

## 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 BigDye™ 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

- <b>BGH-R</b>	5'-TAG AAG GCA CAG TCG AGG-3'
- <b>CMV-F</b>	5'-CGC AAA TGG GCG GTA GGC GTG-3'
- <b>EF-1a Forward</b>	5'-TCA AGC CTC AGA CAG TGG TTC-3'
- <b>EGFP-C</b>	5'-CAT GGT CCT GCT GGA GTT CGT G-3'
- <b>EGFP-N</b>	5'-CGT CGC CGT CCA GCT CGA CCA G-3'
- <b>Gal4-AD</b>	5'- AAT ACC ACT ACA ATG GAT-3'
- <b>Gal4 N-term</b>	5'- GAG TAG TAA CAA AGG TCA A-3'
- <b>GFP-F</b>	5'- GGT CCT TCT TGA GTT TGT AAC-3'
- <b>GFP-R</b>	5'- CCA TCT AAT TCA ACA AGA ATT GGG ACA AC-3'
- <b>IRES-F</b>	5'- TGG CTC TCC TCA AGC GTA TT-3'
- <b>IRES-R</b>	5'- CCT CAC ATT GCC AAA AGA CG-3'
- <b>LKO.1 5'</b>	5'- GAC TAT CAT ATG CTT ACC GT-3'
- <b>M13 F (-20)</b>	5'-GTA AAA CGA CGG CCA GTG-3'

- <b>M13-F (-40)</b>	5'-GTT TTC CCA GTC ACG AC-3'
- <b>M13 R (-27)</b>	5'-CAG GAA ACA GCT ATG AC-3'
- <b>M13 R (-48)</b>	5'-AGC GGA TAA CAA TTT CAC AC-3'
- <b>PGEX-3'</b>	5'-CCG GGA GCT GCA TGT GTC AGA GG-3'
- <b>PGEX-5'</b>	5'-GGG CTG GCA AGC CAC GTT TGG TG-3'
- <b>PolyA-B</b>	5'-AAA AAA AAA AAA AAA AAA AAA AAA B-3'
- <b>PolyT-V</b>	5'-TTT TTT TTT TTT TTT TTT TTT TTV-3'
- <b>RVprimer3</b>	5'- CTA GCA AAA TAG GCT GTC CC-3'
- <b>Sp6</b>	5'-ATT TAG GTG ACA CTA TAG-3'
- <b>SV40pA-R</b>	5'-GAA ATT TGT GAT GCT ATT GC-3'
- <b>SV40pro-F</b>	5'-TAT TTA TGC AGA GGC CGA GG-3'
- <b>T3 Promoter</b>	5'-ATT AAC CCT CAC TAA AGG-3'
- <b>T7-EEV</b>	5'-ATG TCG TAA TAA CCC CGC CCC G-3'
- <b>T7 Promoter</b>	5'-AAT ACG ACT CAC TAT AGG-3'
- <b>T7 Reverse</b>	5'-TAG TTA TTG CTC AGC GGT G-3'
- <b>T7 Terminator</b>	5'-GCT AGT TAT TGC TCA GCG G-3'
- <b>U6 Primer</b>	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.