

F Sequencing

Sequencing Service





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Sequencing Service ●●●

Sequencing Service3

Overview

Bioneer offers our valued customers a high-throughput sequencing service to meet their needs. You can count on Bioneer to provide fast and highly reliable DNA Sequencing data. In order to make it effective Bioneer has an infrastructure of automatic genetic analysis machine ABI 3730XL, which can do sequencing of various template DNA including plasmid DNA, PCR product etc, as well as of well-trained people. 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.

■ 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

- High-reliable Sequence Analysis:
Our Sequencing service provides ≥ 20 Phred Score (QV): as well as ≥ 700 bp of guaranteed read lengths.
- Fast Turnaround Time:
In most cases, the sequence is complete within 24 hours of customer's request.
- Convenience - Universal primer is provided for free:
Over 40 universal primers are provided free-of-charge. Oligo Synthesis service is also available together with DNA sequencing.
- Competitive Pricing:
Bioneer offers the best value for customer's research dollar.

■ BIONEER Sequencing Service Procedure



■ Sequencing Service Information

1. Standard Sequencing Service

The standard sequencing service provides customers with a fast sequencing results of read length up to 700 bp using plasmid DNA or PCR product. Discounted price could be available for orders with more than 2,000 reactions per order or 20,000 reactions per year. Please contact us for details.

2. Full Length Sequencing (primer walking) Service

This service is for sequence analysis of plasmid longer than 2 kb including a long insert or PCR product. Its turnaround time is approximately one week for a 3 kb plasmid and can vary depending on a template length. Assembled analysis data will be delivered to a customer along with an aligned file, single config file as well as custom primers.

3. Difficult Sequencing

This service is aiming for templates that have difficult sequencing such as GC/AT rich, repeat sequence, secondary structure. Difficult sequencing process is optimal with our own know-how and is used for condition of analysis and buffer that are different from standard sequencing.

■ Additional Service Information

This additional service is selectively available for different sample types. All materials that are used for additional service are provided with our own product.

- Cell stock → Cultured cell → Plasmid DNA
- Non-purified PCR product → Purified PCR product

1. Plasmid DNA extraction

It performs plasmid DNA extraction if you send cells that are cultured in liquid media. It could be ordered in stocked cell type and be charged with additional fee.

2. PCR clean-up

① PCR purification:

When direct sequencing of PCR product is performed, PCR clean-up process must be proceeded in advance in order to get rid of impurities. It is important to get rid of non-specific band for obtaining high quality data.

This is a purification method using spin column and is suitable for a case without non-specific band. It performs with PCR Purification Kit (*AccuPrep*® PCR Purification Kit, Bioneer).

② Agarose gel extraction:

This is a separation method for extracted band in agarose gel. It is suitable for a case with non-specific band. It performs with PCR purification kit (*AccuPrep*® Gel Purification Kit, Bioneer).

3. PCR & Purification

The PCR & Purification service reaches PCR (Template and primer including PCR conditions, product size etc. should be sent), size check-up of PCR product, purification to sequencing. Also, Bioneer's primer synthesis service is available. It performs with high fidelity PCR premix (*AccuPower*® *ProFi Taq* PCR PreMix) and PCR purification kit.

■ Ordering Information

Cat. no.	Service Description	Unit	Price
Sequencing Service			
S-3010-1	Standard Sequencing	<96 rxn	\$6.00/rxn
S-3010-2	Standard Sequencing	≥96 rxn	\$5.00/rxn
S-3010-6	Full Length Sequencing	kb	60,000
S-3010-13	Difficult Sequencing Service	rxn	12,000

* 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.

● Service Guidelines

Fill out Bioneer Sequencing Service Order Sheet and send us sample and primer according to following direction.

1. Notice for Sequencing Order Sheet.

- ① Download the "Order sheet" at Homepage (<http://eng.bioneer.com/products/Genomics/SequencingService-overview.aspx>) and fill out customer's information and sample information and etc.
- ② Bioneer recommend that you use simple name for your sample, if the sample/primer name is too long, please mark the tubes and leave a note on the order sheet.
- ③ Email the order sheet to sequencing@bioneer.com

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	Purity
Universal primer	Over 40 free primers available		Over 40 free primers available
Specific primer	5 pmol/μl (5 μM)	5 μl	Sequencing primer: 18-22 mer, T _m 50-56 °C
Ordered primer	Oligo synthesis service is also available together with DNA sequencing		

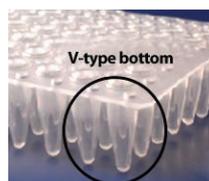
3. Preparation for Sample Types

- 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.

Label on the top of tube



- 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

4. Shipping Guidelines

- Please place samples and primers in separate bags and write down your name on each bag.
- Please send your samples to us via FedEx (preferred), DHL or TNT. When your order is confirmed, you will receive an account number of a carrier service that you chose to create a shipping label.

[Shipping address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Korea]

③ Charge for sample shipment

Bioneer itself pays for the sample shipment with more than 20 rxns per order (The additional shipping cost for the ice or dry ice packing is not covered by us). The shipment can be selectively sent using our account in FedEx (preferred), DHL or TNT, which should be clearly written on the airway bill. This is only available for the package in ambient condition with less than 1 kg.

5. Universal Primer List

Primer name	Sequence (5' -> 3')	Length [bases]
T7 promoter	TAATACGACTCACTATAGGG	20
T7 terminator	GCTAGTTATTGCTCAGCGG	19
T3	AATTAACCCCTACTAAAGGG	20
SP6	ATTTAGGTGACACTATAG	18
EBV-R	GTGGTTTGTCCTAACTCATC	20
BGH-rev	CTAGAAGGCACAGTCGAGGC	20
M13F(-40)	GTTTTCCCAAGTCACGAC	17
M13R(-40)	CAGGAAACAGCTATGAC	17
M13F(-20)	GTAAAACGACGGCCAGT	17
M13R(-20)	GCGGATAACAATTCACACAGG	22
pGEX5	GGCAAGCCACGTTTGGTG	18
pGEX3	GAGCTGCATGTGTGAGAGG	19
pQE-forward	CCCGAAAAGTGCCACCTG	18
pQE-reverse	GTTCTGAGGTCATTACTGG	19
EGFP-C	CATGGTCTGCTGGAGTTCGTG	22
EGFP-N	CGTCGCCGTCCAGCTCGACCAG	22
RVprimer3	CTAGCAAAATAGGCTGTCCC	20
RVprimer4	GACGATAGTCATGCCCGCG	20
GLprimer1	TGTATCTTATGGTACTGTAAGT	23
GLprimer2	CTTTATGTTTTGGCGTCTTCCA	23
CMV-F	CGCAAATGGGCGGTAGGCGTG	21
CMV30	AATGTCGTAATAACCCCGCCCC GTTGACGC	30
CMV24	TATTAGGACAAGGCTGGTGGG CAC	24
Gal4AD	TACCACTACAATGGATG	17
Gal4BD-F	TCATCGGAAGAGAGTAG	17
Gal4BD-R	TTTCTTTGGAGCACTTGAGC	20
MATCHMAKER 3	GTGAACTTGCGGGGTTTTCA GTATCTACGAT	32
pBAD-For	ATGCCATAGCATTTTTATCC	20
pBAD-Rev	GATTTAATCTGTATCAGG	18

SV40-pArev	CCTCTACAAATGTGGTATGG	20
SV40-pAF	AAATAAAGCAATAGCATCAC	20
malEF	GGTCGTCGACTGTGCGATGAA GCC	24
pCold-F	ACGCCATATCGCCGAAAGG	19
pCold-R	TCCCCGCCAAATGGCAGGGA	20
3AOX	GCAAATGGCATTCTGACATCC	21
5AOX	GACTGGTTCCAATTGACAAGC	21
pBabe-F	TGACCTGGGAAGCCTTGCT	20
pBabe-R	TTGCTGACTAATTGAGATGCAT GCTTT	27
V5_Reverse	ACCGAGGAGAGGGTTAGGGA	20
pJET1.2F	CGACTCACTATAGGGAGAGCGG C	23
pJET1.2R	AAGAACATCGATTTTCCATGGC AG	24
27F	AGAGTTTGATCMTGGCTCAG	20
1492R	TACGGYTACCTTGTACGACTT	22
518F	CCAGCAGCCGCGTAATAC	19
800R	TACCAGGGTATCTAATCC	18
ITS1	TCCGTAGGTGAACCTGCGG	19
ITS2	GCTGCGTCTTCATCGATGC	20
ITS3	GCATCGATGAAGAACGCAGC	20
ITS4	TCCTCCGCTTATTGATATGC	20
ITS5	GGAAGTAAAAGTCGTAACAA GG	22
LCO1490	GGTCAACAAATCATAAAGATA TTGG	25
HCO2198	TAACTTCAGGGTGACCAAAA AATCA	26

■ Technical Support & Service Inquiry

- e-mail: sequencing@bioneer.com / sales@bioneer.com
- Time: AM. 09:00 - PM. 06:00 (Monday - Friday)

■ FAQ

[Ordering]

Q: What are required for sequencing?

A: Send us sample and primer, download and fill out sequencing order sheet. Send it to sequencing@bioneer.com as soon as sample arrives it is accepted.

Q: Where can we obtain order sheet?

A: Download at <http://eng.bioneer.com/products/Genomics/SequencingService-overview.aspx>.

Q: What are universal primers that Bioneer offer?

A: Refer to Universal primer list (Page 6).

Q: What is Re-test?

A: Re-test is automatically provided with one time free service for possible improvement test. It is performed with the same sample and primer used in previous test. You can check on result report and receive the result within two days.

Q: What is different from additional reaction and Re-test?

A: Additional reaction is a sequencing using previous test sample and different primer used in previous test. The service will be charged even though the same sample is used. Because re-test is provided with one time free service for possible improvement test, give attention to order.

Q: How can order additional reaction?

A: Fill out previous test sample no. in sequencing order sheet, and send additional order primer.

[Results]

Q: How long is length using sequencing service?

A: In single extension, it is analyzed up to 1.2 kb in general and 700 bp is guaranteed. You can receive high quality data about 1.2 kb in duplex analysis.

Q: How long does it take to analysis?

A: You can receive the result within 48 hours.

Q: What types of the result is offered?

A: You can receive the three formats (ab1/seq/PDF file) of sequencing result per single extension, seq merge text file and report.

- Ab1 file: It is chromatogram file. You can open in such as chromas/ Finch TV.
- Seq file: Seq file is a sequence listing and is open in notepad.
- PDF file: It can be easily confirmed the signal that displays sequence listing.
- Seq merge text file: It is a text file that combined all the analysis sample sequence.
- Report: It is a report that contains information, notification on result analysis and improvement methods.

Q: What are group ①, ②, ③, ④ on sequencing report?

A: Low quality data is divided according to data type so that it can be easily understandable.

① group is possible improvement group through Re-test. It is automatically ordered with one-time free service.

② group is non-analyzed group. It needs to be checked through Re-test. It automatically received one-time free service from Bioneer.

③ group could not get normal service, because of inhibition of analysis with the sample structure. No one-time free service is provided.

④ group does not perform Re-test. It does not improved with the same reaction due to other reasons.

[Others]

Q: Do you keep samples after service performs? If so, how long do you keep them?

A: Yes. Both sample and primer can be divided according to ordered no. keep them up to one month. If you want to order

additional reaction, fill out previous ordered no. We are not responsible for degradation due to long term keeping. We encourage you to exchange every month in case for keeping the primer in a long term period.

Q: How can use the additional service?

A: Please check the order information on order sheet.

Q: We would like to know buffers and instruments

A: Please follow information below.

◎ Sequencing Process Information

1) Material & Instruments

- ① *AllInOneCycler*TM 96 well PCR system (Block PCR instrument, Bioneer)
- ② BigDye[®] Terminator V3.1 sequencing kit (Applied Biosystems, USA)
- ③ ABI3730XL (Applied Biosystems, USA)

2) Method

- ① Sequencing PCR

<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

