LABORATORY OF BIORESOURCES

Specially Appointed Professor
NARUSE, Kiyoshi

Research Staff:	*KANEKO, Hiroyo* *HARA, Ikuyo* *YANOGAWA, Azusa*
Visiting Scientist:	*SATO, Tadashi* *NITTA, Masato*
Visiting Graduate Student:	*IMAEDA YASSUMOTO, Tamiris*
Technical Assistant:	*AJIOKA, Rie* *KOIKE, Chieko* *KOIKE, Yukari* *TESHIMA, Yuuko* *TORII, Naoko* *YAMAZAKI, Toko*
Admin Support Staff:	*SUZUKI, Tokiko*

The medaka is a small egg-laying fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on the dynamics of transcriptional and chromatin accsssibility landscape during medaka development, the understanding of pigment cell differentiation using body color mutants, genome sequence of the Javanese medaka, *Oryzias javanicus* and the molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species, In addition to these activities, our laboratory was charged with the responsibility of leading the National BioResource Project Medaka (NBRP Medaka) from 2007.

I. Daynamic transcriptional and chromatin accesibikity landscape during medaka development.

A high-quality genome sequence and a variety of genetic tools are available for medaka. However, existing genome annotation is still rudimentary, as it was mainly based on computational prediction and short-read RNA-seq data. To overcome this situations, we conducted long-read RNA-seq, short-read RNA-seq, and ATAC-seq coraboration with Dr. Tu at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. This work constructed a much-improved gene model set including about 17,000 novel isoforms and identified 1600 transcription factors, 1100 long non-coding RNAs, and 150,000 potential cis-regulatory elements as well. The work provides the first comprehensive omics datasets of medaka embryogenesis. The data portal (http://tulab.genetics.ac.cn/medaka_omics) will serve as a daily reference tool for the entire medaka community.

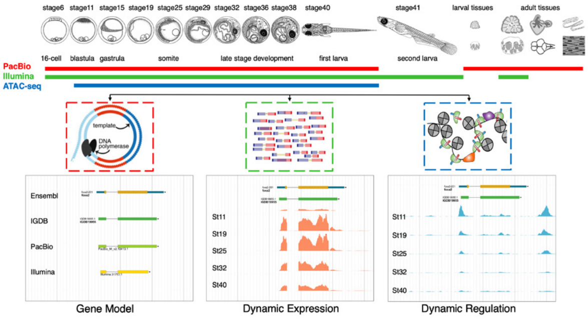


Figure 1. Overview of medaka multi-omics anslysis during development

II. The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel and their genomic variation and epigenomic landscape

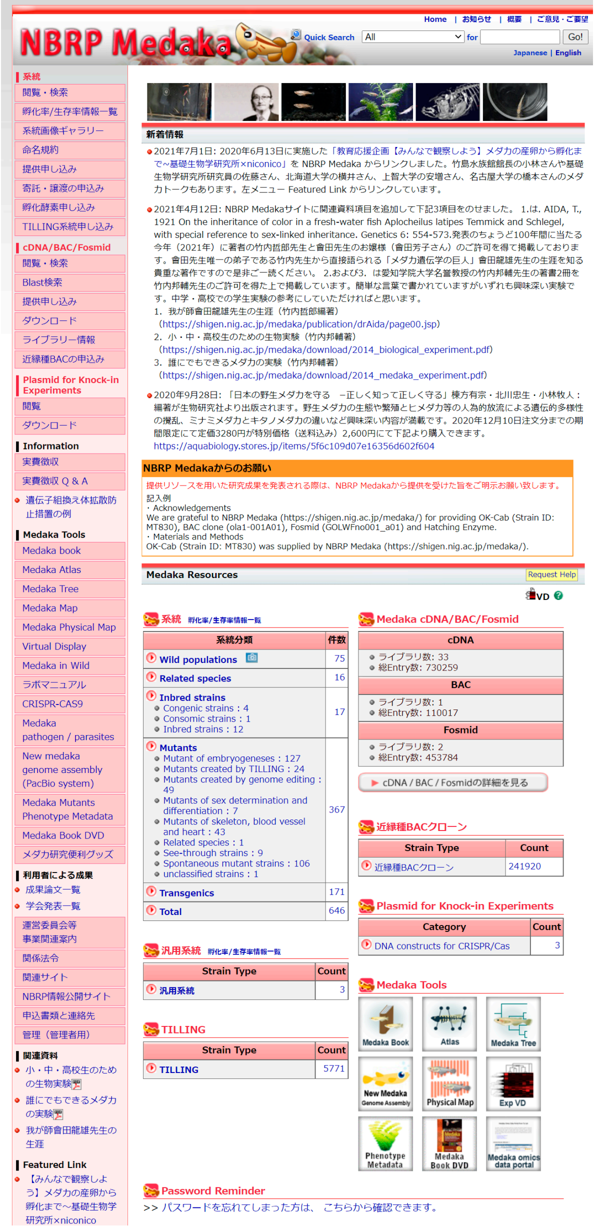
The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel is the first near-isogenic panel of 80 inbred lines derived from a wild founder population collected from Kiyosu, Toyohashi. Inbred lines provide fixed genomes that make it possible to repeat studies, to vary both genetic information and environmental conditions in controlled studies, as well as to conduct functional studies. MIKK will thus make it possible to investigate phenotype-to-genotype association studies of complex genetic traits while carefully controlling interacting factors. Its applications are numerous, and include genetic research, human health, drug development, and fundamental biology. Using short reads from Illumina, we further characterise each of the 80 MIKK lines according to their genetic composition, using the Southern Japanese medaka inbred strain HdrR as a reference genome. We used Oxford Nanopore long read sequencing technology to analyze 12 representative lines from MIKK to analyze the larger and more complex structural variations. We also investigate line-specific CpG methylation and performed differential DNA methylation analysis across these 12 lines.

III. Genome sequence of the Javanese medaka, *Oryzias javanicus*, as a model for studying seawater adaptation

Medaka fish in the genus *Oryzias* are an emerging model system for studying the molecular basis of vertebrate evolution. This genus contains approximately 35 species and exhibits great morphological, ecological and physiological differences among it's species. Among these species, the Java medaka, *Oryzias javanicus*, is the species that has most typically adapted to seawater. We sequenced and assembled the whole genome of *O. javanicus*, as a model fish species for studying molecular mechanisms of seawater adaptation. In teleost fish, the major osmoregulatory organs are the gills, intestine and kidney, and these play different roles to maintain body fluid homeostasis. Many genes encoding hormones, receptors, osmolytes, transporters, channels and cellular junction proteins are potentially involved in this osmotic regulation. In addition to the osmoregulation, hatching enzyme activity dramatically changes in different salt conditions. At the hatching stage, fish embryos secrete a specific cocktail of enzymes in order to dissolve the envelope. In the medaka *O. latipes*, digestion of the envelope occurs after the cooperative action of two kinds of hatching enzymes: (i) the high choriolytic enzyme (HCE) and (ii) the low choriolytic enzyme (LCE) (Yasumasu *et al*., 2010).The HCE shows higher activity in freshwater than in brackish water (Kawaguchi *et al*., 2013). Thus, availability of the high-quality reference genome in *O. javanicus* would facilitate further research for investigating the molecular basis of physiological differences including the osmotic regulation and the hatching enzyme activity among *Oryzias* species.

IV. National BioResource Project Medaka

Our laboratory has been acting as main facility of the Medaka BioResource Project (NBRP Medaka) in the fourth phase of NBRP, which started in 2017. We are providing wild strains, and related species, genomic resources (approximately 400,000 cDNA clones from 30 different cDNA libraries (containing approximately 23,000 different sequences) and BAC/Fosmid clones covering the entire medaka genome), and hatchery enzymes essential for embryo manipulation and live imaging. These bioresources are available on our website (Figure 3. https://shigen.nig.ac.jp/medaka/). These bioresources can be searched by various methods such as keywords, sequence homology, and expression profiles using the database on the website. In addition, genome browsers for the three inbred strains, phylogenetic relationships between wild strains of medaka and related species, and experiment manuals are also available. With the approval of the second supplementary budget for FY2020, we were able to install a cabinet-type fish tank washing machine. This has freed up our technical support staff from the need to wash the tanks by hand, and has allowed us to focus more on breeding and management, which requires more human work. In addition, a system to remotely monitor the temperature, humidity, and illumination in the medaka breeding rooms and the water temperature in the breeding tanks was installed. The air conditioner in the breeding room was also upgraded. We have continuously monitored the medaka breeding conditions using these systems.



新着情報

- 2021年7月1日: 2020年6月13日に実施した「教育応援企画【みんなで観察しよう】メダカの産卵から孵化まで～基礎生物学研究所×niconico」を NBRP Medaka からリンクしました。竹島水族館館長の小林さんや基礎生物学研究所研究員の佐藤さん、北海道大学の横井さん、上智大学の安増さん、名古屋大学の橋本さんのメダカトークもあります。左メニュー Featured Link からリンクしています。
- 2021年4月12日: NBRP Medakaサイトに関連資料項目を追加して下記3項目をのせました。 1.は. AIDA, T., 1921 On the inheritance of color in a fresh-water fish Aplocheilus latipes Temmick and Schlegel, with special reference to sex-linked inheritance. Genetics 6: 554-573.発表のちょうど100年間に当たる今年（2021年）に著者の竹内哲郎先生と會田先生のお嬢様（會田芳子さん）のご許可を得て掲載しております。會田先生唯一の弟子である竹内先生から直接語られる「メダカ遺伝学の巨人」會田龍雄先生の生涯を知る貴重な著作ですので是非ご一読ください。 2.および3. は愛知学院大学名誉教授の竹内邦輔先生の著書2冊を竹内邦輔先生のご許可を得た上で掲載しています。簡単な言葉で書かれていますがいずれも興味深い実験です。中学・高校での学生実験の参考にしていただければと思います。
  1. 我が師會田龍雄先生の生涯（竹内哲郎編著）
  （https://shigen.nig.ac.jp/medaka/publication/drAida/page00.jsp）
  2. 小・中・高校生のための生物実験（竹内邦輔著）
  （https://shigen.nig.ac.jp/medaka/download/2014_biological_experiment.pdf）
  3. 誰にでもできるメダカの実験（竹内邦輔著）
  （https://shigen.nig.ac.jp/medaka/download/2014_medaka_experiment.pdf）
- 2020年9月28日: 「日本の野生メダカを守る　－正しく知って正しく守る」棟方有宗・北川忠生・小林牧人：編著が生物研究社より出版されます。野生メダカの生態や繁殖とヒメダカ等の人為的放流による遺伝的多様性の攪乱、ミナミメダカとキタノメダカの違いなど興味深い内容が満載です。2020年12月10日注文分までの期間限定にて定価3280円が特別価格（送料込み）2,600円にて下記より購入できます。
https://aquabiology.stores.jp/items/5f6c109d07e16356d602f604

NBRP Medakaからのお願い

提供リソースを用いた研究成果を発表される際は、NBRP Medakaから提供を受けた旨をご明示お願い致します。
記入例
・Acknowledgements
We are grateful to NBRP Medaka (https://shigen.nig.ac.jp/medaka/) for providing OK-Cab (Strain ID: MT830), BAC clone (ola1-001A01), Fosmid (GOLWFno001_a01) and Hatching Enzyme.
・Materials and Methods
OK-Cab (Strain ID: MT830) was supplied by NBRP Medaka (https://shigen.nig.ac.jp/medaka/).

Medaka Resources

系統　孵化率/生存率情報一覧

系統分類	件数
Wild populations	75
Related species	16
Inbred strains Congenic strains : 4 Consomic strains : 1 Inbred strains : 12	17
Mutants Mutant of embryogeneses : 127 Mutants created by TILLING : 24 Mutants created by genome editing : 49 Mutants of sex determination and differentiation : 7 Mutants of skeleton, blood vessel and heart : 43 Related species : 1 See-through strains : 9 Spontaneous mutant strains : 106 unclassified strains : 1	367
Transgenics	171
Total	646

汎用系統　孵化率/生存率情報一覧

Strain Type	Count
汎用系統	3

TILLING

Strain Type	Count
TILLING	5771

Medaka cDNA/BAC/Fosmid

cDNA
ライブラリ数: 33 総Entry数: 730259
BAC
ライブラリ数: 1 総Entry数: 110017
Fosmid
ライブラリ数: 2 総Entry数: 453784

近縁種BACクローン

Strain Type	Count
近縁種BACクローン	241920

Plasmid for Knock-in Experiments

Category	Count
DNA constructs for CRISPR/Cas	3

Medaka Tools

Password Reminder

>> パスワードを忘れてしまった方は、 こちらから確認できます。

Figure 2. NBRP Medaka website

Publication List:

〔Original papers〕

- Fitzgerald, T., Brettell, I., Leger, A., Wolf, N., Kusminski, N., Monahan, J., Barton, C., Herder, C., Aadepu, N., Gierten, J., Becker, C., Hammouda, O.T., Hasel, E., Lischik, C., Lust, K., Sokolova, N., Suzuki, R., Tsingos, E., Tavhelidse, T., Thumberger, T., Watson, P., Welz, B., Khouja, N., Naruse, K., Birney, E., Wittbrodt, J., and Loosli, F. (2022). The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel. Genome Biol. *23*, 59. DOI: 10.1186/s13059-022-02623-z
- Leger, A., Brettell, I., Monahan, J., Barton, C., Wolf, N., Kusminski, N., Herder, C., Aadepu, N., Becker, C., Gierten, J., Hammouda, O.T., Hasel, E., Lischik, C., Lust, K., Sokolova, N., Suzuki, R., Tavhelidse, T., Thumberger, T., Tsingos, E., Watson, P., Welz, B., Naruse, K., Loosli, F., Wittbrodt, J., Birney, E., and Fitzgerald, T. (2022). Genomic variations and epigenomic landscape of the Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel. Genome Biol. *23*, 58. DOI: 10.1186/s13059-022-02602-4
- Seleit, A., Ansai, S., Yamahira, K., Masengi, K. W., Naruse, K., & Centanin, L. (2021). Diversity of lateral line patterns and neuromast numbers in the genus *Oryzias*. Journal of Experimental Biology, *224*, jeb242490. DOI: 10.1242/jeb.242490