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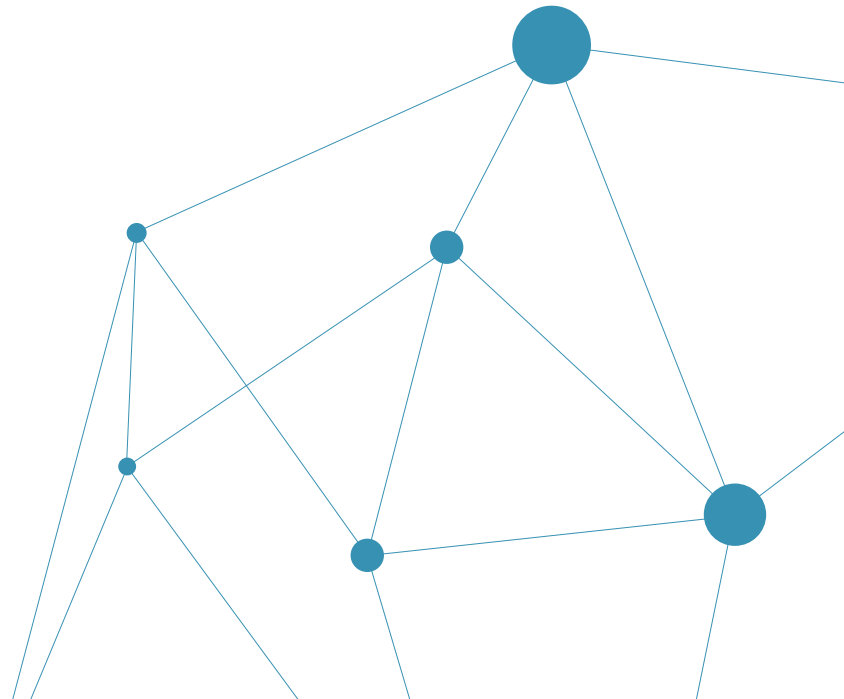
Sequencing

01. Sanger Sequencing Service
02. Next-Generation Sequencing Service(NGS)



01. Sanger Sequencing Service

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Sanger Sequencing Service

Overview

Bioneer started Sequencing Service for the first time in Korea and provides improved high-throughput sequencing service every year. We provide reliable data to researchers with ABI3730XL, automatic gene analysis equipment capable of sequencing various kinds of templates including Plasmid DNA, PCR product, etc. that enables various kinds of template sequencing, and professional personnel.

Additionally, since we are a major oligo manufacturer, our custom oligos can be provided faster than when using other sequencing services: this is especially relevant for primer walking and Virus/Vector sequence validation which can be done faster than other services. Bioneer treats your samples like they are our own, and do our best to provide the best service and deliver satisfaction.

Service Description

Bioneer Sequencing Service is a fast and highly reliable service performed on the ABI 3730XL DNA Analyzer, which provides high-quality of sequence analysis data (Phred Score (QV): ≥ 20, Guaranteed read lengths: ≥ 700 bp) within 24 hr from the arrival of sample. Bioneer's long-standing expertise in oligo synthesis technology supports custom primer order and your DNA sequencing needs.

Features and Benefits

- **Accuracy**

High quality data with accurate results through automated process (Phred Score ≥20, Guaranteed read lengths ≥700bp).

- **Free universal primer**

Supply of commonly used universal primer capable of being selected upon order.

Procedure

Sanger Sequencing Service Information

1. Standard Sequencing

Standard Sequencing Service undergo analysis of desired plasmid or PCR product regions using a primer optimized for most of the normal samples.

2. Full length Sequencing (primer walking)

Full Length Sequencing Service utilizes primer walking to analyze plasmids, long inserts, or PCR products. The service time depends on the size, but it takes about a week for a plasmid being about 3 kb long. The data will be provided in the form of contig file for each single data and assembly while the primer and its design will be sent in a dried form after the analysis.

3. Difficult Sequencing

This product is for analyzing templates difficult to be done using the Standard Sequencing service such as those having GC/AT rich regions, repeated sequences, secondary structures, etc. This service is optimized using our long time experiences and techniques by utilizing different conditions and reagents from the Standard Sequencing Service.

Additional Service Information

Depending on the requested samples, additional services can be chosen.

- Genomic DNA → PCR → Non-purified PCR product → PCR purification/Agarose gel extraction → Purified PCR product
- Genomic DNA → Primer design → PCR optimization → PCR → Non-purified PCR product → PCR purification/Agarose gel extraction → Purified PCR product: **Custom Sequencing**

1. Purification

A purification process must be done to remove impurities prior to the direct sequencing step of PCR products. This is especially crucial when high-quality data is required to eliminate non-specific bands.

① **PCR Purification:** This method uses a spin-column method, which is suitable when it is certain that no non-specific band will occur. This service uses our PCR Purification Kit. (*AccuPrep*® PCR/Gel DNA Purification Kit).

② **Agarose Gel Extraction:** This method extracts DNA fragments from an agarose gel to acquire only the desired bands, which is suitable when non-specific bands occur in the PCR product. This service uses our PCR Purificaiton Kit (*AccuPrep*® PCR/Gel DNA Purification Kit).

2. PCR & Purification

If you send the genomic DNA and specific primers, along with background information such as optimized PCR conditions, product size, etc., we can confirm the size to undergo the suitable purification process after PCR. Primer synthesis service is also available if needed. This service uses our high-fidelity PCR premix (*AccuPower*® *ProFi Taq* PCR PreMix)and PCR Purification Kit.

Ordering Information

Cat. No.	Service Description	Unit
S-3010-1	Standard Sequencing	<96 rxns
S-3010-2	Standard Sequencing	≥96 rxns
S-3010-6	Full Length Sequencing	bp
S-3010-13	Difficult Sequencing Service	rxn

*The sequencing service pricing for the order with more than 2,000 reactions at a time or 20,000 rxns per year can be negotiated.

Cat. No.	Service Description	Unit
S-3010-3	PCR Purification	ea
S-3010-4	Agarose Gel Extraction	ea
S-3010-5	PCR & Purification	ea
S-3010-7	Custom Sequencing Primer Synthesis (Based on 25nmol)	mer

Service Guidelines

After ordering online, send us sample and primer according to following direction.

1. How to order online

① Please login before filling out form to save your data.
② Simply fill out the sample and primer name using the alphabets, numbers, parentheses, and hyphens(-).
③ If you enter all required form and proceed to the payment completion, the order will be completed.

2. Sample Submission Guidelines

① Sample preparation

Type	Concentration	Volume/rxn	Purity
Plasmid DNA	≥100 ng/μl	≥10 μl	$A_{260/280}$: ~ 1.8 $A_{260/230}$: 2.0 ~ 2.2
PCR product	≥50 ng/μl	≥10 μl	
Non-purified PCR product	≥50 ng/μl	≥20 μl	
Genomic DNA	≥30 ng/μl	≥30 μl	

* The data in the above is based on 1 rxn, and please provide us sufficient amount of samples in the 1.5 ml tube or plate for unexpected sequencing circumstances.

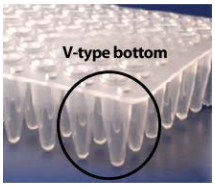
② Primer preparation

Type	Concentration	Volume/rxn	Details
Universal primer	Universal primers are available to use.		
Specific primer	5 pmol/μl (5 μm)	5 μl	Sequencing primer titration conditions: 18~22 mer, Tm 50~56℃
Ordered primer	Sequencing and oligos can be ordered at the same time.		

3. Preparation for Sample Types

① Single tube type: Please label your samples clearly on the top of 1.5 ml centrifuge tubes. Labeled names should match the ones listed on your order sheet. Seal the tube to prevent leakage.

② Plate type: Seal the plate to prevent cross-contamination. Please arrange the samples in sequence vertically (A01→H01).



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Sanger Sequencing Service

4. Shipping Guidelines

① Please place samples and primers in separate bags and write down your name on each bag.

② Please send your samples to us via FedEx (preferred), DHL or TNT. [Shipping address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Sequencing Team]

5. Universal Primer List

Primer name	Sequence (5' → 3')	Length [bases]
T7 promoter	TAA TAC GAC TCA CTA TAG GG	20
T7 terminator	GCT AGT TAT TGC TCA GCG G	19
T3	AAT TAA CCC TCA CTA AAG GG	20
SP6	ATT TAG GTG ACA CTA TAG	18
EBV-R	GTG GTT TGTCCA AAC TCA TC	20
BGH-rev	CTA GAA GGC ACA GTC GAG GC	20
M13F(-40)	GTT TTC CCA GTC ACG AC	17
M13R(-40)	CAG GAA ACA GCT ATG AC	17
M13F(-20)	GTA AAA CGA CGG CCA GT	17
M13R(-20)	GCG GAT AAC AAT TTC ACA CAG G	22
pGEX5	GGC AAG CCA CGT TTG GTG	18
pGEX3	GAG CTG CAT GTG TCA GAG G	19
pQE-forward	CCC GAA AAG TGC CAC CTG	18
pQE-reverse	GTT CTG AGG TCA TTA CTG G	19
EGFP-C	CAT GGT CCT GCT GGA GTT CGT G	22
EGFP-N	CGT CGC CGT CCA GCT CGA CCA G	22
RVprimer3	CTA GCA AAA TAG GCT GTC CC	20
RVprimer4	GAC GAT AGT CAT GCC CCG CG	20
GLprimer1	TGT ATC TTA TGG TAC TGT AAC TG	23
GLprimer2	CTT TAT GTT TTT GGC GTC TTC CA	23
CMV-F	CGC AAA TGG GCG GTA GGC GTG	21
CMV30	AAT GTC GTA ATA ACC CCG CCC CGT TGA CGC	30
CMV24	TAT TAG GAC AAG GCT GGT GGG CAC	24
Gal4AD	TAC CAC TAC AAT GGA TG	17
Gal4BD-F	TCA TCG GAA GAG AGT AG	17
Gal4BD-R	TTT CTT TGG AGC ACT TGA GC	20
MATCHMAK-ER3	GTG AAC TTG CGG GGT TTT TCA GTA TCT ACG AT	32
pBAD-For	ATG CCA TAG CAT TTT TAT CC	20
pBAD-Rev	GAT TTA ATC TGT ATC AGG	18
SV40-pArev	CCT CTA CAA ATG TGG TAT GG	20
SV40-pAF	AAA TAA AGC AAT AGC ATC AC	20
malEF	GGT CGT CAG ACT GTC GAT GAA GCC	24
pCold-F	ACG CCA TAT CGC CGA AAG G	19
pCold-R	TCC CCG CCA AAT GGC AGG GA	20
3AOX	GCA AAT GGC ATT CTG ACA TCC	21
5AOX	GAC TGG TTC CAA TTG ACA AGC	21
pBabe-F	TGA CCT GGG AAG CCT TGG CT	20
pBabe-R	TTG CTG ACT AAT TGA GAT GCA TGC TTT	27
V5_Reverse	ACC GAG GAG AGG GTT AGG GA	20
pJET1.2F	CGA CTC ACT ATA GGG AGA GCG GC	23
pJET1.2R	AAG AAC ATC GAT TTT CCA TGG CAG	24
27F	AGA GTT TGA TCM TGG CTC AG	20
1492R	TAC GGY TAC CTT GTT ACG ACT T	22
518F	CCA GCA GCC GCG GTA ATA C	19
800R	TAC CAG GGT ATC TAA TCC	18
ITS1	TCC GTA GGT GAA CCT GCG G	19
ITS2	GCT GCG TTC TTC ATC GAT GC	20
ITS3	GCA TCG ATG AAG AAC GCA GC	20
ITS4	TCC TCC GCT TAT TGA TAT GC	20
ITS5	GGA AGT AAA AGT CGT AAC AAG G	22
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	25
HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	26
pET-Up-stream	ATG CGT CCG GCG TAG A	16
pBA-F	ATT GTC TCA TGA GCG GAT AC	20
pBH-R	GCG TTA TCC CCT GAT TCT GT	20
pBIC_F	CTC ATG AGC GGA TAC ATA TTT G	22
pBIC_R	GCC GCA GCC GAA CGA CCG AG	20

Technical Support

- E-mail: sequencing@bioneer.co.kr
- Time: GMT +9 AM. 09:00 - PM. 06:00 (Monday - Friday)

Sanger Sequencing Service FAQs

Receipt

1. What do I need to prepare to use this service?

After placing your order, you must prepare the sample and the primer and send them to us.

2. Can I order oligos for sequencing?

Yes you can. You can order them together on the Sequencing order page. After their synthesis, they will be used immediately for the sequencing.

3. Can you provide more information on shipping methods?

Please send the samples to the address below.
(Address: 8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Sequencing Team)

4. What is a re-reaction service?

Re-reaction service provides one more sequence check for samples and primers for those have been previously analyzed, but require another run for further improvements of sequencing results. Data such as sequencing reports can be recorded and delivered for free. After receiving the first report, the re-analysis results can be obtained within two days.

5. What is different from additional analysis and re-reaction service?

Re-reaction, as explained above, uses the same sample and primers for another analysis. While our company guarantees at least 700 base readings, we will provide one more sequencing service for free if the readings are less than that.
On the other hand, additional analysis service provides another sequencing for the same samples, but using different primers. Thus, additional analysis will cost a service fee.

6. I would like to request an additional analysis.

In this case, as we need to keep checking the remaining amount of samples, you must write your initial receipt number in the comment section or select "Previous Order Sample Search" when entering the reaction information.

7. I would like to request sequencing using the primer I have used before.

You must have requested to store the used primers separately at the time of the order. Otherwise, they would be discard after 1 month. To request sequencing using the stored primer, you can select the check box in the order page.

Results

1. How many read lengths can I get through the sequencing service?

We guarantee 700 bp for single extension, but we usually get about 1.2 kb of the reading. If you request both direction analysis, you can receive high quality data up to about 1.4kb.

2. How is the results provided?

Reports will be provided in three formats (ab1/seq/PDF file) with seq merge text file and reports per single extension.

- Ab1 file: Electropherogram file viewable with viewer program
- Seq file: Base calling text file
- PDF file: Ab1 file converted to PDF format.
- Seq merge text file: File combined with analyzed sequence of all the requested samples into a text file.
- Report: A report that contains information and forwarding information on how to analyze and improve results.

3. What do ⓐ, ⓑ, ⓒ, and ⓓ group imply on the sequencing report?

Results in low quality are categorized into four groups depending on the data aspects for easy understanding.

ⓐ Data with low QV values which may be improved through re-analysis which can be done free for once.

ⓑ No electropherogram; this case may be made from the analyst's mistake, so we will undergo re-analysis free for once.

ⓒ Sequencing limited by the DNA sequences; re-analysis service will not be available as improvements cannot be made.

ⓓ Other causes such as primer mis-matches; re-analysis service will not be available as improvements cannot be made.

Payment

1. where can I contact for payment inquiries?

We support online payments. For more information, please contact Customer Support Center (Tel. +82-42-930-8777) and we will kindly reply you.

2. Can I get the printed quotation?

You can simply print it out as you make your online order. Contact our Customer Support Center (Tel. +82-42-930-8777) if you want to issue the quotation separately.
In case of mass ordering, price adjustment is possible.

Sanger Sequencing Service

Etc

1. How long will the analysis take?

Send the result within 48 hours when the reception is completed.

2. What is the retention period for the samples requested?

Samples are kept for one month after the order date.
The primers can be kept, but Bioneer is not responsible for degradation caused by long-term storage. It is recommended to freeze in several tubes and change it every 1 month for long-term usage.

3. How can I use the additional services?

You can choose the desired service according to your requesting sample type when ordering through our online page.

4. Is it possible to collect the remaining sample after analyzing the results?

Yes you may.

5. We would like to know buffers and instruments.

Please follow information below.

◎ Sequencing Process Information

1) Material & Instruments

① *AllInOneCycler*™ 96 well PCR system
(Block PCR instrument, Bioneer)

② BigDye® Terminator V3.1 sequencing kit
(Applied Biosystems® for Thermo Fisher Scientifics, USA)

③ ABI3730XL
(Applied Biosystems® for Thermo Fisher Scientifics, USA)

2) Method

① Sequencing PCR Cyclic

<Refer to table below for quantity of template used in PCR>
- DNA size/ DNA amount

Template	DNA amount
PCR product	
100~200 bp	5~10 ng
200~500 bp	10~20 ng
500 bp~1 kb	20~50 ng
1~2 kb	50~100 ng
≥2 kb	100~200 ng
Double strand	200~500 ng
Cosmid, BAC	300~600 ng
Genomic DNA	2~3 μg

② Purification

③ ABI3730XL Running

02. Next-Generation Sequencing Service(NGS)

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Next-Generation Sequencing Service(NGS)

Overview

Unlike sanger sequencing, NGS(Next-Generation Sequencing) by using high-throughput sequencing technology which can rapidly undergo massive parallel sequencing by fragmenting genes and reading them at once, then using advanced analysis systems to quickly decode genetic information.

Service Description

Bioneer's NGS service provides wide range of NGS applications for various research purposes. We can provide gene decoding and analysis for whole genome, exome, transcriptome, and epigenome. Analysis based on *De novo* sequencing and reference genes can be done not only for humans, but also for various species such as marine animals/plants and bacteria with high GC contents.

Features and Benefits

- Various service for NGS applications and customized research project consultations
- Highly reliable data
- Analysis for basic/customized bioinformatics
- Associated service: Sanger Sequencing, RNA profiling

Procedure

NGS Service Information

1. Whole Genome Sequencing

Whole Genome Sequencing(WGS) is a method that can gain genetic information of the whole genomes of a model organism and human by reading them all at once. By utilizing the data gained through WGS, analysis of various variants such as single nucleotide polymorphisms(SNPs), insertions/deletions (InDels), and copy number variant(CNVs), and structural variation can be done.

2. Whole Exome/Targeted Sequencing

Whole exome sequencing(WES) undergoes selective gene analysis of exons only, which are protein coding regions comprising only 1% of the whole genome (~30 Mb). Targeted sequencing can selectively analyze regions of a specific area of a gene. As the specific kit is used for capturing the target region before undergoing the sequencing process, this not only increases the sequencing depth, but also can be more economic than the whole genome sequencing. This sequencing technology can be also used for clinical researches, diagnostics, and forensics to distinguish variants and genes.

3. Transcriptome Sequencing

RNA sequencing(transcriptome) can provide information for genes activated in a specific point which can be used for quantification and analysis for their structures and functions. Also, this technology can be applied in the fields of general RNA studies, medical researches, pharmacogenetics, and customized medical researches(gene expression profiles, post-translational modifications, SNPs or mutation over time). This service can be also done for total RNA, small RNA(miRNA, tRNA), *de novo* sequencing.

4. Epigenome Sequencing

All epigenetic gene regulation mechanisms for their activation are controlled by DNA methylation and histone modifications. Those can be inherited, but can also be affected by diets, habits, and environments. Epigenetic gene modifications are being widely studied as those have been known to be related to occurrence or progress of various diseases, including cancers. Data gained from those types of analysis can be useful for not only diagnostics, but also surgeries to predict the response and conditions after the treatments or the progress of a disease.

5. Metagenome Sequencing

Metagenome sequencing is not only for a single species or an organism, but for samples collected in a specific environment to analyze their genes to compare the differences in the pattern or interactions of genes for a microbial community. This service is widely used for analysis in clinical researches, biotechnology, or a microbial biogeography of gut microbiota, which have interests of many researchers through 16S rRNA or 18S rRNA, which are widely used as markers for species identification.

Ordering Information

Cat. No.	Service Description
S-3100	Whole Genome Sequencing
S-3110	Whole Exome Sequencing
S-3120	Transcriptome Sequencing
S-3140	Targeted Sequencing
S-3150	Metagenome Sequencing
S-3160	Epigenome Sequencing
S-3170	Bioinformatics Service

Technical Support

- E-mail: NGS_support@bioneer.com
- Time: GMT +9 AM. 09:00 - PM. 06:00 (Monday - Friday)