The MGI Automation System Integrated with DNBSEQ Platform Enables Environmental DNA detection

The ATOPlex Technology Compatible with MGISP-960 and DNBSEQ-E25 & DNBSEQ-G99 Facilitates Fish Diversity Monitoring

MGI has developed environmental DNA (eDNA) metabarcoding sequencing package based on ATOPlex technology. This study comfirmed that this sequencing package is capable of accurate identification and dynamic monitoring of fish ecological communities in natural aquatic environments based on simulated fish community samples and real environmental samples.

Recommended applications: Fish ecology, eDNA detection

Recommended models: DNBSEQ-E25RS, DNBSEQ-G99ARS (Sequencing platform)

MGISP-NE32RS, MGISP-960RS, MGISP-100RS (Automated system)

- ATOPlex technology can fully enables fish ecology research

  MGI's unique ATOPlex multiplex PCR technology can meet the needs of detecting different eDNA. It can also detect trace amounts of eDNA with high sensitivity, suitable for monitoring rare species and early invasive species.

- MGI's automation system is highly compatible with the whole process of nucleic acid extraction and library preparation

  MGISP automated systems such as MGISP-NE32, MGISP-960, and MGISP-100 can accurately, stably, and efficiently complete the whole process of nucleic acid extraction and library preparation, ensuring the uniformity and accuracy of libraries.

- Efficient and high-quality sequencing data output

  DNBSEQ sequencing technology exhibits many outstanding features such as high accuracy, low duplication rate and low index hopping rate. DNBSEQ-E25 is portable and lightweight, and PE150 sequencing can be completed within 20 hours. DNBSEQ-G99 can finish PE150 sequencing in just 12 hours and PE300 sequencing in 30 hours, respectively.

- Ultra-fast and full-process solution

  MGI's eDNA metabarcoding sequencing package covers nucleic acid extraction and library preparation kits, automation systems, DNBSEQ sequencing platforms and bioinformatics analysis software. The whole process from DNA extraction to bioinformatics analysis can be completed in as little as 3 days. Compared with traditional morphological identification methods, this eDNA sequencing package has many advantages such as no damage to the organism and no limitation of the target species' life stage, fully empowering fish ecology research.

Background

Environmental DNA (eDNA) sequencing is a rapidly emerging method for studying biodiversity and monitoring ecosystem changes. As organisms interact with their surroundings, DNA from the organism continuously enters the surrounding environment in the form of blood, tissue, mucus, cells, scales, etc., and forms eDNA[1]. Each organism has a unique DNA sequence or barcode called metabarcoding. This DNA barcode is a highly variable region between conserved genomic regions and can be used for biodiversity assessment[2]. Common barcodes include prokaryotic 16S ribosomal DNA (for bacteria and archaea), eukaryotic 18S ribosomal DNA (for various eukaryotes, such as plants, protozoa, and fungi), eukaryotic ITS ribosomal DNA (for fungi), mitochondrial COI gene (for various eukaryotes, such as animals and protozoa), and mitochondrial 12S DNA (for fish)[3,4]. In eDNA metabarcoding technology, PCR is utilized to amplify these specific barcode regions , followed by massively parallel sequencing (MPS) to obtain a comprehensive view of biodiversity[4].

Through the analysis of eDNA, information about target species can be obtained without damage to the ecosystem[5]. Compared with traditional morphological monitoring methods, such as underwater visual inspection and catching[6], eDNA sequencing is a non-invasive detection method with low professional requirements[7]. In fact, eDNA sequencing is a highly efficient, sensitive, and relatively simple solution for aquatic biological monitoring[8].

In recent years, non-local fish's increasing invading is threatening local rare species[9], and the global freshwater and marine ecology is facing huge challenges. eDNA sequencing is applicable as a non-invasive genetic monitoring tool for various fields of fish biology[6,10]. Some high-quality fragments with high species identification rate have been screened out. Among them, MiFish is a short fragment from the fish mitochondrial 12S rRNA gene, with an amplification length of about 170 bp[11], capable of detecting hundreds of fish species.

In this study, MiFish (171bp) and 12S (385bp) barcodes are utilized for the rapid and accurate analysis of fish community structure. MGI provides the products package for eDNA fish diversity detection, from automated extraction and library preparation, DNBSEQ sequencing to bioinformatics analysis. The self-developed MGISP-NE32, MGISP-100 & 960 could meet the demands of automatic handling of nucleic acid extraction and library preparation, respectively. The DNBSEQ-E25 sequencer utilizes a unique self-luminescence sequencing technology that generates self-luminescence signals without the need for an external excitation light source. The accompanying microfluidics based flow cell integrates CMOS modules, enabling rapid reading of DNA sequences. Thus, the DNBSEQ-E25 can efficiently support research in various fields in ordinary laboratory environments and also in mobile scenarios such as airports, customs, and field investigation. DNBSEQ-G99, as one of the fastest medium and small throughput sequencers, is based on MGI's DNBSEQ sequencing technology and can produce high-quality and efficient data in a short time, meeting the research needs of eDNA detecting.

MGI provides a complete end-to-end solution for fish biodiversity monitoring. The procedure includes: 1) DNA extraction using MGIEasy Stool Microbiome DNA Extraction Kit on MGISP-NE32; 2) Library preparation using ATOPlex library preparation workflow on MGISP-100 or MGISP-960; 3) DNA sequencing on DNBSEQ-G99 or DNBSEQ-E25; 4) Bioinformatics analysis on MetaSIS software.

To evaluate the feasibility and performance of MGI's MiFish and Ac12S barcode primers for fish diversity monitoring, this study used simulated fish communities and environmental water samples for evaluation. Identification and diversity monitoring of fish species were achieved by performing the whole process from sample extraction to bioinformatics analysis.

Materials and methods

Sample collection and DNA extraction

Simulated fish communities preparation

The fish muscle tissue used in this study was provided by cooperator and DNA was extracted from 10~20 mg of fish muscle tissue using the MGIEasy Magnetic Beads Genomic DNA Extraction Kit. The detailed procedures can be referred to in the animal tissue extraction protocol. The extracted DNA was quantified by Qubit Fluorometer and its purity was determined by A260/280.The full-length mitochondrial 12S rDNA of each fish was amplified with primers (Table 1) and quantified, followed by mixing with designed ratio to simulate 3 different fish communities(Table 2).

Primer name	Sequence (5′ → 3′)	Expected amplicon length (bp)
MifishU-Forward	GTCGGTAAAWCTCGTGCCAGC	670-740
AcMDB-Reverse	GTACACTTACCATGTTACGACTT	

Table 1. PCR primers for fish full-length mitochondrial 12S rDNA amplification[12].

DNA extraction from eDNA sample

Real samples (0.6~5 L/sample) were collected from East Lake (Wuhan, China). The vacuum filtration apparatus (enriched filter membranes stored at -20 ℃ and DNA preservation reagents can be added to extend the storage time) or eDNA sampler (enriched filter membranes stored at room temperature) was used to collect and enrich the water sample. All filter membrane were transferred to 5 mL centrifuge tubes for DNA extraction within 7 days.

Subsequently, the MGIEasy Stool Microbiome DNA Extraction Kit was used to extract DNA from the filter membrane, following the procedure for water samples. The samples were extracted by magnetic bead-based method , followed by measuring of the concentration, purity and DNA integrity. The beads binding, purification, washing, and PB elution processes were performed on the MGISP-NE32. In this test, the manual DNA extraction process was also carried out parallelly to evaluate the feasibility of the automated extraction solution.

Library preparation and sequencing

In this study, the library input was50 ng for all real samples (recommended input: 50-100 ng) and 1 ng for simulated samples. Libraries were prepared using the ATOPlex MiFish/Ac12S mtDNA Library Prep Set, which contain two PCR steps and two purification steps. The first PCR step used ATOPlex MiFish or Ac12S mtDNA primer pool to amplify the target fragment with 30 cycles. Detailed instructions could be referred to related manuals. The above steps can be completed on MGISP-960 or MGISP-100 with corresponding scripts. In addition, manual library preparation was performed in parallel to evaluate the feasibility of the automatic library preparation solution. Subsequently, different libraries were mixed in equal mass. The MiFish libraries were prepared into DNA nanoballs (DNBs) using the DNBSEQ OneStep DNB Make Reagent Kit. Ac12S mtDNA libraries were circularized into single-stranded circular DNA (ssDNA) using the MGIEasy Dual Barcode Circularization Kit and then DNBs were made through a standard rolling circle amplification (RCA) process. Before sequencing, these DNB samples

NO.	Order	Family	Species	Environment	Expected abundance-1	Expected abundance-2	Expected abundance-3
1	*Perciformes*	*Sparidae*	*Acanthopagrus schlegelii*	Seawater	15.16%	3.79%	1.94%
7	*Cypriniformes*	*Cyprinidae*	*Carassius cuvieri*	Freshwater	2.61%	21.00%	17.19%
9	*Anabantiformes*	*Channidae*	*Channa maculata*	Freshwater	6.14%	14.73%	15.92%
6	*Cypriniformes*	*Xenocyprididae*	*Ctenopharyngodon idella*	Freshwater	10.03%	10.32%	13.03%
8	*Cypriniformes*	*Cyprinidae*	*Hypophthalmichthys nobilis*	Freshwater	4.67%	11.47%	9.88%
2	*Acanthuriformes*	*Sciaenidae*	*Larimichthys crocea*	Seawater	16.04%	4.01%	2.01%
3	*Perciformes*	*Lateolabracidae*	*Lateolabrax maculatus*	Seawater	16.88%	4.24%	6.13%
10	*Cichliformes*	*Cichlidae*	*Oreochromis niloticus*	Freshwater	4.54%	18.49%	15.98%
4	*Acanthuriformes*	*Sciaenidae*	*Sciaenops ocellatus*	Seawater	11.90%	3.97%	5.95%
5	*Scorpaeniformes*	*Scorpaenidae*	*Sebastiscus marmoratus*	Seawater	12.02%	7.99%	11.97%

Table 2. The list of 10 fish species and relevant abundance for preparing simulated fish communities

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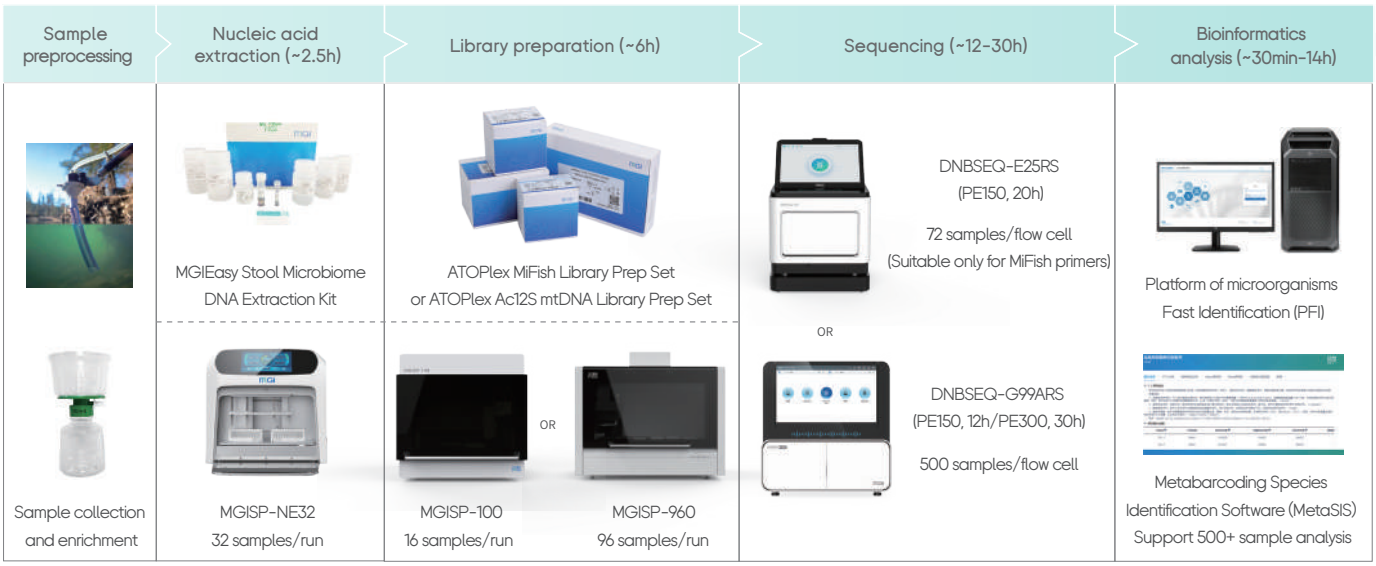
were spiked with DNBs made by the standard library reagents in a certain ratio. For DNBSEQ-E25, the mass ratio of the sample library to the standard library reagent (PCR product) V4.0 is 2:1. For DNBSEQ-G99, the mass ratio of the sample library to the ATOPlex E450 double-label balanced library reagent is 4:1. Then, these mixed DNBs were loaded onto the flow cell of DNBSEQ-E25 (PE150) or DNBSEQ-G99 (PE150/PE300) for subsequent sequencing.

In this study, the MiFish libraries were sequenced by DNBSEQ-E25/DNBSEQ-G99 (PE150), and the Ac12S mtDNA libraries were sequenced by DNBSEQ-G99 (PE300).

Bioinformatics analysis

The MGI Metabarcoding Species Identification Software (MetaSIS) was utilized for bioinformatics analysis in this study, which can perform OTU analysis, species composition analysis (including species distribution stack plots, Krona charts, etc.), Alpha diversity (including Shannon-Wiener curves, rank abundance curves, etc.), and Beta diversity (including inter-group distance analysis, principal component analysis, etc.) based on metagenomic sequencing data. After sequencing, each set of data was accurately down-sampled to 50,000 reads for analysis. For genetic sequencers with built-in bioinformatics modules, such as DNBSEQ-G99ARS, FastQ files can be analyzed directly in the installed MetaSIS software. For genetic sequencers without built-in bioinformatics modules, like DNBSEQ-E25, the bioinformatics analysis process can be performed using Platform of microorganisms Fast Identification (PFI, with MetaSIS software installed). The analysis process is as follows: reads merging, tags filtering, chimera removal, operational taxonomic unit (OTU) clustering and filtering, generating OTU table (this software clusters reads with an identity of 100% into one OTU), and then using the QIIME 2 software to compare and annotate the OTUs with annotation databases, performing species composition analysis, Alpha diversity analysis, and Beta diversity analysis subsequently[13].



Results

High-quality DNA can be extracted from aquatic environment with MGI nucleic acid extraction kit

The researchers utilized relevant kits from MGI and Vendor X to extract DNA from samples collected from the same sampling site of the East Lake of Wuhan to evaluate the quality of the MGIEasy Stool Microbiome DNA Extraction Kit for extracting DNA from real aquatic environment

The DNA yield of the sample extracted with this kit reached 5720 ng (qualified yield > 100 ng) (Fig. 1A), and A260/280 was ~1.8, indicating high purity (Fig. 1B). Electrophoresis results showed that DNA size was as expected, without obvious degradation (Fig. 1C).

Further analysis showed that the sequencing quality of the constructed library based on this kit was high, with Q30 reaching 97.31%. Feature reads is the sequence used for subsequent ASV (OTU, feature) analysis. The feature reads rate was as high as 78.65%. OTU number is the number of operational taxonomic unit (OTU) clusters. In this study, the sequences with 100% similarity were clustered into one OTU, and the number of OTUs was 70. The OTU sequences were compared and annotated with the annotation database to obtain the species identification results at different taxonomic levels, including kingdom, phylum, class, order, family, genus and species numbers. The number of species obtained by MGI's solution was 22. The above sequencing and analytical parameters of this kit were slightly better than those of the Vendor X's extraction kit (Fig. 1D).

Conclusively, the MGIEasy stool genomic DNA (meta) extraction kit has high extraction quality and was comparable to the kit of Vendor X.

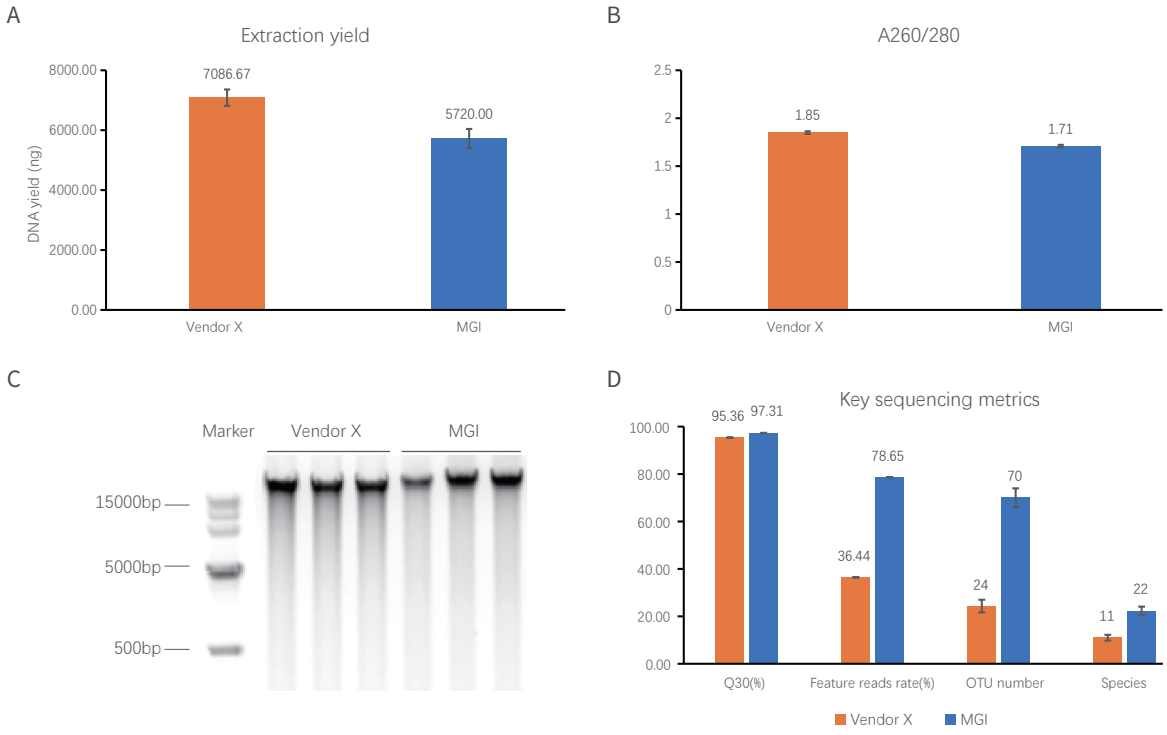


Fig. 1. The MGIEasy Stool Microbiome DNA Extraction Kit was comparable to that of Vendor X. (A-C)The yield (A), purity (A260/280) (B) and size distribution (C) of DNA extracted by those kits; (D) Sequencing results from libraries prepared with the extracted products; feature reads rate = (number of merged reads - number of chimeric reads)/number of down-sampled reads. The above sequencing results were obtained on DNBSEQ-G99 after manual library preparation using ATOPlex MiFish library preparation set. The samples were collected from the same sampling site of the East Lake of Wuhan, with three replicates for each kit.

MGISP system facilitates automatic and high-quality nucleic acid extraction and library preparation process

MGI can provide automatic solutions for nucleic acid extraction and library preparation of environmental samples. In this study, we used both manual and automatic solutions (MGISP-NE32 and MGISP-960) for DNA extraction from real aquatic environments and library preparation.

The yields of the manual and automatic extraction solutions were not compared because only half of the product was loaded onto the MGISP-NE32 after RNA removal. A260/280 of DNA from automatic solution was 1.63 (Fig. 2A), and the high-quality DNA also exhibited expected size distribution (Fig. 2B). DNA quality from automatic solution was comparable to manual solution (Fig. 2A,2B). The library yield of automatic library preparation was 969 ng with 50 ng DNA input (Fig. 2C). Additionally, the library was high-quality and comparable to manual solution with a size of 329 bpno non-specific peaks such as primer dimers as expected(300-400 bp) (Fig. 2D). (NE32, 960) refers to sample extracted using MGISP-NE32 and library prepared using MGISP-960, other operations are named in the same manner. The Q30, feature reads rate, OTU number and the number of detected species of (NE32, 960) was 96.79%, 69.35%, 54 and 18, respectively. The sequencing quality was comparable to (Manual, 960) (Fig. 2E). Similarly, the sequencing results of (Manual, 960) were comparable to (Manual, Manual).

As a conclusion, MGI's automatic system could facilitate high-quality nucleic acid extraction and library preparation from environmental samples, and the quality was comparable to manual solution.

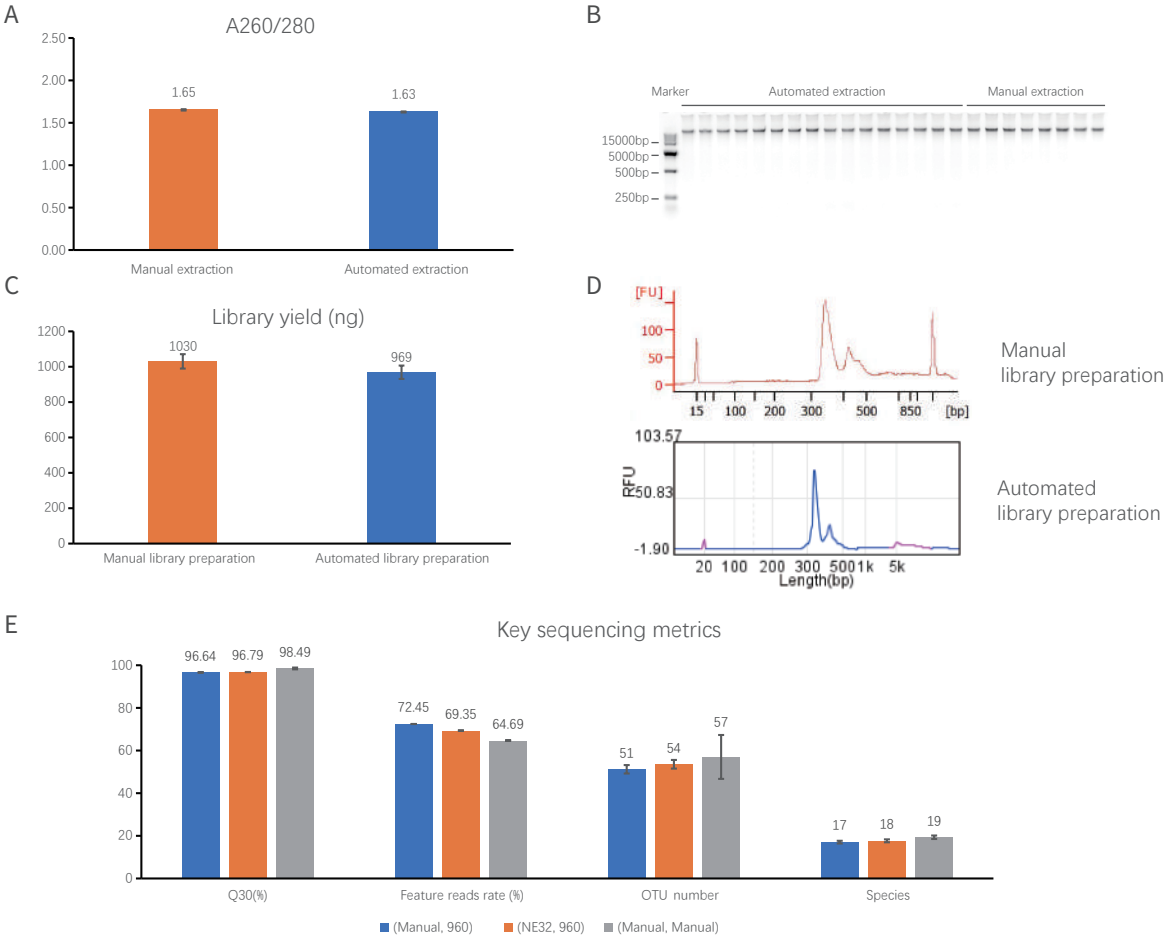


Fig. 2 MGI's automatic solution for sample extraction and library preparation was comparable to manual operation. (A-B) DNA purity (A260/280) (A) and size distribution (B) of manual and automatic extraction solutions; (C-D) library yield (C) and size distribution (D) of manual and automatic library preparation solutions; (E) sequencing results of automatic or manual solutions. All of the above results were from real samples, with library prepared with MiFish panel, and sequencing on DNBSEQ-G99.

eDNA metabarcoding sequencing package can accurately identify fish species

Researchers prepared three simulated fish community samples to verify the accuracy of the package for fish diversity detection. (Manual, MiFish, 960)-1/2/3/4 were four replicates of the same mixing ratio of 10 fish species; (Manual, 12S, 960)-1/2 and (Manual, 12S, 960)-3/4 were samples of two different mixing ratios. (Manual, MiFish, 960) represents that the samples were extracted manually, with library prepared using MiFish panel and MGISP-960, other groups are named in the same manner.

All expected species were detected in both (Manual, MiFish, 960) and (Manual, 12S, 960) (Fig. 3A, B). The measured abundance was highly consistent with the theoretical abundance. The Pearson correlation coefficients (PCC) of those three samples were 0.904, 0.871, and 0.838, respectively (Fig. 3 C, D, and E).

Conclusively, both MiFish panel with DNBSEQ-E25 and Ac12S panel with DNBSEQ-G99 can facilitate accurate fish species detection in the fish diversity detection study.

Dynamic monitoring of fish diversity could be fulfilled with eDNA metabarcoding sequencing package

Currently, eDNA technology has been used in many studies to analyze the diversity of fish in the Yangtze River basin. In this study, the fish diversity in the East Lake of Wuhan was monitored based on eDNA metabarcoding sequencing package. D3-D8 represent six different sampling sites in the East Lake of Wuhan (Fig. 4A). Statistical analysis of the top 18 abundant species showed that different replicates at the same site were highly consistent. The proportion of *Hypophthalmichthys nobilis* was higher in D5, D6, and D7 than the rest (Fig. 4B). Krona chart shows the detailed relative proportions of fish species in D3 at different taxonomic levels (Fig. 4C). The Shannon-Wiener curve showed that the curves for all samples became flat when 30,000 reads were intercepted for analysis, indicating 50,000 reads was sufficient to detect the majority of the biological species (Fig. 4D). The rank-abundance curve showed that the species abundance and homogeneity were higher at D3, and lower at D7 and D8 (Fig. 4E). The inter-group distance analysis diagram

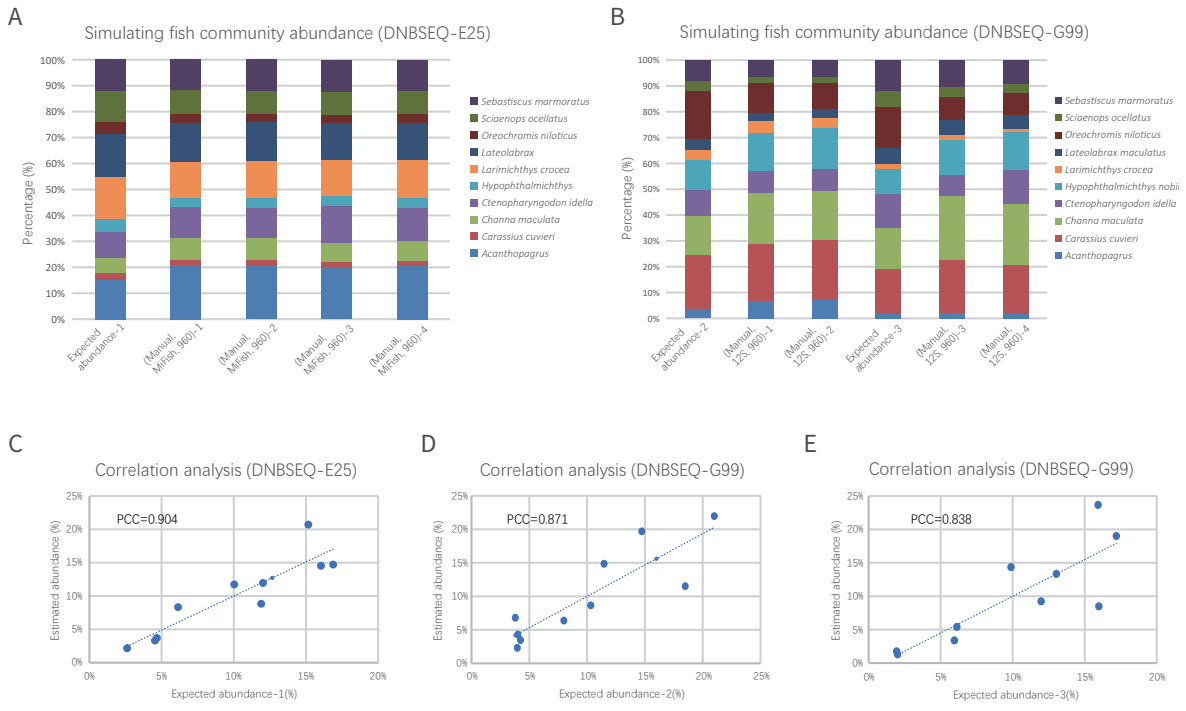


Fig. 3. The measured abundance of the simulated fish community samples was consistent with the theoretical abundance. (A-B) Measured abundance of three simulated fish community samples with different proportions; (C, D, E) correlation analysis of the abundance of three simulated fish community samples with different proportions. The above MiFish libraries were sequenced on DNBSEQ-E25.

showed that D3 and D8 were similar, while the rest sites had greater difference in fish species composition (Fig. 4F).

Principal component analysis showed that D3 and D8 were closer (Fig. 4G) and the fish species composition at these 2 sites was similar, which was consistent with the conclusion of the inter-group distance analysis.

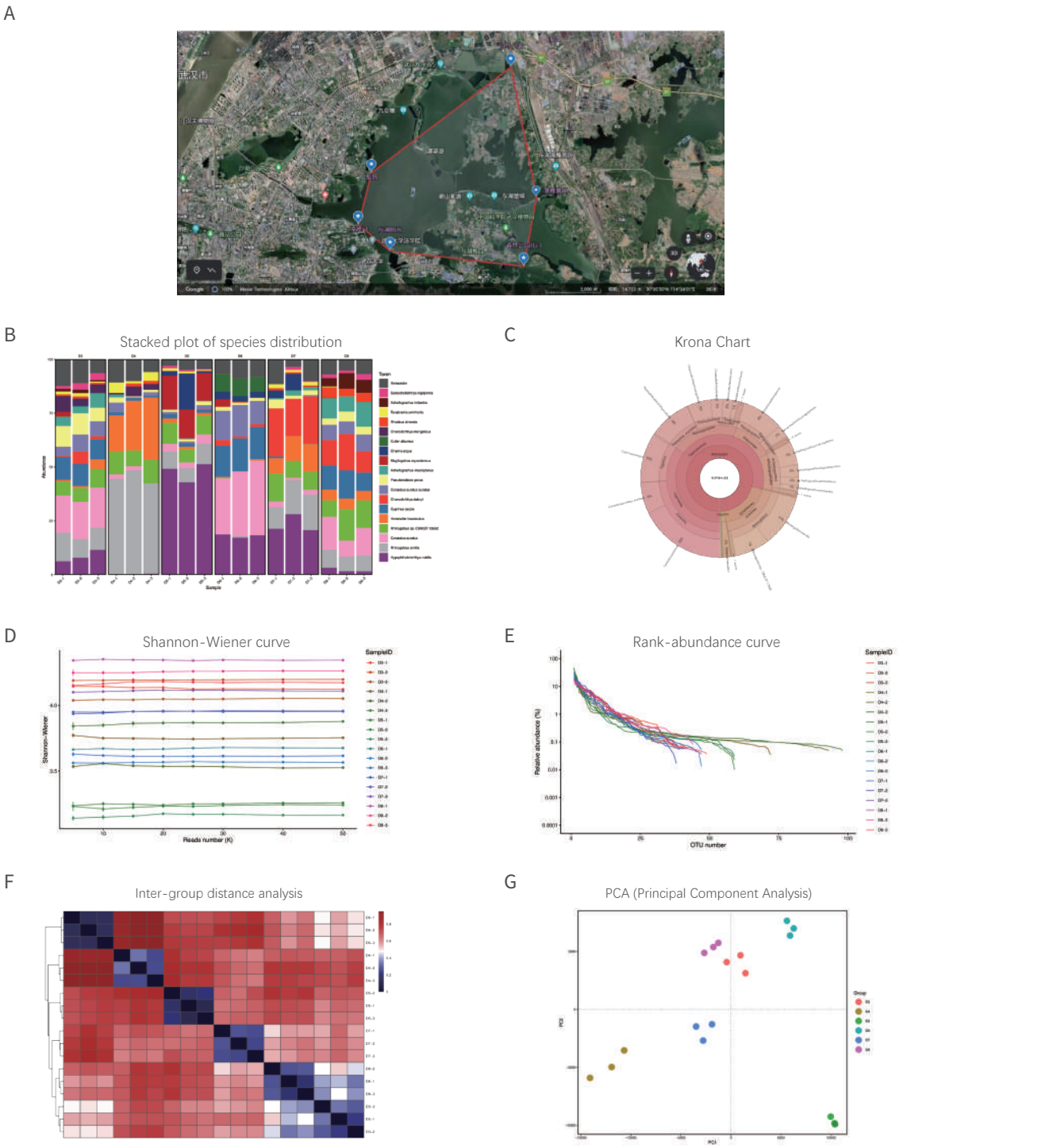


Fig. 4. MetaSIS report of environmental samples from the East Lake of Wuhan. (A) D3-D8 were 6 different sampling sites in the East Lake of Wuhan, with sampling repeated 3 times at each site. (B) Stacked plot of species distribution of the top 18 abundant species. (C) Krona chart of D3. Circles represent the relative proportions of species at different taxonomic levels (phylum, class, order, family, genus, and species) from inside out. (D) Shannon-Wiener curve. Horizontal coordinates represent different sequencing data sizes, vertical coordinates represent biodiversity index. The curve becomes flat for all samples when 30,000 reads are intercepted for analysis. (E) Rank-abundance curves for all samples. Horizontal coordinates represent the OTU number after ranking, and vertical coordinates represent the relative abundance of each OTU. The wider range on the x-axis indicating higher species abundance. The smoother curve indicating a more even distribution of species. (F) Inter-group distance analysis for each sample. The horizontal and vertical coordinates are both samples, which can reflect the similarity and difference of multiple samples by color gradient and degree of similarity. (G) Principal component analysis. Each point represents a sample, the points of the same color come from the same subgroup, and a smaller distance between two points indicates smaller difference.

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Table 3 shows the fishes detected by the MGI eDNA metabarcoding sequencing package in the East Lake. A total of 59 species of fish were identified, belonging to 9 orders, 17 families and 38 genera (Table 3). *Gambusia affinis* is invasive specie[14].

Order	Family	Genus	Species
Anabantiformes	*Channidae*	*Channa*	*Channa argus*
			Channa maculata
Centrarchiformes	*Sinipercidae*	*Siniperca*	*Siniperca chuatsi*
Clupeiformes	*Engraulidae*	*Coilia*	*Coilia nasus*
Cypriniformes	*Acheilognathidae*	*Acheilognathus*	*Acheilognathus barbatulus*
			Acheilognathus hypselonotus
			Acheilognathus imberbis
			Acheilognathus macropterus
			Acheilognathus rhombeus
		Rhodeus	*Rhodeus ocellatus*
			Rhodeus sinensis
	Cobitidae	*Misgurnus*	*Misgurnus bipartitus*
		Paramisgurnus	*Paramisgurnus dabryanus*
	Cyprinidae	*Carassius*	*Carassius auratus*
			Carassius auratus auratus
			Carassius carassius
		Cyprinus	*Cyprinus acutidorsalis*
			Cyprinus carpio
	Cyprinoidei	*diploid*	*diploid Xenocypris davidi x Megalobrama amblycephala*
	Gobionidae	*Abbottina*	*Abbottina rivularis*
		Hemibarbus	*Hemibarbus labeo*
			Hemibarbus maculatus
		Microphysogobio	*Microphysogobio sp. ZF-11935*
		Paracanthobrama	*Paracanthobrama guichenoti*
		Pseudorasbora	*Pseudorasbora parva*
		Sarcocheilichthys	*Sarcocheilichthys nigripinnis*
			Sarcocheilichthys sinensis
			Sarcocheilichthys soldatovi
		Saurogobio	*Saurogobio dabryi*
			Saurogobio lissilabris
		Squalidus	*Squalidus argentatus*

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Order	Family	Genus	Species
Cypriniformes	*Xenocyprididae*	*Chanodichthys*	*Chanodichthys dabryi*
			Chanodichthys mongolicus
		Ctenopharyngodon	*Ctenopharyngodon idella*
		Culter	*Culter alburnus*
			Culter compressocorpus
		Hemiculter	*Hemiculter leucisculus*
		Hypophthalmichthys	*Hypophthalmichthys molitrix*
			Hypophthalmichthys nobilis
		Megalobrama	*Megalobrama amblycephala*
			Megalobrama terminalis
		Mylopharyngodon	*Mylopharyngodon piceus*
		Parabramis	*Parabramis pekinensis strenosomus*
		Plagiognathops	*Plagiognathops microlepis*
		Toxabramis	*Toxabramis swinhonis*
Cyprinodontiformes	*Poeciliidae*	*Gambusia*	*Gambusia affinis*
Gobiiformes	*Gobiidae*	*Mugilogobius*	*Mugilogobius myxodermus*
		Rhinogobius	*Rhinogobius cliffordpopei*
			Rhinogobius fluviatilis
			Rhinogobius nagoyae
			Rhinogobius similis
			Rhinogobius sp. CBM:ZF:13662
	Odontobutidae	*Micropercops*	*Micropercops swinhonis*
		Odontobutis	*Odontobutis sinensis*
Osteoglossiformes	*Notopteridae*	*Chitala*	*Chitala ornata*
Siluriformes	*Bagridae*	*Tachysurus*	*Tachysurus fulvidraco*
	Pimelodidae	*Rhamdia*	*Rhamdia cf. jequitinhonha NS 3056B*
	Siluridae	*Silurus*	*Silurus asotus*
Synbranchiformes	*Mastacembelidae*	*Macrognathus*	*Macrognathus aculeatus*

Table 3. Community composition of detected fishes in the East Lake.

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Conclusion

This study showed that the MGI's eDNA metabarcoding sequencing package was capable of dynamic monitoring of fishes. Our preliminary data demonstrated that the MGIEasy Stool Microbiome DNA Extraction Kit was comparable to Vendor X's kit in terms of the exaction results, the automatic extraction and library preparation process was comparable to the manual, the data obtained from this package was of high quality and the fish species identification was accurate.

Conclusively, this MGI's package provides a complete self-developed solution for the whole workflow of sample extraction, library preparation, sequencing and bioinformatics analysis integrated with automatic system, providing a powerful tool for ecological environment monitoring and environmental scientific research.

DNBSEQ-E25 is a compact and portable genetic sequencer with a size of only 0.1m^2. It has many features like fast speed, low laboratory requirements, easy to install and maintain, and can complete PE150 sequencing within 20h (25M reads/flow cell). The DNBSEQ-G99 is able to run up to two flow cells (80M reads/flow cell) simultaneously. It takes only 12h for PE150 and 30h for PE300 sequencing. Both sequencers can be equipped with a built-in computing module to integrate sequencing and bioinformatics functions, which could maximize the sequencing efficiency.

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References

1. Bohmann K, Evans A, Gilbert M T P, et al. Environmental DNA for wildlife biology and biodiversity monitoring[J]. *Trends in ecology & evolution*, 2014, 29(6): 358- 367.
2. Hebert P D N, Cywinska A, Ball S L, et al. Biological identifications through DNA barcodes[J]. *Proceedings of the Royal Society of London. Series* B: Biological Sciences, 2003, 270(1512): 313-321.
3. Creer S, Deiner K, Frey S, et al. The ecologist's field guide to sequence - based identification of biodiversity[J]. *Methods in Ecology and Evolution*, 2016, 7(9): 1008- 1018.
4. Ruppert K M, Kline R J, Rahman M S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA[J]. *Global Ecology and Conservation*, 2019, 17: e00547.
5. Stewart K A. Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA[J]. *Biodiversity and Conservation*, 2019, 28(5): 983- 1001.
6. Jerde C L, Chadderton W L, Mahon A R, et al. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program[J]. *Canadian Journal of Fisheries and Aquatic Sciences*, 2013, 70(4): 522-526.
7. Wilcox T M, McKelvey K S, Young M K, et al. Robust detection of rare species using environmental DNA: the importance of primer specificity[J]. *PloS one*, 2013, 8(3): e59520.
8. Takahara T, Minamoto T, Yamanaka H, et al. Estimation of fish biomass using environmental DNA[J]. *PloS one*, 2012, 7(4): e35868.
9. Takahara T, Minamoto T, Doi H. Using environmental DNA to estimate the distribution of an invasive fish species in ponds[J]. *PloS one*, 2013, 8(2): e56584.
10. Thomsen P F, Kielgast J, Iversen L L, et al. Detection of a diverse marine fish fauna using environmental DNA from seawater samples[J]. 2012.
11. Miya M, Gotoh R O, Sado T. MiFish metabarcoding: a high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples[J]. *Fisheries Science*, 2020, 86(6): 939- 970.
12. Zhang S, Zhao J, Yao M. A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish[J]. *Methods in Ecology and Evolution*, 2020, 11(12): 1609-1625.
13. May2023 reference datasets for Mitohelper: https://zenodo.org/record/7925541.
14. Li Y, Tang M, Lu S, et al. A Comparative Evaluation of eDNA Metabarcoding Primers in Fish Community Monitoring in the East Lake[J]. *Water*, 2024, 16(5): 631.

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G99ARS	900-000609-00
	Genetic Sequencer DNBSEQ-E25RS	900-000537-00
	Automated Nucleic Acid Extractor MGISP-NE32RS	950-000020-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS High-throughput Automated Sample Preparation System-Custom Configuration 9-V7	900-000154-00
Software	Metabarcoding Species Identification Software（MetaSIS）	970-000417-00
	Platform of microorganisms Fast Identification (PFI)	900-000393-00*
	Platform of microorganisms Fast Identification and assembly evolution	900-000399-00*
Extraction reagents	MGIEasy Magnetic Beads Genomic DNA Extraction Kit	1000010524
	MGIEasy Stool Microbiome DNA Extraction Kit	940-000122-00
	MGIEasy Tissue Grinding Beads Kit	940-000136-00
Library prep reagents	ATOPlex MiFish Library Prep Set(ATOPlex MiFish Panel+ATOPlex DNA Dual BC Library Prep Set)	940-002203-00
	ATOPlex Ac12S mtDNA Library Prep Set(ATOPlex Ac12S mtDNA Panel+ATOPlex DNA Dual BC Library Prep Set)	940-002199-00
DNB Making Reagents	MGIEasy Dual Barcode Circularization Kit	1000020570
	DNBSEQ OneStep DNB Make Reagent Kit	1000026466
Standard Library Reagents	ATOPlex E450 Dual Barcode Balanced Library Reagent	940-000637-00
	Standard Library Reagent (PCR Product) V4.0	1000027585
Sequencing reagents	DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE150)	940-000410-00
	DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE300)	940-000415-00
	DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150)	940-000567-00***
Cleaning Reagent	DNBSEQ-G99RS Cleaning Reagent Kit (G99 SM FCL)	940-000624-00

*You can freely choose one type.
**For more ATOPlex customized products, please visit MGI's official website: https://en.mgi-tech.com/products/atoplex/.
*** DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150) includes DNBSEQ OneStep DNB Make Reagent, no additional purchase required.

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