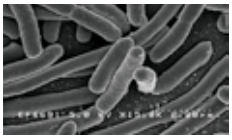
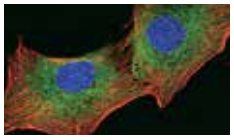
Project Leader **Hisao Masai** Genome Dynamics Project

Genome Replication and Maintenance:
In search of unexplored messages in the genome

Precise duplication of genetic materials is central to the stable maintenance of genomes through generations. Defects in genome copying processes would generate genomic instability which could ultimately result in various diseases including cancer. The goal of our studies is to understand the molecular basis of how huge genomes are accurately replicated and precise copies of genetic materials are inherited to the next generation. Three billion base pairs of the human genome (2 meter long) are replicated with almost no errors during a 6-8 hr time span in the cell cycle. This requires an extreme level of coordination of temporal and spatial arrangements of chromatin organization and signaling events for initiation of DNA replication.





"We are trying to decipher 'unexplored messages' of the genome that are crucial for shaping the chromosomes, copying and reading out genetic information, and even for causing detrimental diseases."

We recently discovered novel and crucial roles of non-standard DNA structures in regulating of DNA replication and transcription. Notably, we found that G-quadruplex structures, which are widely present on genomes (estimated to be present at more than 370,000 locations on the human genome), regulate organization of chromatin architecture and initiation of DNA replication. Our major goal is to establish a novel principle of genome organization by elucidating the fundamental and universal functions of G-quadruplex and other non-B type DNA structures in the regulation of various genome functions. We will also explore the possibility that mutations found in various diseases including cancer and neurodegenerative diseases are related to alteration and mal-formation of these non-B DNA structures, which are likely to be essential components of genomes.

Ito S, et al. (2019) "Cdc7 kinase stimulates Aurora B kinase in M-phase." ***Sci. Rep***. in press

Kobayashi S, Fukatsu R, Kanoh Y, et al. (2019) "Both a unique motif at the C terminus and N-terminal HEAT repeat contribute to G4 binding and origin regulation by Rif1 protein." ***Mol Cell. Biol.*** 39(4). pii: e00364-18. (Cover photo)

Masai H, et al. (2019) "Rif1 promotes self-association of G-quadruplex (G4) by its specific G4 binding and oligomerization activities." ***Sci. Rep.*** 9(1), 8618.

Masai H, et al. (2018) "Molecular architecture of G-quadruplex structures generated on duplex Rif1 binding sequences." ***J. Biol. Chem.*** 293, 17033-17049.

Moriyama K, Yoshizawa-Sugata N, and Masai H. (2018) "Oligomer formation and G-quadruplex binding by purified murine Rif1 protein, a key organizer of higher-order chromatin architecture." ***J. Biol. Chem.*** 293,3607-3624.

You Z, and Masai H. (2017) "Potent DNA strand annealing activity associated with mouse Mcm2~7 heterohexamer complex." ***Nucleic Acids Res.*** 45, 6495-6506.

Yang C-C, Suzuki M, Yamakawa S, Uno S, Ishii A, Yamazaki S, Fukatsu R, Fujisawa R, Sakimura K, Tsurimoto T, Masai H. (2016) "Claspin recruits Cdc7 kinase for initia-tion of DNA replication in human cells." ***Nature Commun.*** 7, 12135.

Kanoh Y, Matsumoto S, Fukatsu R, Kakusho N, Kono N, Renard-Guillet C, Masuda K, Iida K, Nagasawa K, Shirahige K, and Masai H. (2015) "Rif1 binds to G-quadruplexes and suppresses replication over long distances." ***Nature Struct. Mol. Biol.*** 22, 889-897.

Yamazaki S, Ishii A, Kanoh Y, Oda M, Nishito Y, and Masai H. (2012) "Rif1 protein is a key regulator of the genome-wide DNA replication timing in human cells." ***EMBO J.*** 31, 3167-3177.

Hayano M, Kanoh Y, Matsumoto S, Shrahige K, and Masai H. (2012) "Rif1 is a global regulator of timing of replication origin firing in fission yeast." ***Genes and Development*** 26, 137-150.

Matsumoto S, Hayano M, Kanoh Y, and Masai H. (2011) "Multiple path-ways can bypass the essential role of fission yeast Hsk1 kinase in DNA replication initiation." ***J. Cell Biol.*** 195, 387-401.

Masai H, et al. (2010) "Eukaryotic DNA replication; where, when and how?" ***Annual Rev. Biochem.*** 79, 89-130.

Genome Dynamics

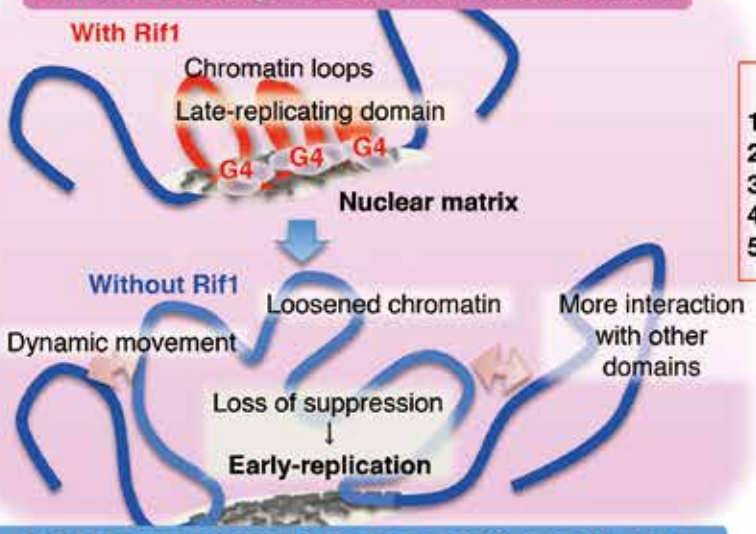
Cdc7 kinase as a modulator of chromosome transactions

Dbf4/ASK

Cdc7

- Mcm: Replication initiation
- Claspin/Mrc1: Replication Checkpoint
- Rad18: Trans-lesion DNA synthesis/ repair
- AuroraB: Trans-lesion DNA synthesis/ repair
- Mer2: Meiotic recombination
- Rec8: Lsr4: Meiotic cell division
- HP1: Heterochromatin formation
- H3 T45: Histone modification
- Caf1: Chromatin reconstruction
- Mrc1, Ams2, Eco1: Protein degradation
- Top2A: Centromere regulation
- TDP43: Protein aggregation
- Mus4-Mus81: Homologous recombination

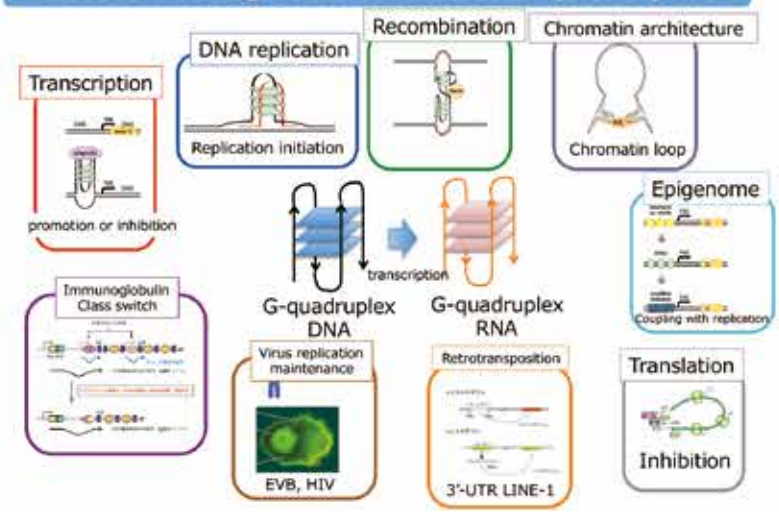
Rif1 and G4 as organizers of chromatin architecture



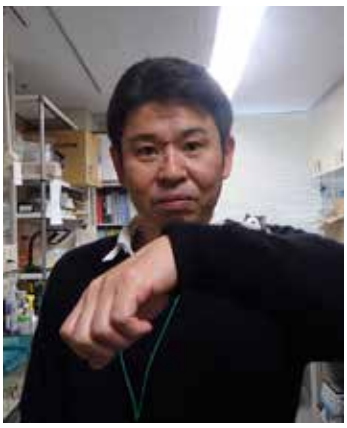
Our Major Projects

1) Universal mechanisms of DNA replication
2) Cellular responses to replication stress
3) Unusual DNA structures (G-quadruplex etc.)
4) DNA replication and development
5) Novel drugs and therapies for cancer

Diverse biological functions of G-quadruplex



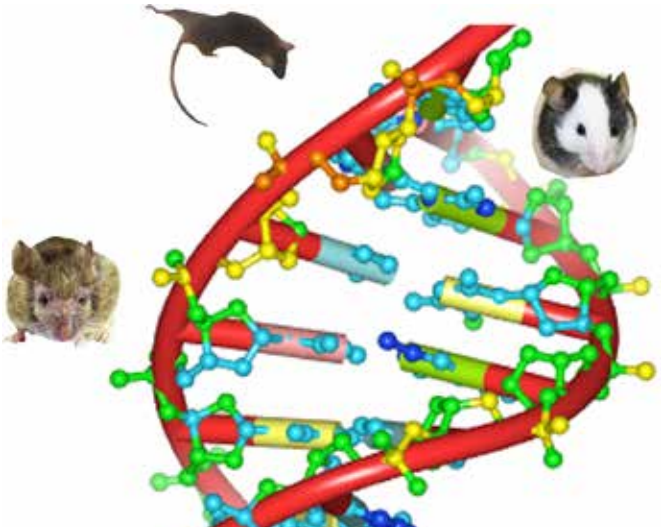
Genome Dynamics



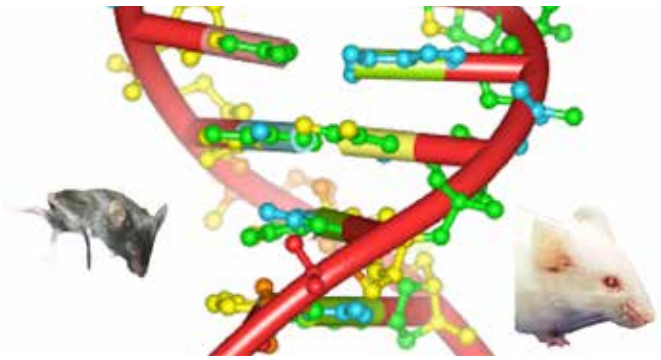
Project Leader **Yoshiaki Kikkawa** Mammalian Genetics Project

Gene discovery: Phenotype- and gene-driven approaches to identify disease-associated genes in mice

The genetic factors and molecular mechanisms behind many human genetic diseases are still unknown. Mouse disease models are important tools for identifying genes that are responsible for genetic diseases. They are also important for studying the processes that regulate the onset of genetic diseases and for evaluating the effectiveness of new drugs. We aim to develop novel mouse models of human genetic diseases via forward and reverse genetics to understand disease pathogenic mechanisms.



"We are identifying genes associated with human diseases and developing new mouse models for human diseases."



Yasuda SP, Miyasaka Y, and Kikkawa Y. (2018) "Effects of genetic background on susceptibility and the acceleration of hearing loss in mice." ***An Excursus into Hearing Loss*** 3–23.

Wada K, Saito J, Yamaguchi M, Seki Y, Furugoria M, Takahashi G, Nishito Y, Matsuda H, Shitara H, and Kikkawa Y. (2018) "*Pde6b*^{rd1} mutation modifies cataractogenesis in *Foxe3*^{rct} mice." ***Biochem. Biophys. Res. Commun.*** 496: 231-237.

Seki Y, Miyasaka Y, Suzuki S, Wada K, Yasuda SP, Matsuoka K, Ohshiba Y, Endo K, Ishii R, Shitara H, Kitajiri SI, Nakagata N, Takebayashi H, and Kikkawa Y. (2017) "A novel splice site mutation of myosin VI in mice leads to stereociliary fusion caused by disruption of actin networks in the apical region of inner ear hair cells." ***PLoS One*** 12, e0183477.

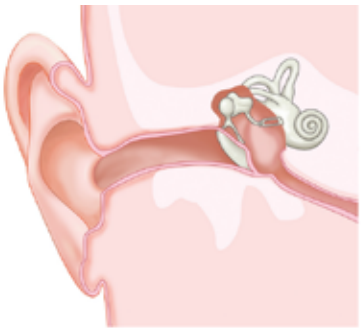
Miyasaka Y, Shitara H, Suzuki S, Yoshimoto S, Seki Y, Ohshiba Y, Okumura K, Taya C, Tokano H, Kitamura K, Takada T, Hibino H, Shiroishi T, Kominami K, Yonekawa H, and Kikkawa Y. (2016) "Heterozygous mutation of *Ush1g/Sans* in mice causes early-onset progressive hearing loss, which is recovered by reconstituting the strain-specific mutation in *Cdh23*." ***Hum. Mol. Genet.*** 25: 2045-2059.

Kikkawa Y, and Miyasaka Y. (2016) "Genetic modifiers of hearing loss in mice: The case of phenotypic modification in homozygous *Cdh23*^{ahl} age-related hearing loss." ***Monogr. Hum. Genet.*** 20: 97–109.

Suzuki S, Ishikawa M, Ueda T, Ohshiba Y, Miyasaka Y, Okumura K, Yokohama M, Taya C, Matsuoka K, and Kikkawa Y. (2015) "Quantitative trait loci on chromosome 5 for susceptibility to frequency-specific effects on hearing in DBA/2J mice." ***Exp. Anim.*** 64: 241-251.

Mammalian Genetics

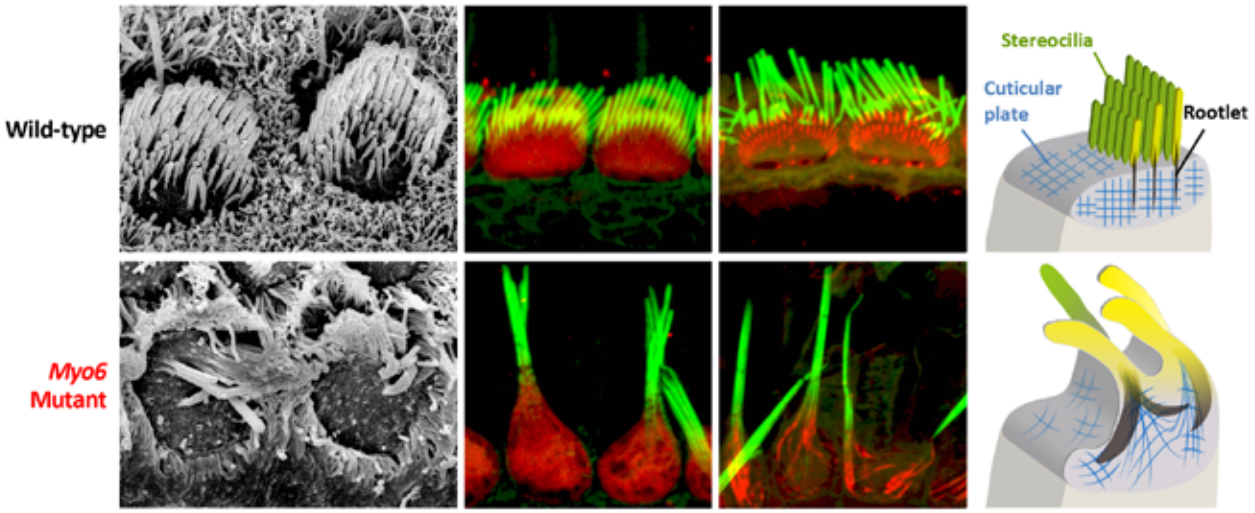
Main project: Genetics of deafness



Hearing loss is the most common sensory disease in humans, and severely affects one's quality of life. We continue to make significant advances in understanding the development, transduction, and homeostasis of the auditory system by studying corresponding mouse mutants. We exploit the similarities between the mouse and human genomes, physiology, and auditory system anatomy to identify and characterize genes related to deafness.

Current focus

Stereociliary fusion in Myo6 mutant mice caused by a disruption of actin networks in the apical region of inner ear hair cells

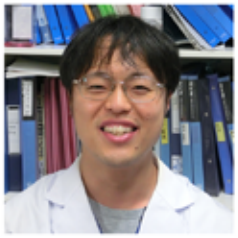


An unconventional myosin encoded by *MYO6*, a myosin VI gene, contributes to hearing loss in humans. Homozygous *Myo6* mutant mice exhibit congenital hearing defects caused by the fusion of stereocilia. We recently identified morphological changes at the base of the stereocilia in *Myo6* mouse mutants by scanning electron microscopy and analysis of the marker proteins of the cuticular plate and rootlet. In wild-type mice, stereocilia have dense rootlets that extend through the taper region of stereocilia to anchor them into the actin mesh of the cuticular plate. These structures are maintained when MYO6 is normally expressed in the stereociliary taper region, cuticular plate, and cytoplasm of the hair cells, but a reduction of MYO6 leads to stereociliary fusion accompanied by deformations of the cuticular plates and the extension of rootlets.

By Yuta Seki

Members

Kunie Matsuoka



Shumpei Yasuda

Xuehan Hou

Yuki Miyasaka

Kenta Wada

Mammalian Genetics

Project Leader **Fumihiko Yasui** Viral Infectious Diseases Project

Control of viral infectious diseases: Virology, immunology, vaccinology and therapy

Our project studies the virology, immunology, vaccinology and therapy of incurable viral diseases. We currently focus on liver diseases, influenza and dengue fever. However, the lack of suitable infection models in vitro and in vivo has hampered the clarification of viral pathogenesis. To overcome this problem, we have been developing various animal models including transgenic mice, humanized mice with human liver cells, monkeys and tree shrews. We also investigate the precise mechanisms by which host factors regulate viral growth.

"We are studying the mechanisms underlying development of severe acute inflammation and establishment of chronic infection by viruses through the development of suitable animal models that are capable of being infected by viruses."

Hepatitis

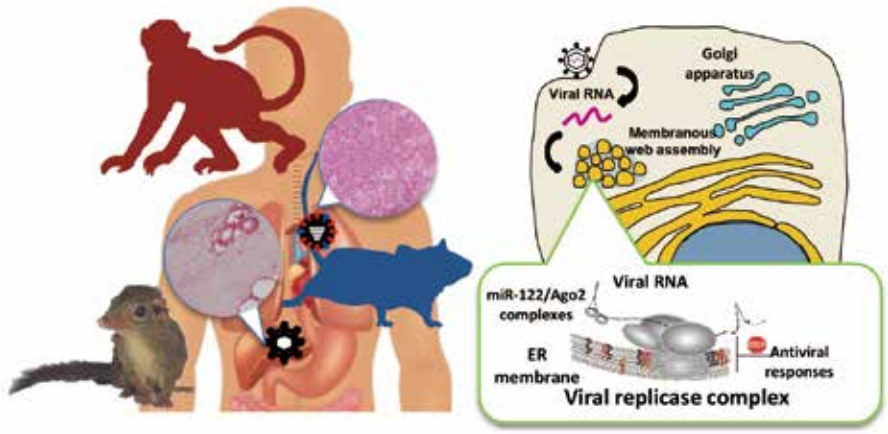
- Identification of host factors regulating virus growth.
- Elucidation of the mechanisms underlying pathogenesis caused by hepatitis virus infection.
- Development of therapeutic vaccine and drug for chronic HBV/HCV infection and other liver diseases.

Influenza

- Elucidation of the mechanisms by which highly pathogenic Flu causes severe pneumonia.
- Development of novel vaccine and therapeutic drug against highly pathogenic Flu and seasonal Flu.

Dengue fever

- Development of suitable animal models to study vaccine efficacy and pathogenesis of dengue fever.
- Development of novel vaccine for all serotypes of DENV.



Sanada T, Yasui F, Honda T, Kayesh MEH, Takano JI, Shiogama Y, Yasutomi Y, Tsukiyama-Kohara K, Kohara M. (2019) "Avian H5N1 influenza virus infection causes severe pneumonia in the Northern tree shrew (Tupaia belangeri)." ***Virology*** 529:101-110.

Tokunaga Y, Osawa Y, Ohtsuki T, Hayashi Y, Yamaji K, Yamane D, Hara M, Munekata K, Tsukiyama-Kohara K, Hishima T, Kojima S, Kimura K, and Kohara M. (2017) "Selective inhibitor of Wnt/-catenin/CBP signaling ameliorates hepatitis C virus-induced liver fibrosis in mouse model." ***Sci. Rep.*** 7: 325.

Sanada T, Hirata Y, Naito Y, Yamamoto N, Kikkawa Y, Ishida Y, Yamasaki C, Tateno C, Ochiya T, and Kohara M. (2016) "Transmission of HBV DNA mediated by ceramidetriggered extracellular vesicles." ***Cell Mol. Gastroenterol Hepatol.***3:272-283.

Yasui F, Itoh Y, Ikejiri A, Kitabatake M, Sakaguchi N, Munekata K, Shichinohe S, Hayashi Y, Ishigaki H, Nakayama M, Sakoda Y, Kida H, Ogasawara K, and Kohara M. (2016) "Sensitization with vaccinia virus encoding H5N1 hemagglutinin restores immune potential against H5N1 influenza against H5N1 influenza virus." ***Sci. Rep.*** 6: 37915.

Sanada T, Tsukiyama-Kohara K, Yamamoto N, Ezzikouri S, Benjelloun S, Murakami S, Tanaka Y, Tateno C, and Kohara M. (2016) "Property of hepatitis B virus replication in Tupaia belangeri hepatocytes." ***Biochem. Biophys Res. Commun.*** 469: 229-235.

Yamamoto N, Sato Y, Munakata T, Kakuni M, Tateno C, Sanada T, Hirata Y, Murakami S, Tanaka Y, Chayama K, Hatakeyama H, Hyodo M, Harashima H, and Kohara M. (2016) "Novel pH-sensitive multifunctional envelopetype nanodevice for siRNA-based treatments for chronic HBV infection." ***J. Hepatol.*** 64: 547-555

Viral Infectious Diseases

Topics of our research

Selective inhibitor of Wnt/β-catenin/CBP signaling ameliorates hepatitis C virus-induced liver fibrosis in mouse model

Chronic hepatitis C viral (HCV) infection is one of the major causes of serious liver diseases, including liver cirrhosis. We investigated the effects of a β-catenin/CBP inhibitor on liver fibrosis. PRI-724, a selective inhibitor of β-catenin/CBP, reduced liver fibrosis in HCV-Tg mice while attenuating αSMA induction. PRI-724 increased matrix metalloproteinase (MMP)-8 mRNA in the liver, and elevated levels of intrahepatic neutrophils and macrophages/monocytes. These results suggest that inhibition of hepatic stellate cell activation and induction of fibrolytic cells expressing MMP-8 contribute to the anti-fibrotic effects of PRI-724.

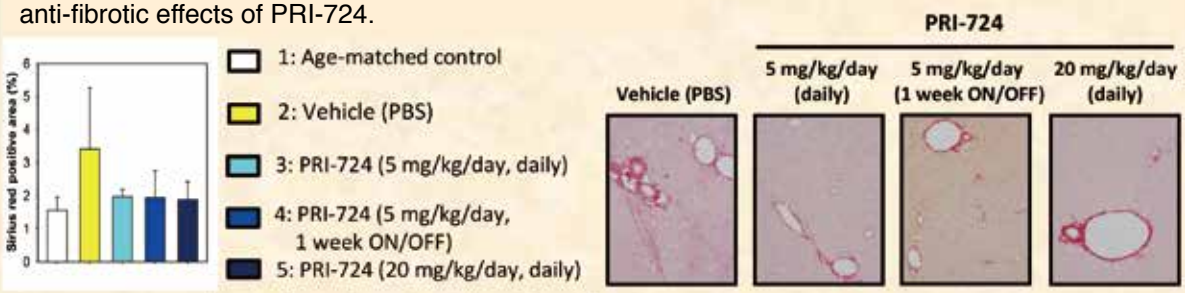
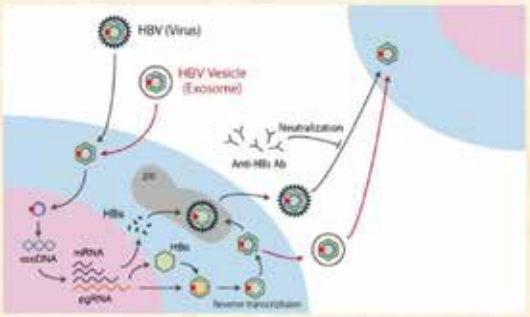


Figure. PRI-724ameliorates hepatitis C virus-induced liver fibrosis.

Transmission of HBV DNA Mediated by Ceramide-Triggered Extracellular Vesicles

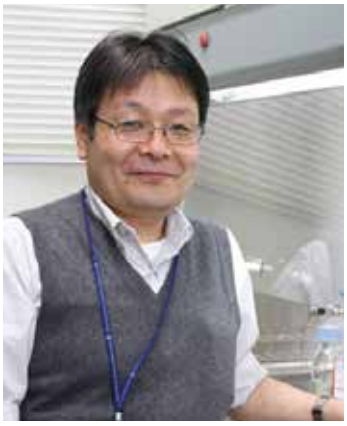
Extracellular vesicles are nanovesicles that shuttle proteins, nucleic acids, and lipids, thereby influencing cell behavior. We showed that ceramide-triggered extracellular vesicles transport hepatitis B virus-DNA and are capable of transmitting viral DNA to naive hepatocytes. Further, we demonstrated that the transmission of hepatitis B virus-DNA via these extracellular vesicles is resistant to antibody neutralization.



Members

Michinori Kohara
Tsubasa Munakata
Daisuke Yamane
Kenzaburo Yamaji
Naoki Yamamoto
Yuko Tokunaga
Takahiro Sanada
Tomoko Honda

Viral Infectious Diseases



Kobayashi K, et al. (2018) "Amino Acid Variation at VP1-145 of Enterovirus 71 Determines Attachment Receptor Usage and Neurovirulence in Human Scavenger Receptor B2 Transgenic Mice." ***J. Virol.***, 92:(15) e00681-18

Fujii K, et al. (2018) "VP1 Amino Acid Residue 145 of Enterovirus 71 Is a Key Residue for Its Receptor Attachment and Resistance to Neutralizing Antibody during Cynomolgus Monkey Infection." ***J. Virol.***, 92:(15)e00682-18

Fujii K, et al. (2013) "Transgenic mouse model for the study of enterovirus 71 neuropathogenesis." ***Proc. Natl. Acad. Sci. USA.***, 110: 14753-14758

Yamayoshi S, et al. (2013) "Functional Comparison of SCARB2 and PSGL1 as Receptors for Enterovirus 71." ***J. Virol.***, 87:3335-3347

Yamayoshi S, et al. (2012) "Human SCARB2-dependent Infection by Coxsackievirus A7, A14, A16 and Enterovirus 71." ***J. Virol.***, 86:5686-5696

Abe Y, et al. (2012) "The Toll-like receptor 3-mediated antiviral response is important for protection against poliovirus infection in poliovirus receptor transgenic mice." ***J. Virol.***, 86:185-194

Yamayoshi S, Koike S. (2011) "Identification of the Human SCARB2 Region That Is Important for Enterovirus 71 Binding and Infection." ***J. Virol.***, 85: (10) 4937-4936

Yamayoshi S, et al. (2009) "Scavenger receptor B2 is a cellular receptor for enterovirus 71." ***Nature Medicine*** 15:789-801

Project Leader **Satoshi Koike** Neurovirology Project

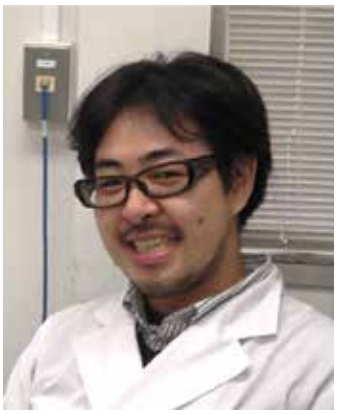
Protecting the Central Nervous System from Infectious Diseases

"The development of vaccines and anti-viral drugs and the evaluation of these agents using experimental models are important for controlling emerging and re-emerging viral infections. We study the basic principles of neurotropic enterovirus infection to further knowledge and technologies to control infectious diseases."

Enterovirus 71 (EV71), a human enterovirus species A of the genus *Enterovirus* within the *Picornaviridae* family, is known to be one of the causative agents of hand-foot-and-mouth disease (HFMD). HFMD is generally a mild and self-limiting disease. However, in some infants and young children, HFMD caused predominantly by EV71 can be complicated by neurological manifestations. Thus, EV71 infection is a serious public health concern. Unfortunately, there is still very little information concerning EV71 pathogenesis, and vaccines or anti-EV71 drugs have yet to be developed.

Members

Kyosuke Kobayashi

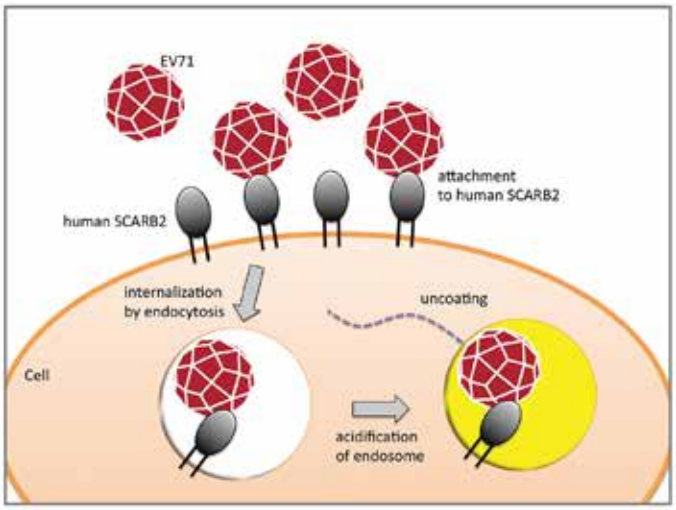


Neurovirology

Research Topics

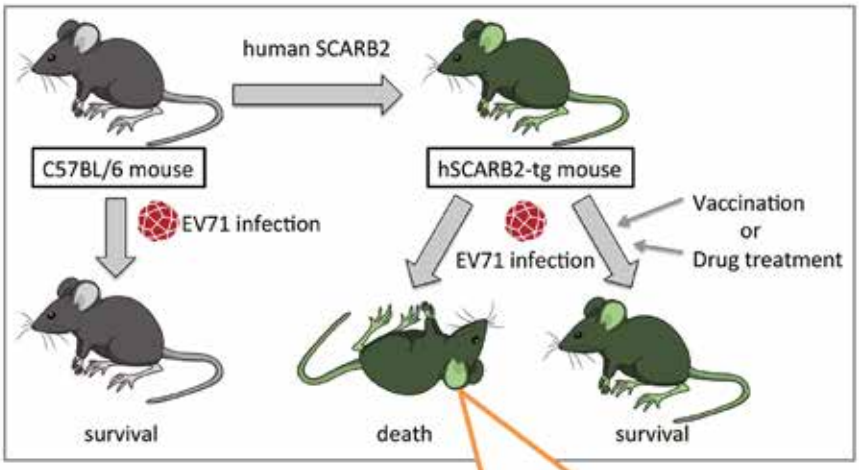
Mechanism of Enterovirus 71 infection

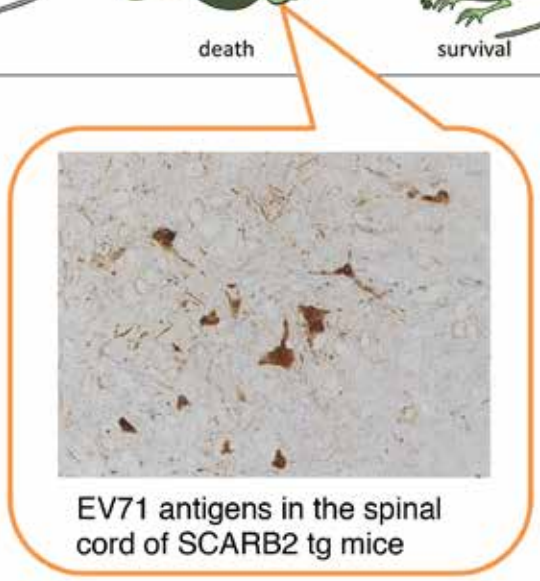
We recently found that Scavenger receptor B2 (SCARB2) is a receptor for EV71. SCARB2 plays a central role in early stages of EV71 infection. SCARB2 is able to mediate binding of the virus at the cell surface, internalization of the virus and initiation of uncoating.



Development of an animal model for Enterovirus 71 infection

Transgenic mice expressing human SCARB2 are susceptible to EV71, and are a useful model for the study of EV71 pathogenesis and vaccine efficacy.





EV71 antigens in the spinal cord of SCARB2 tg mice

Neurovirology

Project Leader **Takachika Hiroi** Allergy and Immunology Project

Allergies and Mucosal Immunology:

Investigating molecular mechanisms of sublingual immunotherapy (SLIT) and developing therapeutic biomarkers for allergic diseases

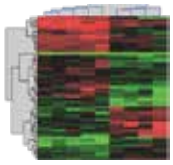
Japanese cedar pollen allergy is the major allergic disease in Japan, affecting approximately 35% of Japanese people. In recent years, sublingual immunotherapy has been recognized as an effective curative treatment for allergic diseases.

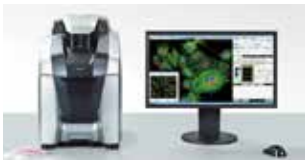
However, the molecular mechanisms of mucosal tolerance still remain unclear. In our laboratory, we focus on the following topics.

"We are developing new diagnostic and treatments for allergies."

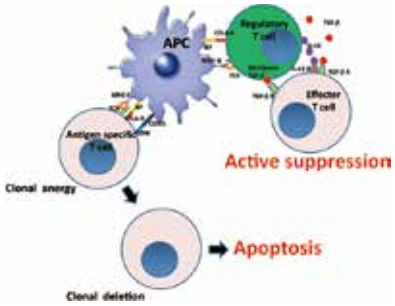
1. Search for effective biomarkers of SLIT

- CNVs
- SNPs
- Epigenome
- Proteome, etc.





2. Elucidation of molecular mechanisms by which SLIT induces immunological tolerance



- iTregs
- Apoptosis
- CTLA-4
- TGF-β
- IL-10 etc.

Gotoh M, Kaminuma O, Nakaya A, Katayama K, Motoi Y, Watanabe N, Saeki M, Nishimura T, Kitamura N, Yamaoka K,Okubo K, and Hiroi T. (2017) "Identification of biomarker sets for predicting the efficacy of sublingual immunotherapy against pollen-induced allergic rhinitis." ***International Immunology*** 29: 291-300.

Nishimura T, Kaminuma O, Saeki M, Kitamura N, Matsuoka K, Yonekawa H, Mori A, and Hiroi T. (2016) "Essential contribution of CD4+ T cells to antigen-induced nasal hyperresponsiveness in experimental allergic rhinitis." ***PLOS ONE*** 11: e0146686.

Yokoyama S, Takada K, Hirasawa M, Perera LP, and Hiroi T. (2011) "Transgenic mice that overexpress human IL-15 in enterocytes recapitulate both B and T cell-mediated pathologic manifestations of celiac disease." ***J. Clin. Immunol.*** 31: 1038-1044.

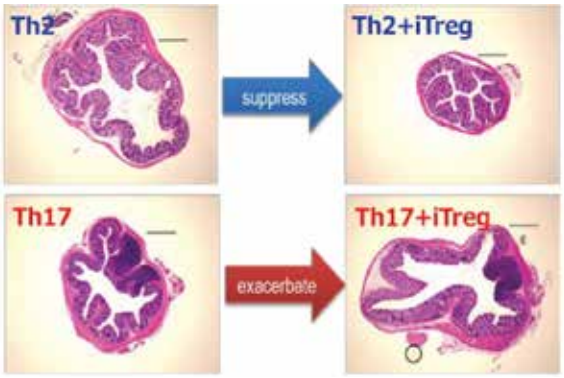
Kaminuma O, Kitamura F, Miyatake S, Yamaoka K, Miyoshi H, Inokuma S, Tatsumi H, Nemoto S, Kitamura N, Mori A, and Hiroi T. (2009) "T-box 21 transcription factor is responsible for distorted TH2 differentiation in human peripheral CD4+ T cells." ***J. Allergy Clin. Immunol.*** 123: 813-823.

Yokoyama S, Watanabe N, Sato N, Filkoski L, Tanaka T, Miyasaka M, Waldmann TA, Hiroi T, and Perera PL. (2009) "Antibody-mediated blockade of IL-15 signaling reverses autoimmune intestinal damage in a mouse model of celiac disease." ***Proc. Natl. Acad. Sci. USA*** 106: 15849-15854.

Allergy and Immunology

Other Research

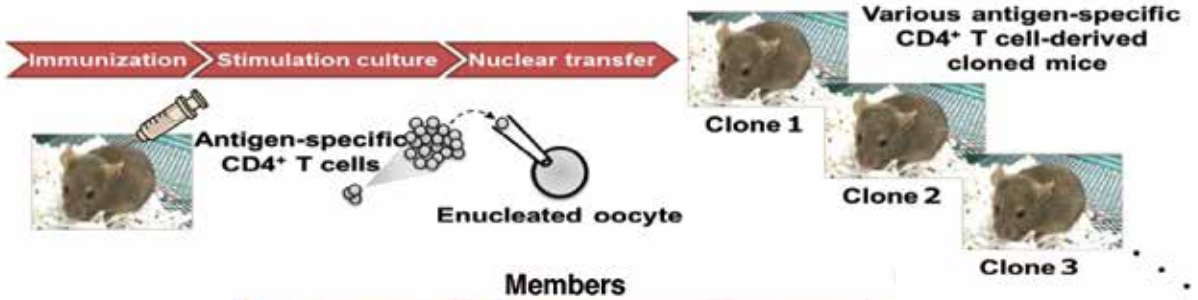
1. Antigen-specific iTreg cells stimulate Th17-mediated colon inflammation



CD4+ helper T cells play a crucial role in allergy and autoimmune diseases including inflammatory bowel diseases (IBDs). Th17 cells and Foxp3+ regulatory T cells (Tregs) are thought to promote and suppress inflammatory responses, respectively. Recently we have developed an antigen-specific and organ-targeted inflammation model by transferring antigen-specific helper T cell subsets followed by antigen administration. By adopting this strategy to colon, we have shown that antigen-specific Tregs stimulate Th17-mediated inflammation in a CTLA4-dependent manner. This finding suggests that Treg/CTLA4-based immunological treatment that are currently in use may be problematic.
(Watanabe N, et al. (2016) *PLOS ONE,* 11: e0150244.)

2. The mechanisms of allergic inflammation investigated using "cloned mice" of antigen-specific CD4+ T cells

Allergens bind to a T-cell receptor (TCR) on CD4+ T cells and induce a series of immune reaction. TCR-transgenic mice are important tools to analyze antigen-response mechanisms, but their non-endogenous TCR might induce immune responses in a manner distinct from those induced by the endogenous TCR. Cloning by the nuclear transfer method enables us to produce animals that retain the donor genotypes in all tissues including germline and immune systems. We generated cloned mice carrying TCR genes of antigen-specific CD4+ T cells that have rearranged in an endogenous manner. These cloned mice express antigen-specific TCR under the intrinsic promoter, and present a unique animal model with which one can investigate CD4+ T cell-mediated pathogenesis and cellular commitment in immune diseases.
(Kaminuma O, et al. (2017) *EMBO Rep.* 18: 885-93.)



Members

Allergy and Immunology

Li Q, et al. (2018) "Int6/eIF3e Silencing Promotes Placenta Angiogenesis in a Rat Model of Pre-eclampsia." ***Sci. Reports*** 12, 8(1):8944

Endo F, et al. (2017) "Development of a simple and quick immun-chromatography method for detection of anti-HPV-16/-18 antibodies." ***PLoS One.*** 12(2):e0171314.

Sakurai A, et al. (2015) "Fluorescent immunochromatography for rapid and sensitive typing of seasonal influenza viruses." ***PLoS One.*** 10(2):e0116715.

Nakano S, et al. (2015) "Immunochromato-graphic Detection of Serum Anti-a-Galactosidase A Antibodies in Fabry Patients after Enzyme Replacement Therapy." ***PLoS One.*** 10(6):e0128351.

Hashimoto T and Shibasaki F. (2015) "Hypoxia-inducible factor as an angiogenic master switch." ***Front. Pediatr.*** 3:33.

Sakurai A, et al. (2014) "Multi-colored immunochromatography using nanobeads for rapid and sensitive typing of seasonal influenza viruses." ***J. Virol. Methods.*** 209:62-68.

Sakurai A, et al. (2013) "Broad-spectrum detection of H5 subtype influenza A viruses with a new fluorescent immunochromatography system." ***PLoS One.*** 8(11):e76753.

Nakano S, et al. (2013) "Development of a highly sensitive immuno-PCR assay for the measurement of a-galactosidase A protein levels in serum and plasma." ***PLoS One.*** 8(11):e78588.

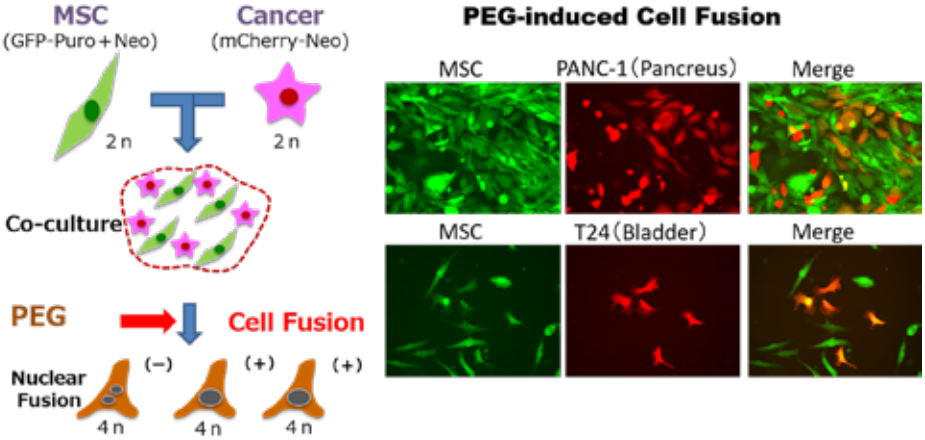
Li Chen, et al. (2007) "Mammalian Tumor Suppressor Int6 Specifically Targets HIF-2alfa To Degradation by Hypoxia- And pVHL- Independent Regulation." ***J. Biol. Chem.*** 282. 12707.

Chen L, et al. (2010) "*Int6/eIF3e* Silencing Promotes Functional Blood Vessel Outgrowth and Enhances Wound Healing by Upregulating HIF2-a Expression." ***Circulation*** 122: 910-919.

Project Leader **Futoshi Shibasaki** Molecular Medical Research Project

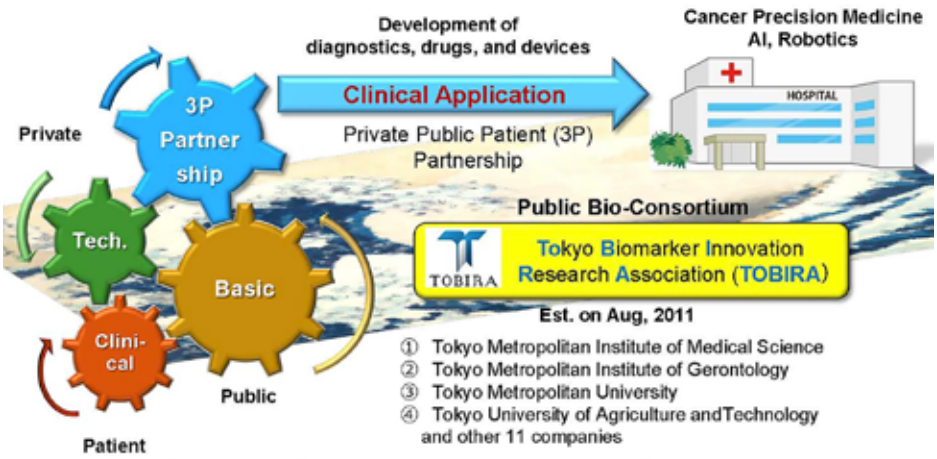
Translational Research for Cancer and Infectious Diseases: Basic to Applied Science

We uncover new mechanisms involved in the pathology of cancers and infectious diseases using novel biomarkers and technologies. This allows us to develop new drugs for the treatment of these diseases. In basic research, we focus on understanding mechanisms of cancer angiogenesis, elucidating how cell fusion induces malignant transformation and metastasis, developing drugs using siRNAs, and developing novel drug treatments for H5 influenza viral infections.



In clinical and translational research, we are establishing a platform to perform "precision medicine" by whole genome analysis using next generation sequencing, in collaboration with metropolitan hospitals. Towards this end, we have already established a bio-consortium, "Tokyo Biomarker Innovation Research Association (TOBIRA) in a private-public partnership (3P) program.

Our general goal is to perform both basic research to identify novel disease targets, and translational research to develop our findings into novel treatments for patients.

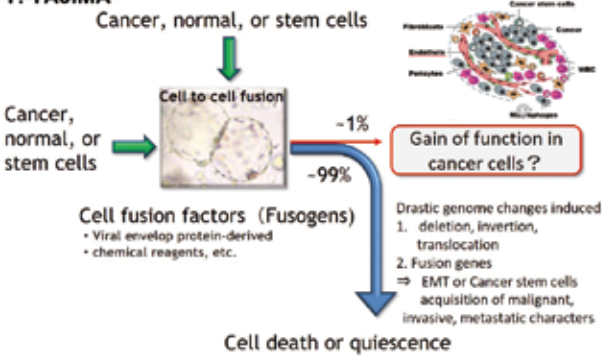


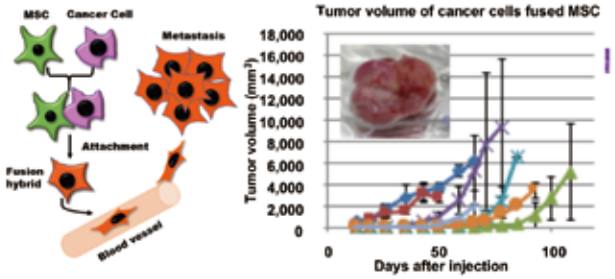
Molecular Medical Research

Malignant cancer progression after cell fusion with stem cells

Y. TAJIMA

Cancer cells fused with mesenchymal stem cell (MSC) in the micro-environment, changes the original character, and often promote dormant, malignant, or metastatic tendency.





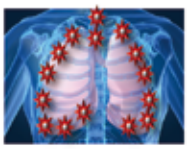
Fused cancer/MSCs promote metastasis than originals

Development of drugs for highly pathogenic H5N1 influenza viruses

N. KAJIWARA

H5N1 has multiple basic amino acids at HA cleavage site.

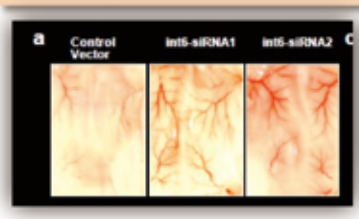
H1N1 Cleavage site
KYVRSTKLRMVTGLRNIPSIQYR----/GLF
H3N2
KYVKQNTLKLATGMRNVPEKQTR----/GLF
H5N1
HA KYVKSNRLVLATGLRNSPQRERRRKKR/GLF

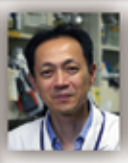


H5N1 highly pathogenic avian influenza virus causes severe pneumonia and multiple organ failure. The mortality rate is about 60%.

We focus on the mechanism of basic amino acid sequence of the split region for discovering new model of the virus entry. The goal of our research is to provide new insights into the molecular mechanism of highly pathogenic avian influenza (H5N1) infection as well as the development of novel antiviral drugs.

Drug development of Int6-siRNA



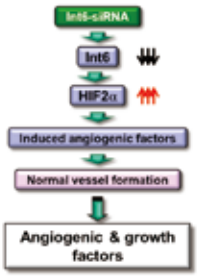


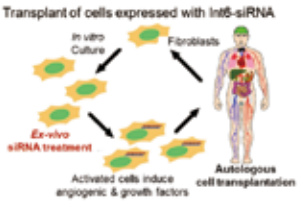
A. IRIE

S. GOTO

Int6 is a key factor to negatively regulate HIF2α-induced angiogenesis and cell protection. The specific siRNA against *int6* would be a possible candidate for cell therapy to treat emic diseases of heart, brain, lower limb, and degenerative and atrophic diseases.

Cell Therapy with *Ex vivo*-siRNA treated Cells





for Ischemia, Degeneration, Atrophy

Diagnostics and device development through Private Public Patient Partnership

Fluoro-IC Chip & Reader

IC Reader

With high sensitive fluoro-beads <15 min, >100 folds sensitive

① **Seasonal A, B Influ IC**
PMDA-approved in 2014
(100 fold higher sensitivity)

② **H5N1 Avian Influ IC**
under development

Rapid & Easy IC Chip

Rapid Colored

Semi-Quantification

Launched in 2016 from LSI Medience

① **Kits for detecting neutralizing Ab in Fabry**

② **Seasonal A, B Influ color IC**
PMDA Approved in 2014
Now on sale

③ **Kits for Cervical Cancer (Plan for sale in 2018)**

Rapid Gene Amp. Devices

Superspeed PCR & Chips

Ver.1 Ver.2 Ver.3

Rapid PCR <8 min for 40 cycles

We aim to develop a rapid and handy device to amplify the target DNAs and RNAs for diagnosis of infectious diseases and cancers.

Molecular Medical Research