Description: Molecular Biology Services and DNA Sequencing

DNA Sequencing Single Pass Sequencing

Purpose	Sequence data only, for plasmids or PCR products
Customer supplies	• Plasmid DNA or PCR products Plasmid DNA: 20 – 100 ng/µl PCR Product: 5 – 50 ng/µl Volume: 20 µl or more (enough for 4 reactions) DNA dissolved in water • Custom primers 2 pmol/µl, minimum of 20 µl
Service includes	• DNA quantification and dilution for optimum sequencing results • Storage of DNA samples and primers for future sequencing reactions for four weeks • Visual inspection of data before release to customer • One repetition of any failed sequencing reaction • Use of a special sequencing reaction mixture, where necessary, for difficult templates (GC-rich sequences, hairpin loops) • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • Sequence data in .ab1 (chromatograms) and text format (.seq) • Read length up to 900 bases • Results within 24 hours
Optional Services	• Plasmid Miniprep or PCR purification • Custom primer design or primer purchase

DNA Sequencing Pre-cycled Sequencing Reactions

Purpose	A fast and economical service giving sequence data only, for plasmids or PCR products
Customer supplies	• Non purified sequencing reactions (Applied Biosystems Big Dye version 3.1) in tubes or in PCR plates • A minimum of 24 reactions
Service includes	• Purification of the sequencing reactions • Analysis of the purified sequencing reactions using an AB3730XL DNA Sequence Analyzer • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • Sequence data in .ab1 (chromatograms) and text format (.seq) • Read length up to 900 bases • Results within 24 hours

DNA Sequencing Double-Stranded Sequencing for Confirmation

Purpose	Complete sequence verification of a DNA sequence of interest on both DNA strands (raw data production, contig alignment and editing, comparison of consensus sequence to customer's reference sequence)
Customer supplies	• Plasmid DNA or plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) • The sequence of the construct or region of interest
Service includes	• Primer design and synthesis • Plasmid template preparation (free upon request) • DNA sequencing (double stranded sequencing) • Storage of DNA samples and primers for future sequencing reactions for four weeks • Sequence data alignment and editing of the resulting consensus • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • A consensus sequence for the sequenced region(s) • A comparison of the consensus sequence to the provided reference sequence • A report listing all observed differences • Results within 24 hours (3-4 days if primers need to be ordered)

DNA Sequencing Single-Stranded Sequencing for Confirmation

Purpose	Complete verification of a DNA sequence of interest on one DNA strand only (raw data production, sequence alignment and editing, comparison of consensus sequence to customer's reference sequence)
Customer supplies	• Plasmid DNA or plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) • The sequence of the construct or region of interest
Service includes	• Primer design and synthesis • Plasmid template preparation (free upon request) • DNA sequencing (single stranded sequencing) • Storage of DNA samples and primers for future sequencing reactions for four weeks • Sequence data alignment and editing of the resulting consensus • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • A consensus sequence for the sequenced region(s) • A comparison of the consensus sequence to the provided reference sequence • A report listing all observed differences • Results within 24 hours (3-4 days if primers need to be ordered)

DNA Sequencing Primer Walking, Non-assembled Single Stranded Sequencing

Purpose	The complete sequence of a DNA sequence of interest on one DNA strand (non assembled single reads)
Customer supplies	• Plasmid DNA or plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) • The sequence of the construct or region of interest
Service includes	• Primer design and synthesis • Plasmid template preparation (free upon request) • Storage of DNA samples and primers for future sequencing reactions for four weeks • DNA sequencing • Visual inspection of raw data before release to customer • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • Sequence data in .ab1 (chromatograms) and text format (.seq) • Read length up to 900 bases • Results within 24 hours (3-4 days if primers need to be ordered)

DNA Sequencing Primer Walking, Non-assembled Double Stranded Sequencing

Purpose	The complete sequence of a DNA sequence of interest on both DNA strands (non assembled single reads)
Customer supplies	• Plasmid DNA or plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) • The sequence of the construct or region of interest
Service includes	• Primer design and synthesis • Plasmid template preparation (free upon request) • Storage of DNA samples and primers for future sequencing reactions for four weeks • DNA sequencing • Visual inspection of raw data before release to customer • Technical support by specialists
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • Sequence data in .ab1 (chromatograms) and text format (.seq) • Read length up to 900 bases • Results within 24 hours (3-4 days if primers need to be ordered)

DNA Sequencing cGMP Sequencing

Purpose	Confirmation of a DNA sequence under cGMP

Description

DNA sequencing under cGMP is based upon a generically validated method. Analysis under cGMP is preceded by product specific verification.

Verification of product specific parameters for cGMP sequencing

Product-specific sequencing conditions are determined via a non-cGMP (ISO9001) study. The conditions are summarized in a QA approved parameter sheet that includes:

- Primer design and primer synthesis
- Double stranded DNA sequencing (at least 2 fold strand coverage)
- Development of product-specific sequencing conditions
- Description of product-specific sequencing conditions in a parameter sheet

cGMP sequencing

Performed under cGMP using the product-specific sequencing conditions to include:

- The usage of qualified equipment
- cGMP trained and qualified personal
- Documentation complying with cGMP guidelines
- Double stranded DNA sequencing with at least 2 fold strand coverage

If the specific sequencing conditions elaborated during verification differ from the validated range, then validation of the specific conditions will be performed.

Customer supplies

- Plasmid DNA: 20 - 1000 ng/µl (at least 5 µg/kb).
- DNA dissolved in water.
- The reference sequence of the construct or region of interest.
- The specification of the product.

Customer receives

- The analytical results under cGMP
- The consensus sequence for the sequenced region
- A sequence alignment with a comparison of the consensus sequence to the reference sequence
- A summary of the DNA sequencing method together with a summary of the verification of product specific parameters
- The parameter sheet
- Results within 4 -6 weeks after receipt of samples

Optional Services

- Plasmid Miniprep or Maxiprep (under ISO9001)
- A full cGMP sequencing report : summary of DNA sequencing strategy, experiments and results; all primer sequences; all sequencing reactions; the sequence alignment; the consensus sequence and the reference sequence.

DNA Sequencing Exon Sequencing

Purpose	Sequence verification of a genomic region of interest in several individuals (i.e., cell lines, diagnostic families members) and comparison to a reference sequence (i.e., SNP analysis)
Customer supplies	• Sequence information concerning the gene or genomic region of interest • Genomic DNA of individuals to be analyzed
Service includes	• PCR amplification of region(s) of interest (maximum 600 nt) • Sequence analysis of resulting PCR product • Comparison of amplicon sequence to customer's reference sequence
Customer receives	• Notification by email that results are available • Results for download from a secure server for up to one month • Detailed report of the SNP analysis

Primer for DNA Sequencing Custom primer

Purpose	For sequencing only
Customer supplies	• Primer sequence
Service includes	• Primer synthesis

Primer for DNA Sequencing Primer design

Purpose	For sequencing only
Customer supplies	• Reference sequence
Service includes	• Primer design • Primer synthesis

DNA Preparations Plasmid Miniprep

Purpose	For sequencing only
Customer supplies	• Plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates)
Service includes	• Purification of the plasmid

DNA Preparations PCR Product Purification

Purpose	For sequencing only
Customer supplies	• A non purified PCR product
Service includes	• Purification of PCR product using QIAGEN method

DNA Preparations Large-Scale, Endotoxin-free Plasmid Prep

Purpose	An endotoxin-free plasmid maxi-prep (100 microgram scale)
Customer supplies	• Plasmid carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) or plasmid DNA
Service includes	• Purification of the plasmid • Photometric quantification of resulting DNA • Identity test by DNA sequencing
Customer receives	• Endotoxin-free plasmid DNA • Minimum of 100 µg of DNA (turnaround time 1 week) • Sequence data and report
Optional Services	• Complete double or single stranded confirmation sequencing.

Transformation *E. coli* Transformation

Purpose	For sequencing only
Customer supplies	• Plasmid DNA
Service includes	• *E. coli* transformation
Optional Services	• Plasmid Miniprep

Site-Directed Mutagenesis Site-Directed Mutagenesis (Single Site)

Purpose	To introduce a change into a sequence of interest (single locus change). To verify the sequence of the mutated site.
Customer supplies	• Plasmid DNA • Reference sequence
Service includes	• Site directed mutagenesis of site of interest • Sequence verification of the mutated site(s) (typically 500 nt around the mutated site will be verified).
Customer receives	• Mutated construct (turnaround time 1-2 weeks) • Sequence data and report
Optional Services	• Complete double or single stranded confirmation sequencing of mutagenised clone (price according to list). • Endo-toxin free plasmid maxiprep

Site-Directed Mutagenesis Site Directed Mutagenesis (Multiple Sites)

Purpose	To introduce multiple changes into a sequence of interest (multiple loci change) To verify the sequence of the mutated sites.
Customer supplies	• Plasmid DNA • Reference sequence
Service includes	• Site directed mutagenesis of sites of interest • Sequence verification of the mutated sites (typically 500 nt around the mutated sites will be verified)
Customer receives	• Mutated construct (turnaround time 1-2 weeks) • Sequence data and report
Optional Services	• Complete double or single stranded confirmation sequencing of mutagenised plasmid (price according to list) • Endo-toxin free plasmid maxi-prep

Clone Screening by Sequencing

Purpose	• A positive clone screened out of multiple clones. • Sequence verification at the site of interest of the positive plasmid • DNA preparation of plasmid template
Customer supplies	• Plasmid DNA of clones to be screened (4 minimum) • Plasmid-carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) of clones to be screened (4 minimum) • The reference sequence
Service includes	• DNA preparation of plasmid templates • Sequencing of each clone with 2 primers of choice (primer design and purchase included • Alignment to the reference sequences
Customer receives	• Sequence data • Report on positive clones identified
Optional Services	• Complete double or single stranded confirmation sequencing of one (or more) positive clones (price according to list) • Endotoxin free plasmid Maxiprep of a positive clone

Subcloning (via PCR or Restriction Enzyme Digestion)

Purpose	Subcloning of a DNA fragment (PCR product or restriction digestion) into a vector of choice
Customer supplies	• Plasmid DNA • Plasmid-carrying *E. coli* culture (i.e., stab culture, glycerol stock, agar plates) • The reference sequences
Service includes	• Subcloning of a DNA fragment (PCR product or restriction digestion) into a vector of choice • Screening for a positive clone • 5 and 3 prime end sequencing and alignment to the reference sequence
Customer receives	• Sequence data • Report on positive clones identified • Plasmid DNA of positive clone
Optional Services	• Complete double or single stranded confirmation sequencing of one (or more) positive clones (price according to list) • Endo-toxin free plasmid Maxiprep of a positive clone

Cloning of cDNA or Genomic DNA

Purpose	Cloning of a DNA fragment or a gene of interest into a vector of choice
Customer supplies	• DNA sequence information
Service includes	• Cloning in a vector of choice of the DNA sequence of interest • Complete sequence verification of the isolated clones
Customer receives	• Plasmid DNA of isolated clone • Sequence data
Optional Services	• Endo-toxin free plasmid Maxiprep of a positive clone

Cloning of Short Hairpin RNAs (shRNAs)

Purpose	Cloning of short hairpin RNA into a vector of choice
Customer supplies	• The reference sequence of the vector • The shRNA sequences
Service includes	• Purchase of sh-Oligos • Cloning of sh-Oligos into a vector of choice • Screening for a positive clone • 5 and 3 prime end sequencing and alignment to the reference sequence
Customer receives	• Sequence data • Report on positive clones identified • Plasmid DNA of positive clone
Optional Services	• Endotoxin-free plasmid Maxiprep of a positive clone