entrance

**SciLifeLab**

**Pick-up time:**

* 10:00 each business day

**Drop box location**

* At the reception desk

 ***External Users*:** Preferably use padded envelope and parafilm over the tube lids.

**Address:**

KIGene

Translational Psychiatry, MMK
CMM L8:00, Karolinska University Hospital
Visionsgatan 18
171 76 Stockholm, Sweden

## Turn-around time

* 10:00 AM: sample-to-result within the same day
* 13:00 PM: pre-made sample-to-result within the same day

**Note**: In rare cases delays may occur if we experience unusually heavy loads or equipment failure.

**Note:** We always reload failed sequence reactions **without charge** in case we assess that they might improve in a second analysis.

## Data storage.

We store all data from pre-made and full-service sequence samples for three years. Therefore, if you lose raw data files we can retrieve these for you if you give us the relevant information (date and sample names).

## Sample storage.

We save primers and templates for full-service sequence samples a month in our freezers.