Technical Report: Bioneer Custom Gene Synthesis and Related Services

1. Introduction

Bioneer offers a comprehensive suite of molecular biology services, with custom gene synthesis positioned as a foundational capability. Leveraging extensive experience dating back to 1995 and significant in-house oligonucleotide synthesis capacity (*HT-oligo*™ synthesizers), Bioneer provides tailored solutions for constructing synthetic DNA sequences. This report details the technical specifications, variations, underlying technologies, quality control measures, and operational aspects of Bioneer's gene synthesis platform. It also examines closely related services, including codon optimization, cloning, mutagenesis, and integrated workflows for mRNA and protein production, highlighting Bioneer's strategy as an integrated provider for molecular construction and functional analysis.

2. Bioneer Custom Gene Synthesis Platform

Bioneer's gene synthesis platform encompasses a core service with several variations, robust technical capabilities, and flexible options tailored to diverse research needs.

2.1 Core Service Offerings and Variations

Bioneer provides multiple gene synthesis options:

- **Standard Gene Synthesis:** This primary service synthesizes custom DNA sequences based on user specifications, guaranteeing 100% sequence accuracy verified by Sanger sequencing using ABI 3730 automated sequencers. The service aims for rapid, economical delivery while maintaining high quality.
- **Rapid Gene Synthesis Service:** Designed for speed, this service synthesizes genes from 1 to 1,000 base pairs (bp), delivering 1-2 µg of the cloned gene in a plasmid. It guarantees delivery within 5 working days (1-500 bp) or 8 working days (501-1,000 bp). A 40% discount is offered if the guaranteed delivery time is exceeded (excluding complex genes). This service is not applicable for sequences deemed complex (e.g., extreme GC content, repeats), which must use the standard service.
- **AccuGeneBlock Service:** This service provides double-stranded DNA (dsDNA) fragments (100-1,000 bp) delivered as PCR products, suitable for synthetic biology applications requiring linear DNA. The standard deliverable is 500 ng to 1 µg, typically with a 3' A-overhang for TA cloning (blunt-end option available). Quality control involves size verification via agarose gel electrophoresis, not full sequence verification. Turnaround time is 7 working days (100-500 bp) or 10 working days (501-1,000 bp). Complex sequences are generally excluded. Due to the nature of dsDNA fragments, fragments not perfectly matching the reference sequence may be present.

Table 1: Bioneer Gene Synthesis Service Comparison

Feature	Standard Service	Rapid Service	AccuGeneBlock Service
Target Product	Cloned Gene in Plasmid	Cloned Gene in Plasmid	dsDNA Fragment (PCR Product, A-tailing or blunt end)
Gene Length Range	1 bp - 100,000 bp+	1 bp - 1,000 bp	100 bp - 1,000 bp
Avg. TAT	Length-dependent	5 or 8 working days	7 or 10 working days

	(5-30+ days)	(guaranteed)	
QC Method	Sanger Sequencing (100% Accuracy)	Sanger Sequencing (100% Accuracy)	Agarose Gel Electrophoresis (Size)
Deliverable	1-2 µg Plasmid DNA (dried)	1-2 µg Plasmid DNA (dried)	500 ng - 1 µg dsDNA (dried)
Complexity Handling	Yes (potential cost/TAT increase)	Excludes complex sequences	Excludes complex sequences
Pricing Basis	Per bp / Fixed (length-dependent)	Fixed (within Rapid service range)	Fixed (within AccuGeneBlock range)
Key Guarantee	100% Sequence Accuracy	TAT Guarantee (with discount policy) & 100% Sequence Accuracy	Size Confirmation

2.2 Technical Capabilities

Bioneer's platform addresses key technical aspects of gene synthesis:

- **Sequence Length:** The service accommodates sequences from 1 bp up to potentially over 100,000 bp. Orders exceeding 15 kb require manual submission via email.
- **Accuracy Guarantee:** Bioneer guarantees 100% sequence accuracy for plasmid-based synthesis, verified by Sanger sequencing. Proprietary analysis software is used to assess sequencing data quality.
- **Handling Complex Sequences:** The service can synthesize challenging sequences (e.g., high/low GC content >70%/<40%, repeats, homopolymers), although this may increase cost and turnaround time. Methods like gene fragmentation and codon optimization are employed. If a gene proves toxic or inhibitory to *E. coli* growth during synthesis, Bioneer may increase the price (up to 20%), cannot guarantee the standard TAT, and might deliver the product as a PCR fragment instead of a plasmid.

2.3 Underlying Technologies

The synthesis process utilizes established and proprietary methods:

- **Gene Assembly Methods:**
    - **Gibson Assembly:** Used for sequences ≤ 3 kb. This method typically employs enzymes like T5 exonuclease, Phusion DNA polymerase, and Taq DNA ligase to join fragments with overlapping ends.
    - **Fragment Assembly:** A proprietary technology used for sequences from 3 kb up to 100 kb. While specifics are not disclosed, related research mentions Bioneer's Pfu polymerase used in Polymerase Chain Assembly (PCA) workflows, suggesting expertise in this area.
- **Oligo Synthesis:** Bioneer utilizes its in-house *HT-oligo*™ synthesizers for the production of the necessary oligonucleotides.

3. Related Services

Bioneer integrates its gene synthesis platform with several complementary services:

3.1 Codon Optimization Service

Bioneer offers codon optimization to potentially enhance protein expression levels in a specified host organism. This service modifies the coding sequence to use codons preferred by the host, aiming to improve translation efficiency and mRNA stability, and can also help resolve sequence complexities like secondary structures or repeats. The optimization is based on Bioneer's "accumulated know-how" rather than a publicly detailed algorithm. Requests are submitted via email with the sequence and target host information.

3.2 Cloning Service

Synthesized genes are typically delivered cloned into Bioneer's standard pBHA vector (high-copy, Ampicillin resistance) at no extra charge.

- **Bioneer Vectors:** Cloning into a selection of other Bioneer vectors (offering different resistance markers, copy numbers, or functionalities like expression tags or *in vitro* transcription promoters) is available for an additional fee ($50.00) and adds approximately 5 working days to the TAT. (See Table 2 for vector details).
- **Customer Vectors:** Cloning into a customer-provided vector requires ordering the Cloning Service separately and incurs a higher subcloning cost ($200.00) on top of the synthesis price.

Table 2: Bioneer Cloning Vector Summary

Vector Name	Key Features	Resistance	Copy No.	Add'l Cost	TAT Impact	Map/Sequence Links
pBHA	Standard vector	Ampicillin	High	None	None	Map,(https://www.bioneer.co.kr/vector-sequence-pbha-1987bp)
pBHK	High copy	Kanamycin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbhk-1867bp)
pBHC	High copy	Chloramphenicol	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbhc-1853bp)
pBHZ	High copy	Zeocin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbhz-1361bp)
pBLA	Low copy	Kanamycin	Low	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbla-1983bp)
pBLK	Low copy	Kanamycin	Low	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pblk-1848bp)
pBLC	Low copy	Chloramphenicol	Low	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pblc-1834bp)
pBLZ	Low copy	Zeocin	Low	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pblz-1342bp)

pBIC-A	*in vitro* transcription (T7/SP6)	Ampicillin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbic-a-2055bp)
pBT7-N-His	Expression (T7 promoter, N-term 6xHis)	Ampicillin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbt7-n-his-3986bp)
pBT7-C-His	Expression (T7 promoter, C-term 6xHis)	Ampicillin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbt7-c-his-3986bp)
pBT7-N-GST	Expression (T7 promoter, N-term GST)	Ampicillin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbt7-n-gst-4613bp)
pBT7-C-GST	Expression (T7 promoter, C-term GST)	Ampicillin	High	+ $50.00	+ 5 working days	Map,(https://www.bioneer.co.kr/vector-sequence-pbt7-c-gst-4614bp)

3.3 Mutagenesis Service

Bioneer offers site-directed mutagenesis, which can be combined with gene synthesis to generate specific sequence variants (e.g., point mutations, insertions, deletions). Customers need to provide sequence information for both the wild-type and desired mutant forms. Introducing small internal sequence changes (≤ 20 bp) during synthesis costs $200.00, equivalent to point mutation pricing.

3.4 mRNA Synthesis Service

Synthesized genes can serve as templates for Bioneer's custom mRNA synthesis service. This service produces various mRNA types and includes options for critical modifications like 5' capping and 3' poly-A tailing. Combined packages ("Gene & Standard mRNA Synthesis" or "Gene & Complete mRNA Synthesis") streamline the process for customers needing both the DNA template and the final mRNA product.

3.5 Gene to Protein Synthesis

This integrated service provides an end-to-end solution, delivering both the sequence-verified gene and the purified protein product. The service aims for a minimum turnaround time of two weeks and utilizes Bioneer's expression vectors and protein synthesis platforms (*ExiProgen*™ automated system or *AccuRapid*™ manual kit).

4. Operational Details

Bioneer provides clear information regarding the operational aspects of its gene synthesis services:

- **Ordering:** Orders are typically placed online via Bioneer's website after login,

requiring sequence input and quote generation. Orders placed before 4 PM KST begin synthesis the same day. Large genes (>15 kb) require submission of an order form via email.

- **Pricing:** Costs are primarily based on gene length, with tiered pricing per base pair or fixed prices for shorter fragments. Additional costs apply for non-standard vectors, complex sequences, or order modifications.
- **Turnaround Time (TAT):** Average TAT for the standard service varies significantly with length (5-10 working days for ≤1,200 bp, up to 25-30 working days for 3-15 kb). Faster, guaranteed TATs apply to the Rapid service (5 or 8 days), and specific TATs apply to AccuGeneBlock (7 or 10 days). TAT can be extended by sequence complexity or vector choice.
- **Deliverables:** The standard deliverable is 1-2 µg of lyophilized plasmid DNA (0.5-1 µg for low-copy vectors) containing the sequence-verified gene, usually in pBHA. AccuGeneBlock delivers 500 ng - 1 µg of dried dsDNA fragment. A QC report with sequencing data is included. Plasmid Increase services for larger quantities are available.
- **Quality Control (QC):** 100% sequence accuracy is guaranteed for plasmid-based synthesis, verified by Sanger sequencing. AccuGeneBlock QC involves size verification by gel electrophoresis.
- **Order Change/Cancellation Policy:** Due to the immediate start of synthesis, changes incur fees ($50.00/end for ≤9 bp changes; $200.00 for internal changes ≤20 bp). Cancellations result in charges (50% within 5 days; 80% after 5 days).

5. Conclusion

Bioneer offers a robust and versatile custom gene synthesis platform, characterized by a 100% sequence accuracy guarantee for plasmid-based synthesis, the capacity to handle a wide range of gene lengths and complexities, and multiple service tiers (Standard, Rapid, AccuGeneBlock) to meet diverse needs regarding speed and product format. The platform is supported by established assembly technologies like Gibson Assembly and proprietary methods for longer constructs. Crucially, Bioneer integrates gene synthesis seamlessly with related services, including codon optimization, cloning into a wide array of proprietary vectors, site-directed mutagenesis, and downstream production of mRNA or purified protein. This integrated approach, combined with clear operational procedures and quality control measures, positions Bioneer as a comprehensive solution provider for researchers requiring synthetic DNA constructs and their functional derivatives.