High-Fidelity Metagenomic Workflows Optimize 16S rRNA Gene Profiling and Genomic Assembly within Complex Salmonid and Aquaculture Matrices

KURA biotech

Codebreaker

Alejandro Bisquertt[1], Catalina Lorca[1] Francisco Fuentes[1], Javier Gimpel[2], Isidora Arriagada[2] Isa Marx[2], Tamara Akentjew[2], Mauricio Vargas[2], M. Rozas[2].
[1]CodeBreaker Bioscience, Santiago, Región Metropolitana, Chile . [2]Kura Biotech, Puerto Varas, Los Lagos, Chile

Introduction

Deciphering salmonid microbiomes is critical for optimizing teleost physiology, nutrition, and identifying ecosystem health indicators. However, these aquaculture ecosystems remain uncharacterized; baseline taxonomic presence and expected community structure are virtually unknown. This unmapped space presents severe analytical bottlenecks due to uncultivable taxa and marine PCR inhibitors, where conventional amplification introduces systemic taxonomic bias and distorted genomic representation. To explore this uncharacterized biosphere, we evaluate DeCodiFi™ High-Fidelity Polymerase for 16S rRNA gene profiling and shotgun metagenomics. This streamlined optimization delivers superior community architecture and high-quality MAGs via an inexpensive, less time-consuming protocol.

Objective

We benchmarked DeCodiFi™ workflows against standard amplification methods utilizing challenging aquaculture matrices by quantifying:

- 16S rRNA Profiling: Amplification yields, primer-dimer formation workflow processing steps, and phylum-level taxonomic fidelity.
- Shotgun Metagenomics: Metagenome-Assembled Genome (MAG) assembly quality based on completeness, contamination and contiguity (N50).

Methods

16S PROFILING
Samples used: Salmon gut mucosa and skin, biofilter water, and marine sediment near salmon farms.
Methods compared: Quick-16S NGS Library Prep Kit vs. homebrew amplification method using DeCodiFi™ and custom-designed primers.
Evaluation: PCR amplification assessed by Fragment Analyzer and phylum-level relative abundance obtained from Illumina sequencing data. Amplicon sequence variants (ASVs) were generated using AmpliSeq, and taxonomy was inferred using the GTDB r220 database.

SHOTGUN SEQUENCING
Samples used: Marine sediment near salmon farms.
Methods compared: Illumina Enhanced PCR Mix and DeCodiFi™ All-In-One Mix.
Evaluation: Order-level relative abundance obtained from Illumina sequencing data, and assessment of assembled genome completeness, contamination, genome size, and contig N50. The BugBuster workflow was used, and MAG taxonomy was inferred using the GTDB r220 database.

16S Profiling using DeCodiFi™ High-Fidelity Polymerase

Amplification of the V3–V4 region in marine sediment prior to cleanup is shown in **Figure 1.** DeCodiFi™ (0.1–0.2 μM primers) yielded a clear peak at the expected size (606–610 bp), while the 16S Zymo kit showed a dominant peak at ~63 bp, consistent with primer dimers (**Figure 1**). Although Table 1 indicates higher yield for the 16S Zymo kit, this is likely driven by dimer formation. In **Figure 2,** fragment analyzer results of the pooled samples are presented. In general, DeCodiFi™ did not require extra cleanup steps to remove primers compared to the 16S Zymo kit, reducing processing time. DeCodiFi™ with 0.2 μM primers showed the highest peak intensity at the expected size

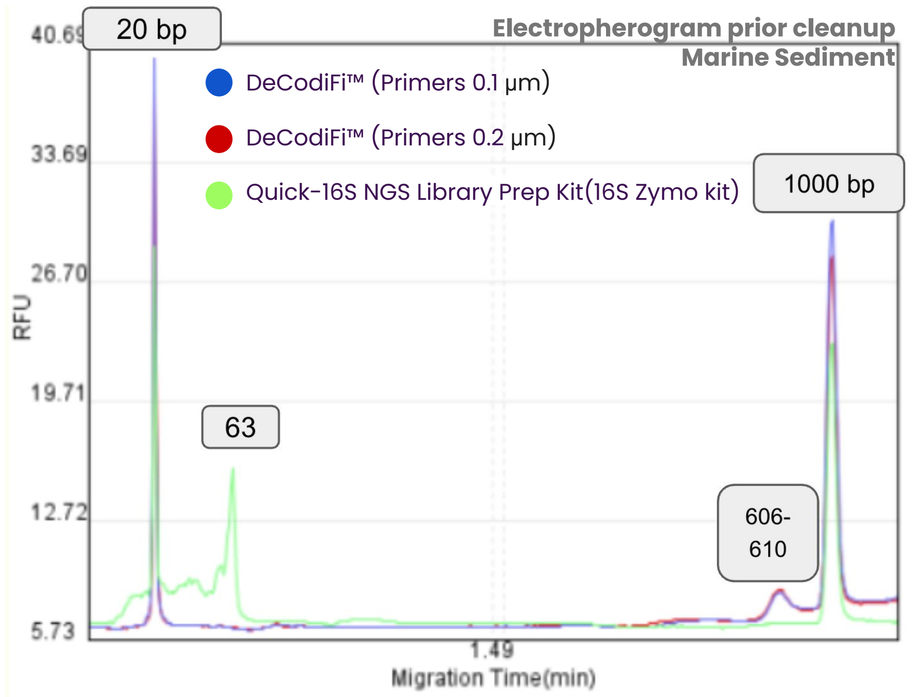

Figure 1. Amplification of marine sediment sample
A signal at ~606–610 bp is observed for DeCodiFi™ , while a peak at ~63 bp and ~606–610 bp is observed for the 16S Zymo kit.

Table 1. PCR quantification of a marine sediment sample.
DNA concentration (ng/μL) before (input DNA) and after PCR amplification (post-PCR DNA) using DeCodiFi™ (0.1 and 0.2 μM primers) and the 16S Zymo kit. Input DNA was 1.83 ng/μL. Post-PCR DNA concentrations are higher with DeCodiFi™ using 0.1 μM primers and the 16S Zymo kit.

Enzyme	Input DNA (ng/μL)	Post-PCR DNA (ng/μL)
DeCodiFi™ (Primers 0.1 μm)	1.83	12.3
DeCodiFi™ (Primers 0.1 μm)	1.83	20.4
16s Zymo kit	1.83	22.4

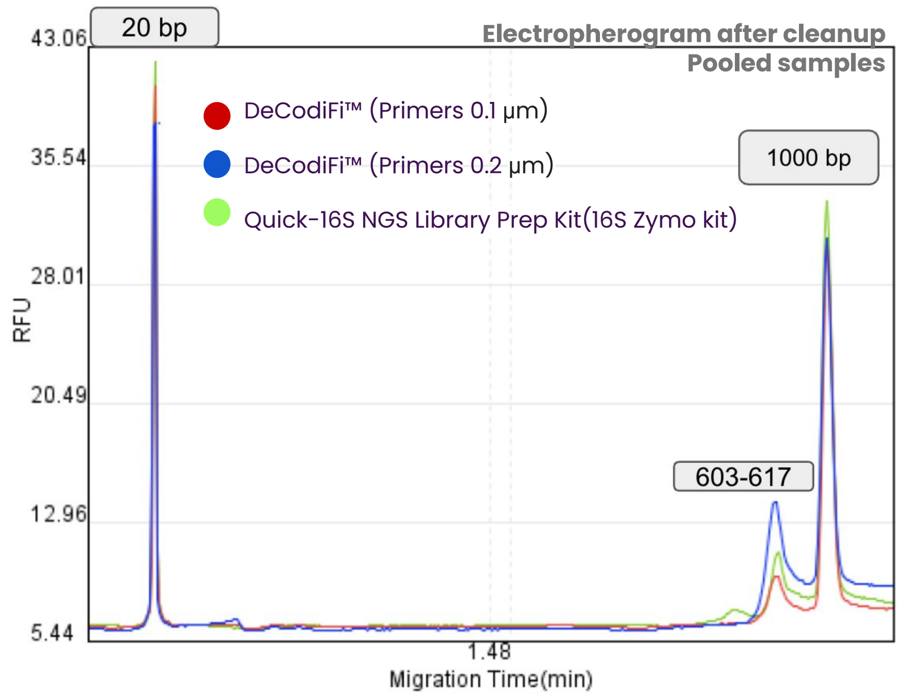

Figure 2. Amplification of the pooled samples.
A signal at ~603–617 bp is observed, with higher intensity for DeCodiFi™ using 0.2 μM primers. The 16S Zymo kit also showed a signal at the expected size. Zymo required prior cleanup steps to remove the primer dimers

Relative abundance of two marine sediment samples obtained with the 16S Zymo kit and DeCodiFi™ High-Fidelity Polymerase is shown in **Figure 3.** Both methods display similar taxonomic profiles at the phylum level, indicating consistent community composition between the two approaches

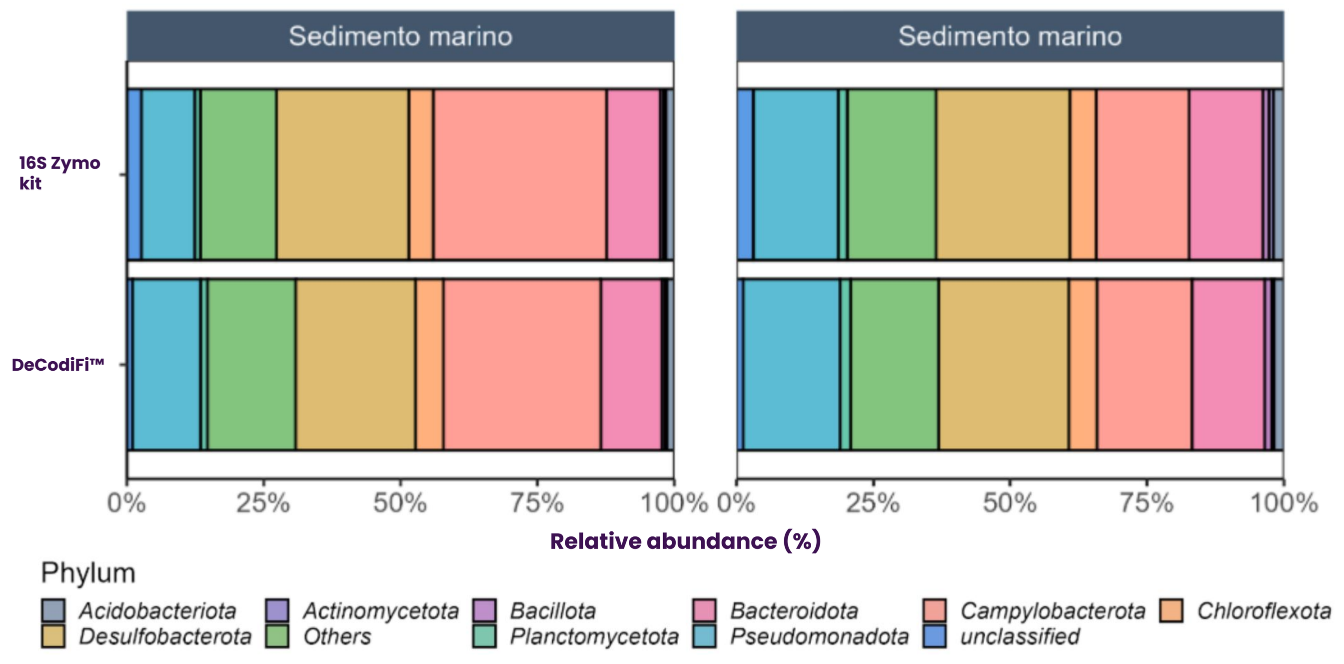

Figure 3. Relative abundance of two marine sediment samples.
A total of 10 phyla were identified using two 16S amplification methods: the 16s Zymo kit and DeCodiFi™

Shotgun sequencing: Higher completeness and lower contamination of MAGs with DeCodiFi™

Two amplification methods were used: the standard (Illumina Enhanced PCR Mix) and DeCodiFi™ All-In-One Mix. By pooling data from five marine sediment samples, 18 MAGs were identified with both methods; however, DeCodiFi™ yielded higher genome completeness (95.8%) and lower contamination (1.1%) than the Illumina Enhanced PCR Mix, as shown in **Figure 4.** Distribution of MAGs is the same between both amplification methods (**Figure 5**).

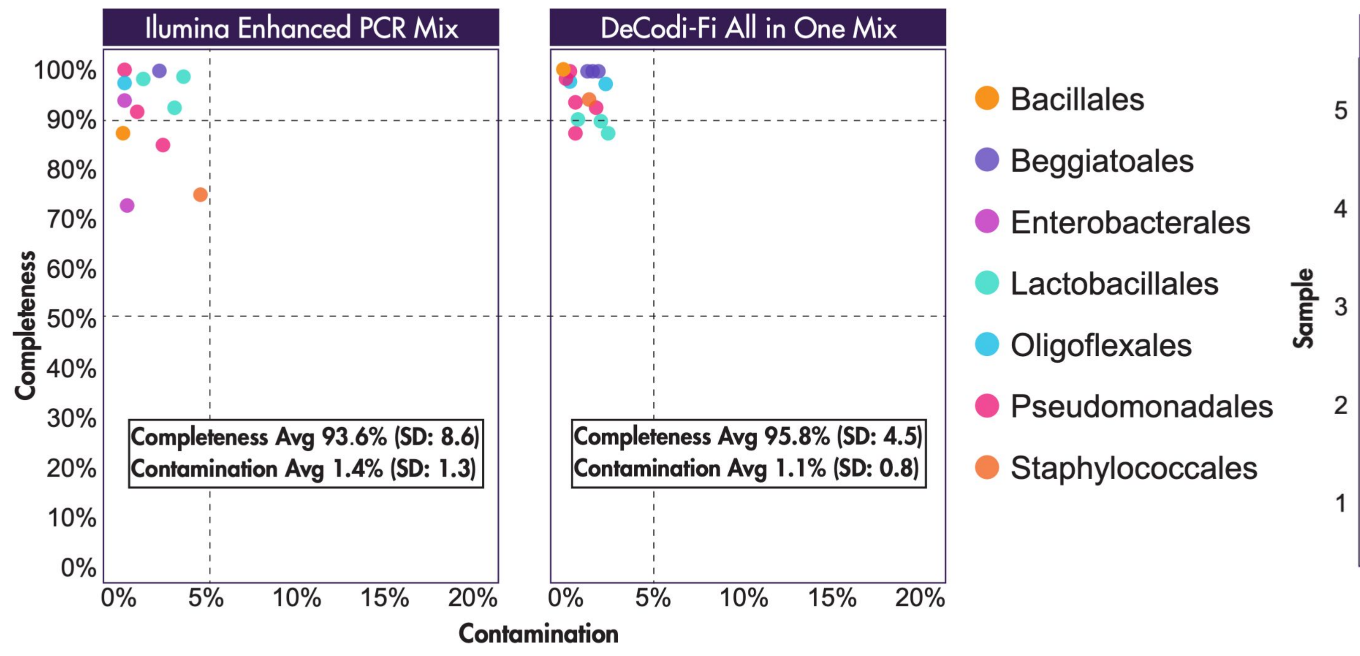

Figure 4: Completeness and contamination of 36 refined MAGs from five pooled samples: 18 amplified with Illumina Enhanced PCR Mix and 18 with DeCodiFi™ All-in-One Mix. Colors indicate MAG orders. Avg=Average. SD=Standard Deviation

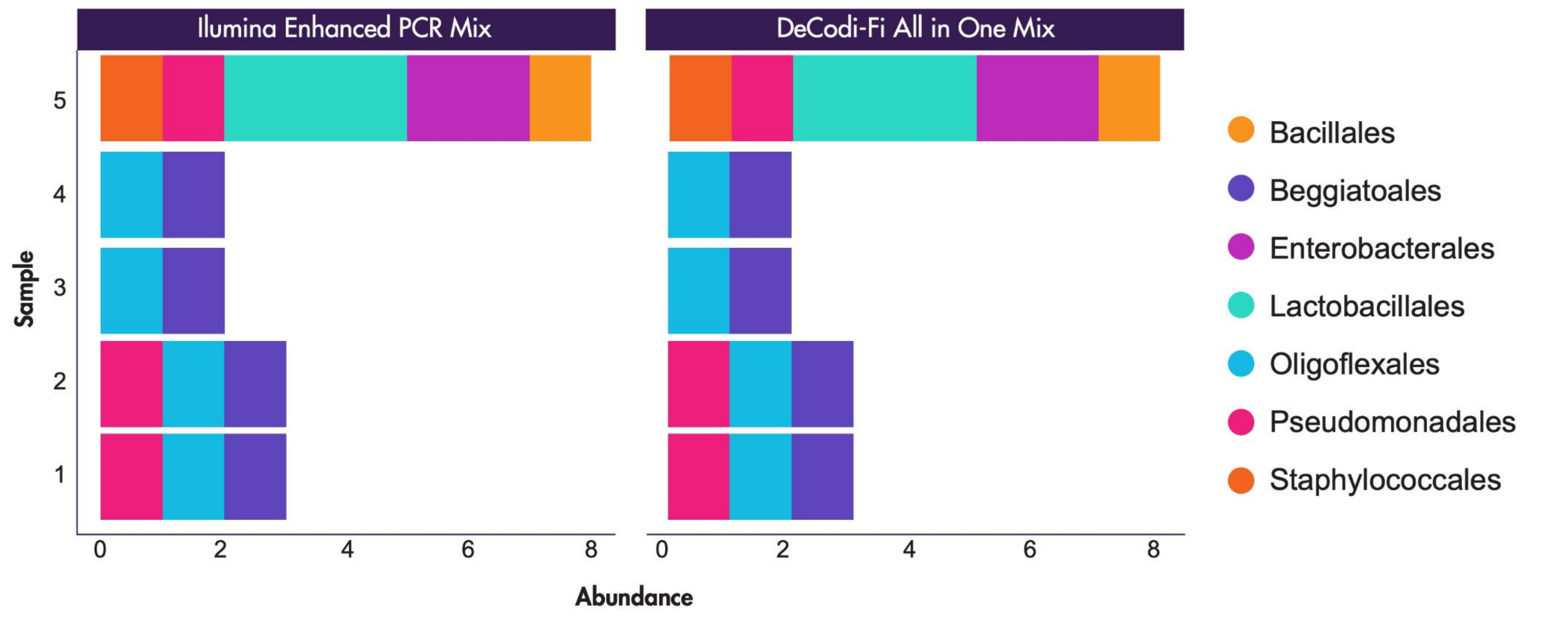

Figure 5: Distribution of MAGs at the order level across five enriched-sediment samples amplified with Illumina Enhanced PCR Mix and DeCodiFi™ All-in-One Mix.

DeCodiFi™ Polymerase: An Effective Choice for good Quality Genome Assemblies

MAGs with completeness below 90% (in both treatments) and one Beggiatoales MAG, included as an Order of interest, are shown in **Table 2.** DeCodiFi™ consistently achieved higher completeness, larger genome sizes, and more total coding sequences.

Table 2: This table compares seven MAGs: six with completeness below 90% (from both treatments) and one Beggiatoales MAG. Genome size, total coding sequences, completeness, contamination, and Contig N50 are presented for each treatment to assess assembly quality. Parameters in which DeCodiFi™ outperforms are highlighted

MAGs	Amplification Method	Genome Size (bp)	Total Coding Sequences	Completeness (%)	Contamination (%)	Contig N50 (bp)
Pseudomonadales	Illumina Enhanced PCR Mix	3,113,425	2,944	84.92	2.26	10,245
	DeCodi-Fi All in One Mix	3,232,124	2,991	88.29	0.66	10,075
Bacillales	Illumina Enhanced PCR Mix	3,445,592	3,456	87.26	0.02	294,549
	DeCodi-Fi All in One Mix	3,971,685	3,967	100	0.02	269,899
Enterobacterales	Illumina Enhanced PCR Mix	3,317,986	3,043	72.44	0.28	93,660
	DeCodi-Fi All in One Mix	4,340,522	4,024	92.61	1.73	83,269
Lactobacillales	Illumina Enhanced PCR Mix	1,765,439	1,972	92.45	2.87	6,515
	DeCodi-Fi All in One Mix	1,586,692	1,726	87.05	2.42	8,742
Lactobacillales	Illumina Enhanced PCR Mix	2,915,935	3,148	98.91	3.38	12,170
	DeCodi-Fi All in One Mix	2,626,467	2,803	89.92	2	12,468
Staphylococcales	Illumina Enhanced PCR Mix	2,162,581	2,670	74.53	4.36	2,424
	DeCodi-Fi All in One Mix	2,395,142	2,415	93.8	1.4	9,987
Beggiatoales	Illumina Enhanced PCR Mix	6,649,855	5,260	100	2.01	27,666
	DeCodi-Fi All in One Mix	6,786,311	5,356	100	1.77	26,811

Conclusions

Accurate profiling and robust genomic reconstruction are critical to characterize uncultivable aquaculture taxa driving teleost health, nutrition, and bioremediation. Within inhibitor-dense matrices, DeCodiFi™ optimized throughput by mitigating primer-dimer artifacts, eliminating post-PCR purification steps and potential errors while maintaining strict taxonomic fidelity. Crucially, in shotgun metagenomics, DeCodiFi™ recovered the identical 18 MAGs at equivalent abundance as standard methods, yet delivered significantly higher-quality assemblies that facilitate deeper downstream genomic characterization.

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