



Sanger Sequencing Services



Xcelris is a trusted name in Sanger sequencing when it comes to accuracy, reliability and high quality. Rechristened as XcelSeq, we now offer rapid and high quality DNA sequencing services with online reporting. Till date, we have completed more than 8000 sequencing projects. We perform DNA sequencing services on ABI 3730xl/96 capillary system using Big Dye Terminator v3.1 kit.

SEQ-1 Plasmid/PCR Sequencing Services

Process

- Quality check of Plasmid/PCR by gel electrophoresis
- DNA Sequencing with client specific primer or universal primers using Sanger Sequencing chemistry
- Q20 Read Length upto 800 bases with ABI or SCF, FASTA File with Quality Control reports

TAT: 2-3 Days

SEQ-2 r-*E.coli* Culture Sequencing Services

Process

- Propagation of r-*E.coli* cultures having plasmid DNA in test tubes or in deep well culture plates (96-wells)
- Plasmid DNA isolation using XcelGen kits followed by Quality Check
- Sequencing of DNA with client specific primers using Sanger Sequencing chemistry
- Q20 Read Length upto 800 bases with ABI or SCF, FASTA File with Quality Control reports

TAT: 5-6 Days

SEQ-04 Primer Walk Sequencing

Process:

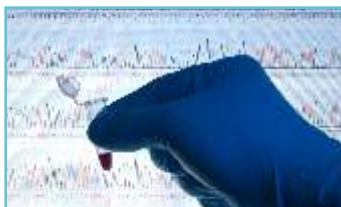
- Primer design and synthesis
- BAC/Fosmid DNA Isolation & sequencing with interim primers
- Universal primers details used for Sequencing
- Q20 Read Length upto 800 bases with ABI or SCF, FASTA File with Quality Control reports

TAT: 10-15 Days

Microsatellite Genotyping

Process

- Assay primer design based on gene sequence from database
- PCR Amplification of desired loci using labelled primers with or without multiplexing
- High throughput fragment analysis of labelled amplicons using ABI 3730xl genetic analyzer system with known standard
- Data capturing and analysis using GeneMapper software



SNP Genotyping by SNaPshot Assay on ABI 3730xl

Process:

- Quality analysis of amplicon
- SNaPshot reaction preparation
- Electrophoresis of samples on ABI 3730xl
- Data analysis with GeneMapper using LIZ100 fragments std

Report consists of SNP identified in each sample along with zygosity information.

SNP Discovery by Sanger Sequencing on ABI 3730xl

Process:

- Assay primer design based on gene sequence
- PCR amplification and purification of amplicon
- High throughput automated DNA sequencing using ABI 3730xl
- Sequence trace alignment and editing

Raw data file consists of chromatogram, FASTA files and report containing SNP list with minor allelic frequency

Microbial Identification Services

Based on sequencing of 16SrRNA gene (bacterial) and ITS/D2 region of 28S Large subunit rRNA gene for phylogenetic analysis

MID-S

- Sequencing of ribosomal genes or other conserved regions upto 1500 bp
- Sequencing data with chromatogram file and FASTA file

MID-C

- Sequencing of ribosomal genes or other conserved regions upto 1500 bp
- Contig report with sequencing data consisting of chromatogram and FASTA file

MID-R

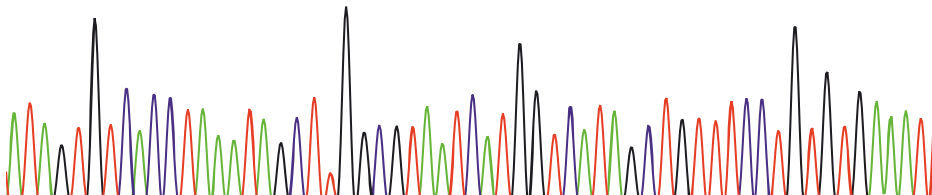
- Genomic DNA extraction from microbial culture & amplification of desired gene by PCR.
- Enzymatic/ Gel based purification of the amplicon.
- Sequence data generation by primer walk using BDTv3.1 chemistry on ABI 3730xl
- Quality check, trimming of low quality sequence from ends and assembly.

Report includes

- Genus and species level identification with top 10 matches and DNA consensus sequence
- Electropherogram, phylogenetic tree and distance matrix using MEGA 4.1
- Organism lineage and taxonomy

TAT: 3-12 Days

ATAGT GTCACCTAAATAGCTTGGCGTAATCAT GGT CAT AGC TGT TTCCTGTGT GAAAT
133 145 157 169 181



Sample Requirements

Template	Sample Requirement	Primer Requirement
SEQ-1 Plasmid/ PCR Sequencing		
Purified PCR Product	20 µl of 10-20 ng/µl per sample	Conc.= 10 pmole/µl (µM), volume - 10 µl and Tm of the primers.
Plasmid	25µl of 30-100ng/µl per sample	
SEQ-2 r-E.Coli Culture Sequencing		
<i>E.coli</i> culture	96-well bacterial culture in 200µl LB media/culture plate/glycerol stock with details of inserted gene size and gene information, vector details	Conc.= 10 pmole/µl (µM), volume- 10 µl and Tm of the primers.
SEQ-4 Primer Walk Sequencing Culture Sequencing		
Culture	Glycerol stock	details of inserted, gene size and gene information, vector details with customer specified primers at conc.= 10 pmole/µl (µM), volume- 20 µl and Tm of the primers.
Purified PCR Product (1-4 kb)	4 to 8 µg	
Plasmid (10 kb)	Insert size upto 3-4 kb	
Microsatellite Genotyping		
Purified PCR	20 µl of 10-20 ng/ µl per sample in 96-well format, allele size information, level of multiplexing, dye used for multiplexing, amplicon size information	
DNA	20µl of 150-200ng/µl of gDNA with quality report, primer information for amplification with standardized conditions for each primer, sizing information and multiplexing details.	
SNP Genotyping by SNaPshot Assay on ABI 3730xl		
PCR Product	20µl of 10-20 ng/µl per sample, SNP information per sample and reference sequence.	
SNP Genotyping by Sanger Sequencing on ABI 3730xl		
gDNA	2-5µg of gDNA with quality report, primer information for amplification of locus in which SNP is to be identified with standardized conditions, SNP information per sample and reference sequence.	
MID - S/C/R Microbial Identification Services		
Culture (Bacteria/ Yeast/ Fungi)	Plate/Slants/Glycerol stock, culture growth conditions (medium / temperature)	
Microbial gDNA	concentration of 100 - 200 ng/µl, vol.-20 µl	
PCR product of 16S/18S rDNA gene	concentration of 20-30 ng/µl, vol. - 20 µl.	

XcelGen[®]
Quality Kits made by **Xperts**

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NXT
NGS Services

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Xcelris Labs Ltd

Old Premchand Nagar Road, Opp. Satyagrah Chhavani, Bodakdev, Ahmedabad-380015, Gujarat, India.
Tel.: +91-79-66197777 | Fax: +91-79-66309341 | e-mail: bdgenomics.corp@xcelrislabs.com
Website: www.xcelrisgenomics.com