Measurements for identifying weak spots in vinyl isolator and breeding room walls

○Muneo Saito, Kuniyasu Imai

1JIC Co., Ltd, Kawasaki, Japan, 2Central Institute for Experimental Animals, Kawasaki, Japan

When vinyl isolators and breeding rooms are used for microorganism control, it is important to identify any weak spots in the walls promptly to ensure continuous airtightness. We aim to establish a method of locating possible weak spots in the walls of vinyl isolators and breeding rooms. Wind speed, air pressure, temperature and humidity were measured in various areas within a vinyl isolator under each of five pressurization conditions induced with a blower. The areas adjacent to the weakest points in the walls were identified based on differences between expected values and the actual measurements in those areas. The maximum margin of error between the estimated and actual areas of weakness was 18%. We judged that this margin of error was within the range of normal prediction. In a breeding room, we estimated areas of weakness by the same method using an instrument that measures airtightness, and were able to identify the areas of weakness easily. We expect that the measurement methods established in this study will be useful in controlling airtightness in vinyl isolators and breeding rooms.

Estimating leak size in vinyl isolators based on air pressure changes

○Kuniyasu Imai, Muneo Saito

JIC Co., Ltd., Kawasaki, Japan

Our object is to establish a leak test enabling us to estimate the sizes of leaks in vinyl isolators. Several vinyl isolators composed of vinyl parts (vinyl cap, sterile lock and sleeve) and with chamber size 115 × 50 × 45 cm were prepared for this study. An artificial hole with a diameter of 0.1, 0.2, 0.3, 0.5, 0.6, or 1.0 mm was made in each vinyl isolator, and the vinyl isolators were pressurized with blowers. An additional chamber without a hole was used as a negative control. The proposed leak test was then performed as follows. The vinyl isolators were pressurized to either 100 or 300 Pa. Temperature, humidity, and air pressure changes in the vinyl isolators with holes were simultaneously measured using a Testo-435 air quality measuring instrument. The severity of the decrease in air pressure resulting from a leak was proportionate to the size of the hole. Furthermore, the final air pressure value depended on the initial pressurization (100 or 300 Pa) induced by the blower. These results suggest that this leak test is a useful means of estimating leak size in vinyl isolators.
P-3  CARD Premium Mouse Bank System

○Mari Iwamoto1, Kiyoko Yamashita1,2, Tomoko Kondo1,2, Yukie Haruguchi1,2, Yumi Takeshita1,2, Yuko Nakamuta1,2, Tomoko Umeno1,2, Ai Miyagawa1,2, Fumi Takahashi1, Wataru Sakamoto1, Yoshiko Nakagawa1, Yuki Yamamoto1, Yuki Yamamoto1, Shuuji Tsuchiyama1, Toru Takeo1, Naomi Nakagata1
1Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan, 2Kyudo Co., Ltd., Tosu, Japan

To facilitate the use of genetically engineered mice, we provide a mouse bank system. Our mouse bank system aids the sharing of genetically engineered mice by supporting the production, preservation and supply thereof. In our system, we archive cryopreserved embryos and sperm derived from genetically engineered mice. Information concerning the archived mice is published on the website of the International Mouse Strain Resource. All researchers are free to use the mice for their experiments under the terms of a material transfer agreement. In addition to the CARD Mouse Embryo and Sperm Bank System described above, CARD offers another type of mouse bank service, called the CARD Premium Mouse Bank System. Our premium mouse bank system enables the efficient preservation and production of genetically engineered mice for an additional charge. Using the system, researchers can produce mice based on an experiment design or recover a mouse line that is affected by infertility or subfertility during natural mating. In this presentation, we will introduce the CARD Premium Mouse Bank System in detail.

P-4  CARD Mouse Embryo and Sperm Bank System

○Fumi Takahashi1, Mari Iwamoto1, Kiyoko Yamashita1,2, Yukie Haruguchi1,2, Tomoko Kondo1,2, Yumi Takeshita1,2, Yuko Nakamuta1,2, Tomoko Umeno1,2, Ai Miyagawa1,2, Shuuji Tsuchiyama1, Wataru Sakamoto1, Yoshiko Nakagawa1, Yuki Yamamoto1, Yuki Yamamoto1, Toru Takeo1, Naomi Nakagata1
1Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan, 2Kyudo Co., Ltd., Tosu, Japan

In 1998, the CARD Mouse Bank was established to share genetically engineered mice by supporting the production, preservation and supply thereof. Mouse banks collaborate on a global scale and share archived strains of genetically engineered mice via the International Mouse Strain Resource. Until now, we have archived more than 2,000 strains of genetically engineered mice in the CARD Mouse Embryo and Sperm Bank System. The system allows researchers to deposit their mice free of charge. Meanwhile, information concerning the archived mice is published on our CARD R-BASE website. Using the mouse bank system, we have supplied mice to domestic (570 strains) and foreign institutes (170 strains). Recently, we have applied new services to the mouse bank system, namely the shipment of cold-stored sperm, the supply of cryopreserved embryos and sperm, and the introduction of the CARD Entry System. In this presentation, we will introduce the latest information concerning the CARD Mouse Embryo and Sperm Bank System.
P-5  Smart Lab application; Development of facility monitoring system ~ Escape detection system ~
Miki Tajima, Kenji Sakuma, Yoshiharu Tanaka, Toshiaki Fukui, Kazuhisa Yatabe
Oriental Giken Inc.

The increased popularity of the smart phones and tablet devices expanded the remoted control system, such as smart appliances, which allowed the users to be able to obtain required information when needed. On the other hand, in the laboratory animal breeding environment in companies and universities, the smartization of breeding environment has not yet been realized, where the people still have to go into the site to obtain required information. Considering the diffusion prevention (Cartagena Law) and animal welfare of transgenic animals in the breeding facilities, it is important to regularly monitor the status of animals. Since there is immeasurable impact on the environment by escaped experimental animals, by any chance when these events occurred, immediate assessment of the incident and action should be taken. There are 4 major cause of animal escape, it is (1) aging of the cage, (2) forgetting to close the lid, (3) absence of mouse-back, (4) escape during handling. In order to accomplish immediate assessment of the escape incident and to take prompt action, we developed an escape detection system of experimental animals. Furthermore, to allow the users to be able to obtain required information instantaneously when needed, an open network system using ICT is also developed and studies.

P-6  Air conditioning system of an animal experiment facility in Shimane University
Takaya Yamada¹, Kouhei Kawakami¹, Yumiko Kirihara¹, Noyo Kajitani¹, Masaki Kitano², Shiniti Yamazaki³
¹Department of Experimental Animals, Interdisciplinary Center for Science Research, Organization for Research, Shimane University, Japan, ²CLEA Japan, Inc. Tokyo, Japan, ³Nitto air tech. Co., Ltd. Kawaguchi, Japan

For facilities performing animal experiments, it is important to maintain a constant environment for experimental animals to obtain reproducible experimental results. The first-stage construction of the Izumo Campus of Shimane University Department of Experimental Animals was completed in 1978 (floor space: 983 m²), and the second (floor space: 910 m²) and third (floor space: 983 m²) stages were completed in 1982 and 1985, respectively. However, the air conditioner installed in the general animal rooms in the 3rd stage used gas for the heat source and its operation was stopped due to a high running cost. We report renewal of the air conditioner installed in the 3rd stage. The adoption of this AQUA-CLEAN system achieved the following improvements: 1) Although it was an estimate, the annual running cost was markedly reduced. 2) The temperature and humidity in the animal rooms were stabilized. 3) The product lives of moderate-performance and HEPA filters were extended. 4) Odor in animal rooms, such as ammonia, was completely removed, achieving a favorable environment for both users and animals. 5) The shower water quality in the water tank is high, and its maintenance work was reduced by use of the unit heat exchanger.
P-7  Long term performance report of cleanliness in a compact facility for breeding SPF mice

Hironori Otomo¹, Kenji Sakuma², Keiko Otokuni¹, Susumu Hayashi², Hideaki Matsuoka¹,
Mikako Saito¹

¹Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan, ²Oriental Giken Inc., Tokyo, Japan

According to the standard operating procedures (SOP), the cleanliness of a compact facility for SPF mice could successfully be maintained for long time. This facility used disposable mouse cages and therefore a usual large autoclave was not necessary. The SPF clean room was 5.7 X 8.1 X 2.7 m³, with a breeding capacity of 1008 cages (168 cages on each of 6 racks). The cleanliness in the SPF clean room was evaluated under the conditions of the occupation rate of 60% to 70% and typically 1 to 3 personnel (maximum, 4 to 6) daily on weekdays. Only personnel that passed the qualification test and were well educated about SOP were permitted to use the facility. The concentration of airborne particles was measured regularly at fixed 5 points 1 time per week. During the study period, the particle concentration level could be maintained below the criteria of ISO 14644-1. No mice exhibited clinical symptoms of infection. Two mice that looked healthy were selected arbitrarily and tested for 16 pathogens including Staphylococcus aureus, Pseudomonas aeruginosa, and Helicobacter bilis. However no pathogen was detected. This report demonstrates the effectiveness of SOP and long term recording of the cleanliness for the maintenance of facility performance.

P-8  Investigation of pH changes in mice feces as an indicator of environmental stress during breeding

Hironori Otomo¹, Kenji Sakuma², Keiko Otokuni¹, Susumu Hayashi², Hideaki Matsuoka¹,
Mikako Saito¹

¹Tokyo University of Agriculture and Technology, Tokyo, Japan, ²Oriental Giken Inc., Tokyo, Japan

The detection of mice that have been accidentally exposed to stresses during breeding and the removal of those mice are essential for reliable experiments using mice. Previously the microflora and the odor of the feces were found to be feasible as the indicator of their stress exposure. This study focuses the pH changes of the feces as a more useful indicator. Various stresses that mice might suffer during breeding have been simulated by the following 4 models: fasting, movement restriction, shaking, and no bedding chips. The fecal pH of the control mice was constant around 7.15-7.44 during 60h breeding period. In contrast, that of the mice exposed to each stress model increased significantly up to 7.60-8.00 at 24h. In the particular case of fasting stress, the pH value further increased up to 8.40 at 48h and then decreased to 7.60 at 60h. In the other stresses, the pH values were maintained at the same level until 60h. Therefore the fecal pH changes of mice are a useful indicator of stress exposure that may be determined by a simple, rapid, and non-invasive manner.
P-9  A study of the properties of chlorine dioxide gas as a fumigant-2

○Fujio Sekiguchi1, Ayumi Matsuura1, Masashi Uekusa1, Keisuke Nakagawa1, Toshiaki Hayashi1, Akira Kasai1, Satoru Sharyo2, Yasufumi Shirasaki2
1Hamri Co., Ltd, 2Bioligical Research Department

Objective: To assess the feasibility of chlorine dioxide (ClO₂) gas for use in animal facilities, we developed a simple method for ClO₂ gas generation. Here we examined the time course of changes in concentration of ClO₂ gas and the antibacterial effect of ClO₂ gas against a bacterial spore, Bacillus atrophaeus (B. atrophaeus).

Methods: ClO₂ gas was generated by mixing 3.35% Na chlorite solution (Purogene) and phosphoric acid with a 10:1 volume ratio. Under high humidity (75-85%), experiments were performed various amounts of Na chlorite ranging from 0.25 to 20 ml/m³. Biological indicator strips preloaded with B. atrophaeus (ACE test) were placed at various sites, including the ceiling, walls and floor. We also used chemical indicator strips (ClorDiSys) to validate ClO₂ gas exposure.

Results and Discussion: The gas concentration increased in a Na chlorite volume-dependent manner and reached peak values of 0.8 to 40.8 ppm at 2-3 h, followed by a decline. Gas exposure at Na chlorite 4 ml/m³ (maximal concentration of 10.6 ppm) achieved complete inactivation of B. atrophaeus. In addition, all chemical indicator strips changed color from a deep purple to a lighter pink when exposed to ClO₂ gas, indicating that gas was equally distributed. Thus, our approach is expected to serve as an alternative method to formaldehyde fumigation in cleaning and disinfecting animal facilities.

P-10  Study on High-level Containment Facilities (1)
Airtightness for Laboratory Animal Facilities

○Hideaki Tani, Mikio Takahashi, Kentaro Amano, Hiroki Takahashi, Taku Hidaka, Hayato Yamamoto, Daizou Yokoyama, Hiromichi Yanagi, Shuichi Numanaka
Takenaka Corporation, Tokyo, Japan

In laboratory animal facilities, airtightness is important because of the prevention from leakage of odors or decontamination gases. We conducted some airtightness tests with two different methods at 23 animal rooms. One is fan depressurization method, evaluated with C-value (corresponding opening area). The other is tracer gas method using CO₂ gas, evaluated with Air Change Rate (ACR). As a result, C-value was within the range of 0.002 to 0.350cm²/m², and ACR was within the range of 0.001 to 0.056 times/h. Otherwise, airtightness of the infected animal lab to research pathogenic mechanism, treatment method, etc. is more important than others. For high-level containment facilities, Pressure Decay Testing (PDT) has been adopted as the airtightness test. According to Canadian Biosafety Standard (CBS), acceptance criteria of airtightness for BSL4 facility is that it doesn’t lose pressure up to 250Pa from an initial 500Pa over a 20min period by PDT. Then, we tried this test with small chamber, appox.4.8m², and the pressure decayed from 500Pa to 50Pa within 260sec. For comparison, C-value of the chamber was 0.027cm²/m². Those show airtightness is far higher than existing animal facilities to achieve the criteria of CBS. Therefore, we will research techniques for highly airtightness facilities using new Lab at TAKENAKA R & D institute, called "Bio-clean/Bio-safety experimentation facility".
P-11  Post-treatment nutritional control in induced-disease model in common marmoset

Eiko Nishinaka, Chiyoko Nishime, Takashi Inoue, Norio Okahara, Ryo Inoue, Tomoko Ishibuchi, Kiyoshi Ando, Hideki Tsutsumi
Central Institute for Experimental Animals

Nutritional control with veterinary and nursing care is indispensable to overcome the acute stage of post-treatment to establish an induced-disease model. In order to improve the nursing care during the acute stage of post-treatment in brain-nervous disease model, we examined the nutritional control. Since it was estimated that 37-92.5 kcal/day would be necessary to keep the animal in good condition and that the activity level after the treatment would reduce to a half, we calculated 46.3 kcal/day would be necessary for the treated animal. Liquid diet and nutritional food (49 kcal) was supplied by gavage to the post-treated animal for a week. As pellet diet (CMS-1M) supply increased, liquid diet supply was reduced; subsequently, only pellet diet was available. Water supplied by gavage and subcutaneous fluid therapies were given to the animals depends on their appetites. Clinical condition, body weight and other factors were considered to judge if the animal was suitable to the induced-disease model. Body weight varied within 5 % range throughout the study. The post-treatment nutritional control including the liquid diet therapy kept animals in good condition. It was proven that post-treatment nutritional control mainly supplying liquid diet was enough to overcome the acute stage. As a result, the appropriate nutritional control increased the number of successful disease models and contributes the animal welfare simultaneously.

P-12  Hematological findings for common marmosets in oocyte collection

Miku Yamasaki1,2, Takashi Inoue1, Mitsuyoshi Togashi1, Tomoko Ishibuchi1, Yoshihisa Sawada1,2, Toshio Ito1, Erika Sasaki1,3
1Central Institute for Experimental Animals, Kanagawa, Japan, 2JAC Inc., Tokyo, Japan, 3Keio University, Tokyo, Japan

The common marmoset (Callithrix jacchus) has been used for generating genetically modified non-human primate model which is advancing knowledge in biomedical research. In the research of developmental engineering, oocyte/embryo collection, and embryo transfer are routinely performed, and veterinary care to these animals must be provided meticulously. In this study, we performed blood examination on these marmosets which were periodically used for oocyte collection by laparotomy. As a result of complete blood cell counts by Sysmex XT2000iV (Sysmex) and blood biochemistry by VetScan VS2 (Abaxis), 10 out of 16 marmosets showed significant evidence of abnormal findings; 5 had hypoalbuminemia (< 2.5 g/dl), 3 had anemia (hemoglobin < 12 g/dl, hematocrit < 40%) and 2 had both hypoalbuminemia and anemia. Paleness in visible mucous membrane and emaciation were observed in 2 out of 5 marmosets with anemia. One out of 7 marmosets with hypoalbuminemia had weight loss (BCS1) associated with diarrhea, but other 6 marmosets showed no clinical signs. This result indicates that the marmosets used for oocyte collection by a laparotomy carry a risk of latent anemia and undernutrition associated with blood loss due to the surgery itself and periodic blood collection for hormonal monitoring. For taking steps to prevent these symptoms, further nutritional support is effective and required for these marmosets.
P-13  Attempt for the Development of Cardiac Surgery Training Program Combined with Live Animal Surgery

○Katsuhiro Ohuchi1, Tomohiro Mizuno1,2, Keiji Ooi2, Masafumi Yamashita2, Tsuyoshi Hachimaru2, Eiki Nagaoka2, Tatsuki Fujiwara2, Hidehito Kuroki2, Dai Tasakii2, Masashi Takeshita2, Syu Endo1, Masami Kanai1, and Hirokuni Arai2
1Advanced Surgical Technology Research and Development, Tokyo Medical and Dental University, Tokyo, Japan, 2Cardiovascular Surgery, Tokyo Medical and Dental University, Tokyo, Japan

We are attempting to develop the cardiac surgery training program combined with live surgical broadcast using the number of live animals reduced to a minimum. A Holstein calf was used. This program was approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. After the premedication, general anesthesia was maintained. Aortic valve replacement was performed after the initiation of cardiopulmonary bypass. The interactive communication system between animal lab and seminar room were connected with video conference system. After the live surgery operation, surgical skill practices with isolated heart preparation were executed in small-group instructions. Observation of expert performing surgical procedure and mutual discussion during the operation were found to be useful to acquire surgical skills that were enhanced the subsequent practices using isolated heart preparation. Our approach utilizing interactive communication system will be effective to reduce the number of animals in a large group cardiac surgical training seminars.

P-14  New Type of Pasteurella pneumotropica Detected by 16S rRNA Gene Sequence

○Mizuno-horikawa Yoko, Andou Rieko, Tanigawa Arisa, Hiraiwa Goro, Kotani Yuko, Kaneko Shiro, Tajima Masaru
The Institute of Experimental Animal Sciences, Faculty of Medicine, Osaka University, Osaka, Japan

Heterogeneous 16S rRNA gene sequence identified to Pasteurella pneumotropica. Pasteurella pneumotropica is well known to cause pneumonia and abscess in immunodeficient mice. Our facility carries out monitoring of P. pneumotropica, because many immunodeficient mice are housed in the facility. We perform nucleotide sequencing of the isolate 16S rRNA gene, because it allows identification of the bacterium origin through the nucleotide sequencing. P. pneumotropica was detected recently from sentinel mouse housed in our facility.

M&M
Laryngopharynx swabs from sentinel mice were incubated on a blood agar. The single colonies were picked up and then subcultured on a chocolate agar three times for cloning. The colonies after cloning were used for examination. PCR was carried out using P. pneumotropica specific primers reported by Kojo to identify the biotype of P. pneumotropica. Then we performed phylogenetic analysis of relationship of selected P. pneumotropica strains using sequence of 16S rRNA gene.

Results
We analyzed each P. pneumotropica strains and isolate with the sequence of 16A rRNA gene. The isolate sequence is approximately different 1% from the sequence of P. pneumotropica ATCC35149 strain.
P-15  Serosurvey of Murine encephalomyelitis virus (TMEV) in laboratory mice and rats in Japan

Tomoko Ishida1, Mai Tanaka1, Narumi Watanabe2, Yu-Ming Hsu3, Nobuhito Hayashimoto1

1Central Institute for Experimental Animals ICLAS Monitoring Center, 2Recruit Staffing Co., Ltd., 3National Taiwan University, College Of Medicine, Laboratory Animal Center

Serosurvey of Murine encephalomyelitis virus (TMEV) in laboratory mice and rats in Japan. Tomoko Ishida, Mai Tanaka, Narumi Watanabe, Yu-Ming Hsu, Nobuhito Hayashimoto

TMEV was a common virus that was observed in laboratory mice and rats in conventional colonies in Japan. However, the information of current status of TMEV in mice and rats is scarce because the virus was known low pathogenic and was not considered as routine test items. To reveal the current status of prevalence of TMEV, we performed serosurvey of the virus in laboratory mice and rats. Material and Methods In total, 16,666 serum samples derived from 2,771 facilities were tested. ELISA was used for screening tests and IFA was used for confirmation tests. Results All 16,666 samples tested were negative. No positive cases were found in this study. Discussion From these results, the prevalence of TMEV seemed to be rare in mice and rats in Japan. In 2012, our group revealed prevalence of TMEV in mice derived from pet shops in Japan. In addition, positive cases of TMEV were confirmed in laboratory mice in Taiwan in 2013. Therefore, we should monitor TMEV in mice and rats in Japan, intermittently.

P-16  Colonization and distribution dynamics of the altered Schaedler flora strains in the gut of NOG mice

Masami Ueno, Yuyo Ka, Ryoko Nozu, Nao Yoneda, Nobuhito Hayashimoto

Central Institute for Experimental Animals, Kanagawa, Japan

The artificial microbiota that consists of about 80 bacterial strains is used for colonization in germfree mice in our institute. Strict control of the colonization of all strains is difficult because of their diversity. The altered Schaedler flora (ASF), consisting of eight bacterial species, is common globally as minimum microbiota that is sufficient to establish normal physiological functions in the gut of mice. Recently, we introduced ASF, and started the basic study for global harmonization. In this study, we investigated the colonization and distribution dynamics of the ASF strains in the gut of gnotobiotic mice (NOG mice with ASF as their only flora).

The colonization change of the ASF strains caused by aging was assessed using fecal samples obtained each week from 10 male and 10 female 3-12-week-old NOG mice. Stomach, small intestine, cecum, colon and fecal samples were collected from same mice at 12-week-old to analyze the distribution patterns of the ASF strains. Extracted DNA from each sample was used for real-time quantitative PCR based on SYBR Green I, and obtained data were analyzed using Thermal Cycler Dice Real Time System.

As a result, colonization of ASF strains has been established in 3-week-old mice. In addition, we will present the data including differences in composition of ASF strains due to factors such as gender or breeding cage.
P-17 EZ-Spot®: Dried-whole-blood Samples for Mice and Rats Serology

○Hanako Kubomura, Kaori Kusano, Moe Yokoyama, Hiroshi Nishida, Kazuyoshi Miyata, Shigeri Maruyama
Charles River Laboratories Japan Inc.

EZ-spot® is the serology system used in Charles River group and the tests can be conducted with the blood sample collected from live animals. With EZ-Spot®, approximately 25 micro liter of blood samples, which are collected directly from laboratory animals and dried on the EZ-Spot® card, is used. Using the dried-blood samples, transporting samples can be simplified. Because the infectious agents can be monitored with live animals (mice and rats) by using EZ-Spot®, it is possible to monitor animals used for a research or quarantine animals without euthanizing those animals. Therefore, the number of sentinel animals, which are generally used for serology test, can be reduced. Time for sample collection and preparing serum can also be reduced. In addition, with PCR tests, it can monitor infectious agents comparative to those in the general health monitoring which euthanizes animals. Therefore, it is beneficial for the animal facility which does not have easy access to the health monitoring laboratory and reduces the transportation costs. We introduce the process of sample collection from mice and rats through serology tests of EZ-Spot® and compare the results between EZ-Spot® and general serology methods in Multiplexed Fluorometric ImmunoAssay and Indirect Fluorescent antibody Assay. *MFIA is a method which can test multiplex items and assay the suitability of samples and reagents. Consequently, more highly trusted results are achieved compare to the ELISA.

P-18 Effect of lactoferrin for the infected sperm

○Takashi Takeuchi, Syunjiro Koizumi
Laboratory of Experimental Animal, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan

Cell death of the sperm is thought to be induced by the recognition of pathogen with the Toll like receptor, which expressed on the cell surface of the sperm.In this study, we examined the effect of Lactoferrin (LF) for the suppression of the induction of cell death.The sperm, which collected from the epididymis of ICR mice, was co-cultured with the LF and lipopolysaccharide (LPS: major ligand of the TLR 4) or the peptidoglycan (PG: major ligand of the TLR2). Thereafter cDNA of the sperm was prepared and used for the real-time PCR.As a result, gene expression of the Tradd, which expressed during the apoptosis, was inhibited by addition of the LF for the co-culture of LPS or PG with sperm.On the other hand, oral admiration of LF suppressed the endogenous expression of the LF in the epididymis.These results suggest that the LF could be suppress the apoptosis of the sperm by the pathogen.
P-19  Rapid and competitive detection of Pseudomonas aeruginosa using Acetamide broth in animal facility

○Soon-Deok Lee¹, Eunseok Auh¹, Hyoung-Jin Kim¹, Jun-Won Yun¹, Yeong-Cheol Kang¹²

¹Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea, ²Graduate School of Translational Medicine, Seoul National University College of Medicine, Seoul, Korea

The prevalence of Pseudomonas aeruginosa in infected colony of normal mice reaches about 5-10%, whereas it may reach 100% in immune-deficient mice. Therefore, an immediate examination is essential to remove infected animals from facilities. In general, P. aeruginosa can be diagnosed by smear and biochemical identification. For this, feces from 1297 mouse cages were cultured on MAC agar, and analyzed by VITEK. As a result, the prevalence of P. aeruginosa was found to be 4.9%. This examination took about 7-8 weeks and cost 8,100,000 for final diagnosis. To test new diagnostic process using Acetamide broth, feces from 1145 cages were incubated in Acetamide for 48 to 72h. The changes in broth color of 95 cages appeared from orange to purple-red. Since the color change can appear at deamination of non-fermenting gram(-) microbes, additional smear and VITEK analysis was conducted for 95 suspected cases to distinguish P. aeruginosa from others. P. aeruginosa was finally diagnosed in 2 of 1145 (0.2%). This diagnostic process took about 3-4 weeks and cost 600,000. Consequently, in comparison to general process, the use of Acetamide reduced analysis time (50%) and expenses (94%), suggesting that it can be useful for a rapid screening of P. aeruginosa infection.

P-20  Comparison of microscopy with PCR for mouse pinworm monitoring method

○Ayako Kajita, Chiimi Ogawa, Hiromi Sakata, Naoki Hirano, Akemi Yasui, Noriko Hiraiwa, Atsushi Yoshiki, Fumio Ike

Experimental Animal Division, RIKEN BRC, Tsukuba, Japan

Syphacia obvelata and Aspiculuris tetraptera are well-known pinworms that parasite intestines of mice. Although these pinworms generally show low pathogenicity, the pinworms parasitism affects cytokine production and their heavy infestation result in pups death. We use microscopy for routine pinworm test at RIKEN BioResource Center (BRC) and have found them in many deposited mouse strains. In this study, we compared the microscopy test with PCR method to monitor mouse pinworms.

[Materials and methods] Cecum and colon specimens were cut in pieces and suspended in saline, then observed under stereomicroscope. DNA was extracted from feces and cecum by FastDNA Kit (MP-Biomedical). PCR was performed using S. obvelata and A. tetraptera specific primers (Parel et al. Vet. Parasitol. (2008) 153: 379-383).

[Results and discussion] S. obvelata and A. tetraptera were observed in conventionally housed mice by using microscope. Cecal PCR results were S. obvelata positive and A. tetraptera negative, and both of them were detected by fecal PCR. It seems that differences of their habitat and life cycle influence results of cecal and fecal PCR. In our hands, one sample positive for A. tetraptera by microscopy was negative for both cecal and fecal PCR. Consequently, careful selection of organs for DNA extraction is quite important to get reliable PCR results. Combination of traditional microscopy and PCR is also required for pinworm monitoring.
**P-21**  
**HEV infection survey of wild Japanese macaques in Fukushima Prefecture**

Hiroshi Yamamoto, Naomi Ishii, Sachie Nakiri, Chinatsu Watanabe, Eri Abe, Minami Ishibashi, Ryohei Suzuki, Shin-ichi Hayama, Hiroshi Sato, Ryohei Suzuki, Shin-ichi Hayama, Hiroshi Sato, Kimiyasu Shiraki, TC Li

The Department of Virology, Faculty of Medicine, University of Toyama, Toyama, Japan, School of Veterinary Medicine, Nippon Veterinary and Life Science University, National Institutes of Natural Sciences, Institute for Physiological Sciences, National Institute of Infectious Diseases, Department of Virology II

**Purpose**  
Hepatitis E virus (HEV) is the causative virus that causes acute hepatitis E, and has attracted attention as a zoonosis. Four genotypes of HEV have been identified from human, HEV or HEV-like virus from animals such as bats, pigs, boar, deer, mongoose, ferrets, rats, are separated. We have reported the HEV separation for the first time from the monkey of natural infection of HEV (Yamamoto H. et al, Emerg. Infect. Dis., 18, 2012). However, HEV infection of the wild monkeys is still unclear. **Materials and Methods**  
Wild Japanese macaques in Fukushima Prefecture were investigated the HEV infection. About the 169 serum samples were examined by ELISA, and RT-PCR. **Result and Discussion**  
Anti-HEV-IgG and IgM antibody were not detected with samples of 200-fold dilution, but detected with 6 samples of 50-fold dilution. HEV genetic testing by RT-PCR for these six samples was negative. These results showed that HEV infection of 169 wild Japanese monkeys were negative and it seems to be low risk of HEV infection for humans from wild Japanese monkeys in Fukushima Prefecture.

**P-22**  
**European wood mice *Apodemus sylvaticus* as a possible model for human neuromuscular diseases**

Goro Kato, Masahiro Yasuda, Akio Shinohara, Chihiro Koshimoto

Division of Bio-Resources, Frontier Science Research Center, University of Miyazaki, Miyazaki, Japan, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

**Human neuromuscular diseases have many different types including intractable disease. For research to seek cures for such diseases, several model animals have been developed. However, most of the neuromuscular diseases have yet to be established standard therapy. In order to make next breakthrough to develop new animal models, unprecedented bio-resource might have a latent potential. Here, we present a spontaneous mutant of European wood mice *Apodemus sylvaticus* as a novel model for human neuromuscular diseases. This mutant have leaned limbs and show an abnormality of gait, trembling of the mandibular and limbs. These locomotive symptoms become progressively worse, eventually they demonstrate an astasia, dysphagia and dyspnea. Those symptoms have usually appeared by 1-2 weeks of age, and usually the animals died before sexual maturation. Histopathologically, mutant wood mice had an apparent atrophied quadriceps muscle, and their muscular cells exhibited a variety of sizes and shapes compared with them in normal sibling. In the breeding test with normal siblings from the same pedigree, 25% of offspring in every generations exhibited morbid phenotype, irrespective of sexes. On the basis of our findings, this mutation might be classified as an infantile neuromuscular disease, and inherited in an autosomal recessive manner.**
P-23  An advanced model of cholestatic liver injury in mice

○ Jeyoung Ryu, Nayeong Gu, Jinguen Rheey, Saet-Byul Hong, Jehoon Yang, Sung Joo Kim

Laboratory Animal Research Center, Samsung Biomedical Research Institute, Seoul, Korea

Common bile duct ligated mice are commonly used for a cholestatic liver injury animal model, because liver enzyme and fibrosis levels are rapidly elevated. However, survival length is drastically reduced in the model so as not be appropriate for long-term experiments. To modify the reduced survival length, we developed an advanced model that presents hepatic cholestasis and regeneration concurrently by double ligation of right biliary branch and left portal branch in mice. In the advanced model, survival length was greatly improved and liver enzyme levels were significantly elevated and remained stable for a long period. Moreover, the fibrosis is developed continuously in the right lateral lobes. These advantages of the advanced model are useful to long-term cholestasis study with a substitute of bile duct ligation models.

P-24  Combined secretase modulation therapy provides cognitive benefit and reduces brain amyloid pathology

○ Naoki Koyama, Takashi Mori

Department of Biomedical Sciences, Saitama Medical University, Saitama, Japan

In Alzheimer’s disease (AD), there has not been a successful disease-modifying therapy. We screened a class of phenolic compounds and have focused on two promising compounds [octyl gallate (OG), an enhancer of α-secretase activity and ferulic acid (FA), a β-secretase inhibitor] that inhibit amyloidogenesis. Here, we examined whether OG plus FA combination therapy might synergistically improve behavioral impairment and mitigate amyloid-β (Aβ)/β-amyloid pathology in the PSAPP transgenic mouse model of cerebral amyloidosis. Beginning at 12 months of age, PSAPP mice were orally administered OG plus FA, OG, FA (all at 30 mg/kg), or vehicle once daily for 3 months. At 15 months of age, each treatment improved PSAPP transgene-associated behavioral impairment of novel object recognition, activity, working memory, and spatial reference memory, but left non-transgenic mouse behavior unaltered. Notably, combination therapy synergistically improved most behavioral outcome measures. Moreover, brain parenchymal and cerebral vascular β-amyloid deposits as well as levels of various Aβ species were synergistically decreased in OG plus FA-treated versus singly-treated PSAPP mice. These effects were due to a shift toward non-amyloidogenic amyloid precursor protein (APP) cleavage via α-secretase enhancement and β-secretase inhibition. Together, these data provide pre-clinical proof-of-concept that secretase modulation via combination therapy is a promising avenue for AD treatment.
**P-25** Mouse B6-\(A^y\)/+, \(W^v\)/+ strain is a new diet-inducible diabetes model

○Tamio Ohno\(^1\), Tomoki Maegawa\(^1\), Misato Kobayashi\(^2\), Takeshi Sube\(^1\), Fumihiko Horio\(^2\)

\(^1\)Graduate School of Medicine, Nagoya University, Nagoya, Japan, \(^2\)Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

The \(A^y\) mutation was caused by a deletion of an upstream region of the agouti gene. Inappropriate constitutive expression of the mutated agouti gene causes hyperphagia and progressive obesity; however, B6-\(A^y\)/+ mice do not develop diabetes. \(W^v\)/+ mice, which contain a point mutation of the \(c\text{-}Kit\) gene, exhibit glucose intolerance associated with impaired development and function of pancreatic \(\beta\)-cells. Here, we generated B6-\(A^y\)/+, \(W^v\)/+ mice that carry both mutant genes on a B6 genetic background, and evaluated these mice as a new diabetic model. On a high carbohydrate diet (MR-DBT), B6-\(A^y\)/+, \(W^v\)/+ male mice exhibited hyperglycemia (>250mg/dl) after 11 weeks of age, with a cumulative incidence of 100% at 17 weeks. By contrast, the majority of B6-\(A^y\)/+, \(W^v\)/+ male mice did not show hyperglycemia on a normal diet (CE-2). We conclude that the B6-\(A^y\)/+, \(W^v\)/+ mice offer a new diet-inducible model of diabetes.

**P-27** Development of dental caries in alloxan-induced diabetic mice

○Takahiro Umeno, Kiyokazu Ozaki, Hayato Maruyama, Yui Terayama, Shiori Yoshida, Tetsuro Matsuura

Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka, Japan

Background: Alloxan (AL) induces severe hyperglycemia and causes dental caries in rodents. However, no decayed crown detected in AL-treated diabetic rats is observed in these diabetic mice. In this study, we investigate the relationship between salivary gland and enhanced dental caries, and estimate the availability of AL-treated mice as a model of diabetic dental caries. Methods: Diabetic conditions were induced in both sexes of ICR mice by a single intravenous injection with 75 mg/kg of AL (DM group). Age-matched untreated mice were used as control group. Mice were sacrificed at 35 weeks after AL treatment, and the molars as well as salivary glands were morphologically examined. Results: Almost male diabetic mice were dead or necropsied due to moribund condition. Meanwhile, two-thirds of female AL-treated diabetic mice survived, and dental caries deeply eroded their crown surface. By soft X-ray examination, dentin and enamel was frequently defected at the molar surface and radiolucent lesion extensively expanded around pulp tissue. Lobular atrophy of the parotid glands was observed in large numbers of female diabetic mice. Conclusion: The dental caries in AL-treated diabetic mice is milder compared to AL-treated diabetic rats, and yet the lesion made progress to dental decay till 35 weeks after AL treatment. Atrophy of the parotid gland may induce the dental caries in diabetic mice.
P-28  Dysfunctional and morphological change in salivary gland in type 2 diabetic db/db mice

Tetsuro Matsuura, Miho Yoshikawa, Yui Terayama, Kiyokazu Ozaki
Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka, Japan

Background: Dysfunctional salivary gland is known to occur in diabetic patient. Dental caries is frequently observed in a model of type 2 diabetes, db/db mice at 40 weeks of age, and the lesion seems to relate to morphological change in salivary gland. However, these aged db/db mice are prone to be susceptible to infection, and to lose its characters, which make it difficult to judge precisely the results. Thus, in this study we investigate the relationship between dental caries and disordered salivary glands in this strain at 30 weeks of age when they certainly shows remarkable hyperglycemia and obesity. Methods: Male and female db/db mice aged 30 weeks along with age-matched db/+ mice were used. The saliva volume was measured by stimulation of pilocarpine. The serial sections of defatted salivary glands in the same mice with stimulation of pilocarpine were subjected to histopathological examination. The molars were examined macroscopically for evaluating dental caries. Results: The incidence and severity of molar caries in db/db strain were much higher than in db/+ strain. Meanwhile, the average saliva volume in db/db mice was remarkably decreased compared to db/+ mice. Morphologically, lobular atrophy of the parotid glands was observed in large numbers of db/db mice in both sexes. Conclusion: Salivary secretion is depressed in disordered parotid gland, and this may induce development of dental caries in db/db mice.

P-29  Role of an extracellular matrix tenascin-X, a causative gene of Ehlers-Danlos syndrome

Naoyo Kajitani¹,², Takaya Yamada¹, Kohei Kawakami¹, Ken-ichi Matsumoto²
¹Department of Experimental Animals, Interdisciplinary Center for Science Research, Shimane University, Izumo, Shimane, Japan, ²Department of Biosignaling and Radioisotope Experiment, Interdisciplinary Center for Science Research, Shimane University, Izumo, Shimane, Japan

Tenascin-X (TNX) is a member of the tenascin family of the extracellular matrix (ECM). ECM plays an important role in cellular response, functional expression and differentiation for mesenchymal cells. TNX is one of the causative genes for type I and type III Ehlers-Danlos syndrome (EDS). EDS is a hereditary disease of connective tissue and it shows genetic disorder of the formation of collagen fibrils. We previously reported abnormalities in the skin of TNX-deficient (TNX-/-) mice compared with that in wild-type (WT) mice. It was found that the level of triglyceride was significantly increased in the skin of TNX-/- mice. So we predicted that TNX is involved in the differentiation of mesenchymal cells. In the present study, significant differences were found in expression of mesenchymal cell markers during osteogenesis, adipogenesis and chondrogenesis in embryonic fibroblasts of WT and TNX-/- mice. Subsequently, we examined bone density in femur from WT and TNX-/- mice, since EDS shows abnormality of osteogenesis. In consequence, significant low bone density was found in trabecular bone of femur in TNX-/- mice. Next, we will purify mesenchymal stem cells (MSCs) from WT and TNX-/- mice and then investigate roles of TNX in the differentiation of MSCs.
P-30  A comparative analysis of the organ-weight between C57BL/10Sc-mdx and C57BL/10ScN

Nao Yoneda¹, Masahiko Yasuda¹, Takuma Mizusawa¹, Yuyo Ka¹, Mika Yagoto¹, Chie Shimomura²,
Motohito Goto¹, Takayuki Goto², Tomoyuki Ogura¹, Kenji Kawai¹, Riichi Takahashi¹

¹Central Institute for Experimental Animals (CIEA), Kawasaki, Japan, ²Technical Service Dept., CLEA
Japan, Inc. Tokyo, Japan

CIEA has maintained Duchenne muscular dystrophy (DMD) model mice including C57BL/10Sc-mdx (mdx),
NOD/Shi-scid, IL-2Rγnull-mdx and DBA/2-mdx mice, and has provided them to the researchers related to
myopathy. In this study, to contribute to DMD researches we compared body-weight and organ-weight (brain,
heart, lung, liver, kidney, spleen and testicle/ovary) and calculated body-weight ratio (mg/100g) of
mdx (18 animals/sex, 7 weeks of age) and C57BL/10-ScN (B10; 15 animals/sex, 7 weeks of age) mice. Those data were
processed by the statistics on Student's t-test, we validated significant difference at p<0.05.

In body-weight, female mdx mice significantly showed high value (mdx vs B10: 22.7g vs 21.4g). The brain
and kidney of mdx mice showed low values in both sexes, furthermore, testis and female lung of mdx mice
showed low values. On the other hand, the liver of mdx mice both sexes, and the heart of male mdx mice showed
high values.

P-31  Generation of the humanized liver mouse using HepaRP3 cells

Yuichiro Higuchi¹, Kenji Kawai¹, Hiroshi Yamazaki², Bree Francoirs³, Guguen-Guillouzo Christiane⁴,
Hiroshi Suemizu¹

¹Central Institute for Experimental Animals, Kanagawa, Japan, ²Showa Pharmaceutical University, Tokyo,
Japan, ³XENOBLIS, Saint Gregoire, France, ⁴Biopredic International, Rennes, France

Humanized-liver mice, in which the liver has been repopulated with human hepatocytes, have been used to
study aspects of human liver physiology such as hepatitis infection. The procurement of human hepatocytes is
a major problem in producing humanized-liver mice because of the finite nature of the patient-derived resource.
We previously reported that the human hepatic cell line HepaRG cells were a possible cell source for generating
humanized-liver mice. Although HepaRG cells differentiate into mature hepatocytes in the liver of TK-NOG
mouse, the replacement index is significantly low. Here we describe the transplantation studies of new cell line,
HepaRP3. HepaRP3 is one of the human hepatic cell line derived from the HepaSC cells carrying a stem cell-
like phenotype, following genomic engineering of the parental HepaRG cells. Transplanted HepaRP3 cells
retained proliferation activity in vivo, and HepaRP3 derivatives replaced about 20% of recipient mouse liver.
Immunohistochemical analysis revealed that HepaRP3 derivatives remain the hepatic stem cell characteristics,
such as duct-like morphology and disappearance of mature hepatocyte markers. Since the HepaRP3 derivatives
express the SLC10A1, we expect that humanized-liver mouse carrying HepaRP3 cells would be an infection
model for hepatitis virus.
P-32 Suppressive effect of Asparagus cochinchinensis on skin inflammation

○ Ji Eun Sung, Ji Eun Kim, Jun Go, Hyun Ah Lee, Woo Bin Yun, Eun Ji Seo, Dae Youn Hwang

Department of Biomaterials Science, Pusan National University, Korea

To quantitatively evaluate the suppression effects of ethyl acetate extracts of A. cochinchinesis (EaEAC) on phthalic anhydride (PA)-induced skin inflammation and investigate the role of IL-4 during suppressing process, alterations in luciferase-derived signal and general phenotype biomarkers were measured in IL-4/Luc/CNS-1 transgenic mice with PA-induced skin inflammation after treatment with EaEAC for 2 weeks. A significant decrease of luciferase signal derived from IL-4 promoter was detected in the abdominal region, submandibular lymph node and mesenchymal lymph node of the PA+EaEAC treated group. Furthermore, the weight of the immune organs, IgE concentration, epidermis thickness and number of infiltrated mast cells were decreased in the PA+EaEAC treated group compared with the PA+Vehicle treated group. Moreover, expression of IL-1β, TNF-α, IL-6 and VEGF also decreased in the PA+ EaEAC cotreated group. Therefore, these results suggest that EaEAC treatment could successfully improve PA-induced skin inflammation of IL-4/Luc/CNS-1 Tg mice, as well as IL-4 cytokine play a key role during therapeutic process of EaEAC.

P-33 Role of Asparagus cochinchinensis as a stimulator of NGF on the brain of Tg2576 mice

○ Hyun Ah Lee, Ji Eun Kim, Ji Eun Sung, Woo Bin Yun, Eun Ji Seo, Dae Youn Hwang

Department of Biomaterials Science, Pusan National University, Korea

The identification of novel NGF stimulator has been considered as one of important strategy for treatment of neurodegenerative disease because NGF is unable to across the blood-brain barrier. In this study, we investigated to determine if aqueous extract of Asparagus cochinchinensis (AEAC) could stimulate the NGF biosynthesis and secretion in the brain of Tg2576 mice including Aβ-42 deposition and behavioral defects. AEAC treatment significantly reduced the number of Aβ-stained plaques and the acetylcholinesterase (AChE) activity. Additionally, the concentration of NGF was dramatically enhanced in Tg+AEAC group although it was lower in Tg+AEAC group than nTg group. Furthermore, the decreased phosphorylation of downstream members in TrkA high affinity receptor signaling pathway in Tg+Vehicle group was significantly recovered in Tg+AEAC group. A similar pattern was observed in p75NTR expression and JNK phosphorylation in NGF low affinity receptor signaling pathway. Therefore, these results suggest that AEAC could exert a wide range of beneficial activities for neurodegenerative disorders through stimulation of NGF biosynthesis.
**P-34**  
**Screening of type 2 diabetes candidate genes in the OLETF rat using Drosophila**  
Tomone Hayashi¹, Takahisa Yamada², Hiroyuki Kose¹  

¹College of Liberal Arts, International Christian University, Tokyo, Japan, ²Niigata University, Department of Agriculture, Niigata, Japan

The rapid advancement of understanding of polygenic traits is underway by WGAS (Whole genome association study). However, many causative genes of spontaneous animal model are yet to be uncovered. The OLETF rat is one of such examples in which none of the hyperglycemic QTL has identified its causative genes. Previously, we demonstrated that a gene called imp, whose homologue is localized in Nidd4/of of the rat chromosome 4, is involved in metabolic regulation. Here we performed a semi-comprehensive analysis of genes that are mapped in Nidd2/of closely associated with obesity. There are two peaks of LOD score in this locus. From the analysis with congenic strains, each peak should contain one causative gene. We focused a distal peak of 0.8 Mbp between D14Rat23 and D14Wox1. In Ensembl database, 83 genes are mapped, for 26 of which we generated mutant strains by RNAi. We first established a method to allow extraction of a very small amount (0.25 μl) of hemolymph from a single third instar larva and quantification of trehalose concentration. As a result, we found that two of the strains showed significant high trehalose levels. They are Hsd17b (Estradiol 17-beta-dehydrogenase 11) and Rpl29 ribosomal protein. We will evaluate this screening method and discuss the potentially novel diabetes genes identified in this study.

**P-35**  
**Quantification of cystine in rat white blood cells with cystinosis using LC/MS/MS**  
Yukiko Shimizu¹, Yoshiaki Okuma²,³, Tadashi Okamura¹,⁴  

¹Department of Laboratory Animal Medicine, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan, ²Graduate School of Medicine, Juntendo University, Tokyo, Japan, ³Department of Pediatrics, Center Hospital, National Center for Global Health and Medicine, Tokyo, Japan, ⁴Section of Animal Models, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

Cystinosis is a rare lysosomal storage disease characterized by the abnormal accumulation of cystine in various organs. Without specific treatment, cystinosis patients develop end stage kidney failure at an early age. The definitive diagnosis is made by the leukocyte cystine content. This study was conducted to establish a high sensitivity method using LC/MS/MS system with Intrada Amino Acid column for determination of the leukocyte cystine. The levels of cystine in the leukocyte of cystinosis model rats were significantly higher than those of control rat. Cystine concentrations in white blood cells were also measured in cystinosis patients. Quantification of cystine using LC/MS/MS with Intrada Amino Acid column provides a high sensitivity method for the diagnosis of cystinosis.
P-36  Can high salt loading spontaneously hypertensive rats be used as a new stroke model?

○Kohei Kawakami1, Hiroyuki Matsuo2, Kaoru Niiya2, Kazuo Yamada1, Takaya Yamada1, Toru Nabika2
1Department of Experimental Animals, Shimane University, Japan, 2Department of Functional Pathology, Shimane University

The characteristic of salt-sensitive hypertension is blood pressure elevation corresponding to salt intake, and complications of brain and kidney impairments readily develop. Although spontaneously hypertensive rats (SHR) were reported to be salt-sensitive and less prone to stroke, its mechanism has not been investigated in detail. We found that high salt loading causes a stroke at a high frequency in SHR. SHR were fed a normal or high salt diet for 12 weeks. The clinical condition of stroke was observed daily, and the body weight and blood pressure were measured. Weight gain started to decrease at 6 weeks in the high-salt diet group, and a significant difference was noted between the groups. The blood pressure elevation-inhibitory effect became marked in the high-salt diet group at 2 weeks after administration, showing a significant difference. The incidences of cerebrovascular disorder were 83% and 0% in the high-salt diet and control groups, respectively. Softening of the brain and infarcts occurred in 67% and hemorrhage in the brain parenchyma occurred in 17% in the high-salt diet group. High-salt diet loading tended to more frequently cause ischemic stroke rather than cerebral hemorrhage in SHR, unlike SHRSP. We are planning to closely investigate whether SHR serve as a new stroke model.

P-37  Analysis of retinoid X receptor in the kidney of model animals for polycystic kidney disease

○Masanori Kugita1, Daisuke Yoshihara1, Yu Kato2, Noboru Ogiso1, Yasuyo Shimomura2, Osamu Nishida2, Shizuko Nagao1
1Education and Research Center of Animal Models for Human Diseases, Fujita Health University, Toyoake, Japan, 2Department of Anesthesiology and Critical Care Medicine, Fujita Health University School of Medicine, Toyoake, Japan

Polycystic kidney disease (PKD) is the most common renal hereditary disease and forms innumerable cysts with abnormal cell proliferation of renal tubular epithelial cells. To elucidate retinoid X receptor (RXR)-mediated cyst formation mechanism indicated from our analysis of kidney in Han:SPRD-Cy rats of PKD model animal, we investigated role of renal RXR in jck mouse and pcy mouse of other PKD model animals, and the therapeutic effect of RXR agonist on PKD in Cy rat.

The expression level of renal RXR in 12-week-old jck mouse and 16-week-old pcy mouse were increased compared with age-matched normal mouse. In both of the two PKD models, RXR and PCNA as a marker of cell proliferation were co-localized in the epithelial cell of renal cyst. RXR was phosphorylated in jck mouse. 4-week-old Cy/+ male rats were treated with 30 mg/kg bexarotene (BEX) as a RXR agonist or vehicle (CON) for additional 6 weeks. Kidney/body weight, renal cyst area and expression level of renal RXR were slightly decreased by BEX treatment. These results indicate RXR might be involved in renal cystogenesis, and RXR agonist might be a therapeutic agent for PKD.
P-38  Laxative effects of Galla Rhois in loperamide-induced constipation of SD rats  
○ Ji Eun Kim, Jiun Go, Ji Eun Sung, Hyun A Lee, Eun Ji Seo, Woo Bin Yun, Dae Youn Hwang  
Department of Biomaterials Science, Pusan National University, Korea  

To investigate the laxative effects and their mechanism of Galla Rhois (GEGR) was using the constipation model induced by loperamide (Lop) injection, alterations in the excretion parameters, histological structure, and related protein levels were measured in the transverse colon of SD rats with Lop-induced constipation after treatment with 250, 500 and 1,000 mg/ml of GEGR. The number and weight of feces increased significantly by 48-79% and 128-159% in the Lop+GEGR treated group relative to the Lop+vehicle treated group. The thickness of mucosa, muscle and flat luminal surface as well as the number of goblet cells and crypt of lieberkuhn were enhanced in the Lop+GEGR treated group. Moreover, mucin secretion increased significantly in a dose dependent manner in the Lop+GEGR treated group. Furthermore, the downstream signaling pathway of the mAChR M2 and M3, Gα expression and IP3 concentration was recovered in the Lop+GEGR treated group relative to the Lop+vehicle treated group. These results of the present study provide strong additional evidence that tannins distributed in various medicinal plants are important candidates for improving chronic constipation induced by Lop treatment in animal models.

P-39  Transmission and expression of heterozygous mutant fibrillin-1 gene in genome edited pigs  
○K Umeyama1, K Watanabe2, M Watanabe1, K Horiuchi2, K Nakano1, M Kitashiro2, H Matsunari1, M Nagaya1, M Matsumoto2, H Nagashima1  
1MUIIBR, Kawasaki, Japan, 2Keio University School of Medicine, Tokyo, Japan  

[Purpose] Marfan syndrome (MFS), an autosomal dominant disorder, is caused by a mutation in the fibrillin-1 gene (FBN1). We created heterozygous mutant FBN1 cloned pigs (+/Glu433AsnfsX98) using zinc finger nuclease via somatic-cell nuclear transfer. The purpose of the present study was to use cloned founders for sexual reproduction in order to transmit the mutant FBN1 to the progeny, and to verify progeny phenotypes.[Methods] Two heterozygous mutant FBN1 cloned pigs (male) were mated naturally with wild-type (WT) female pigs to produce progeny, and the germline transmission of mutant FBN1 and the progeny phenotypes were investigated.[Results] Twenty-eight progeny (male WT: 8, female WT: 6, male mutant (Mut): 8, female Mut: 6) were born to three female pigs. We verified that the mutant FBN1 was transmitted to the progeny by Mendelian inheritance. The weight, length, and height of the body and the circumference and depth of the chest of male Mut pigs were significantly larger than those of male WT pigs. These phenotypes were similar to the phenotypes of MFS patients, who are tall and have long limbs. On the other hand, no significant difference was observed between female WT and Mut pigs. However, one female Mut pig was confirmed to have scoliosis. [Conclusions] Our data demonstrated that the mutant FBN1 was stably transmitted to the progeny, and that MFS phenotypes can also be transmitted.
P-40 Restoration of lymph node development by specific expression of IL-2Rgc in LTi in NOG mice

○Takeshi Takahashi¹, Fumihiro Sugiyama², Seiya Mizuno², Hayato Abe¹, Mamoru Ito¹

¹Central Institute for Experimental Animals, Kawasaki, Japan, ²Laboratory Animal Resource Center

Aim NOG mice are widely used as a platform for engrafting various tissues including xenogeneic materials such as human hematopoietic stem cells (HSC). However, at the expense of the high engraftment, the loss of IL-2Rgc results in the impaired development of mouse lymph nodes (LNs). In this research, we attempted to restore LN development in NOG mice in order to reconstruct physiological environments for human lymphocytes.

Methods Lymphoid Tissue inducer (LTi) cells are essential for inducing development of lymph nodes during embryo. We attempted to rescue LTi by introducing the IL-2Rgc gene in an LTi-lineage specific manner. We used the promoter of a transcription factor, RORgt, a master regulator for LTi differentiation. We constructed a BAC DNA, in which the mouse IL-2Rgc gene was placed under the promoter of RORgt. We generated pRORgt-gc BAC Tg mice by microinjection.

Results We confirmed the restoration of LN development in IL-2Rgc/-pRORgt-gc BAC Tg mice. Those included inguinal LNs, popliteal LNs, para-Aorta LN, iliac LNs. The mice also manifested the enlargement of mesenteric LNs. We transplanted human HSC into NOG-pRORgt-gc BAC Tg after X-irradiation. Human hematopoiesis was detected by flow-cytometric analysis and migration of human lymphocytes into the restored LNs was also confirmed. We are now investigating whether engraftment of human cells is improved in the NOG-BAC Tg compared with NOG-nonTg mice.

P-41 Study of Effects of Drugs on Cynomolgus Monkeys with Type 2 Diabetes in Kunming

○Azusa Seki, Haruya Honda, Nobuo Suzuki

HAMRI Co., Ltd., Ibaraki, Japan

The purpose of this study was to evaluate the effects of the test drugs on Type 2 diabetic cynomolgus monkeys given the test drugs for 6 months in YuMore Biolaboratory Primates Inc., China. In the present study, Type 2 diabetic cynomolgus monkeys aged 20 - 22 years were used, and commercially available preventive and therapeutic drugs for osteoporosis and therapeutic drugs for diabetes due to a decrease in blood glucose levels were used. These animals were allocated to the following groups; Group A (5 animals for therapeutic drugs), Group F (1 animal for PTH), Group V (5 animals for vehicle group) and Group I (5 animals for intact). In these animals, the bone mineral density, Os iliac biopsy and bone histomorphology were examined before and at the end of the administration period and parameters of urine and serum collected from each animal every 3 months were analyzed. In the parameters at 6 months after treatment, the BMD (mg/cm³) of the third lumbar vertebrae by CT increased only in Group F. The blood glucose values (mg/dL) and HbA1c (%) in Group A was nearly equal to those in Group I. In bone morphogenesis, both osteoblast surface (Ob.S/BS:%) and number of osteoclasts (N.Oc/BS:N/mm) were high in Group F.
The Japan Mouse Clinic (JMC) has performed comprehensive phenotyping of mouse lines deposited in RIKEN BRC based on standardized operating protocols (SOPs). We have analyzed 298 mouse strains, and these results have been reported on the Pheno-Pub website (http://mouseclinic.brc.riken.jp/).

In 2011, JMC participated in the International Mouse Phenotyping Consortium (IMPC). The objective of the IMPC is the primary phenotyping of all gene Knockout (KO) mouse lines in the genome developed by ES Cells in KOMP and EuCCOM. The IMPC has analyzed over 2000 mouse lines, and these data have been released to the public (http://www.mousephenotype.org/).

The IMPC has established SOPs; the International Mouse Phenotyping Resource of Standardized Screens (IMPReSS) has been prepared and therefore the parameters of the phenotyping data and meta-data are clearly defined. The obtained data from each phenotyping center were collected in the Data Coordination Center (DCC) and validated before being released to public on website.

In compliance with IMPReSS, JMC has constructed the phenotyping system and has established a Laboratory Information Management System (RIKEN LIMS). The mouse phenotyping data generated at JMC contribute to the worldwide progress of the field of life sciences.

We developed a portal site, "J-phenome" (http://jphenome.info/), for the wider dissemination of phenotype data of experimental animals collected from broad research communities of model animals and genome editing.

We imported data from NBRP Rat, NBRP medaka, NIG consomic mouse and so on. Then we developed databases as derivative works annotated by ontologies as "common vocabularies", helps comprehensive search. Cross-database search can be performed where mice and rat that exhibit the same (or similar) phenotype at a time.

Currently, J-phenome links to BRC mouse (5800 strains), cells (3800 lines) and microorganisms (15,000 strains). We update them monthly to provide latest data. J-phenome also links to MSM-B6 consomic mouse (about 50 strains) developed in National Institute of Genetics, Functional Glycomics with KO mice database (JCGGDB) (40 strains), NBRP Rat (170 strains), and International mouse phenotype Consortium (IMPC) mouse strains. Full datasets are available for downloading for data analyses of user’s sides.

We aim to contribute wide range of researches including disease studies through data dissemination of variety experimental animals.
P-44  Mice Key Bank: Mouse Sharing System to Accelerate Research Collaborations in TMDU
○Hitomi Takahashi, Hitomi Suzuki, Masami Kanai-Azuma

Center for Experimental Animals, Tokyo Medical and Dental University, Tokyo, Japan

Since genetically modified mice are the essential tools for researches in the life science field, researchers are spending much money, effort and time to introduce mice into their laboratories. It is inefficient that researchers in the same university purchase the same mouse lines independently. We have set up the Mice Key Bank System (MKBS) to share mouse lines within our university. This system enables researchers to reduce the mouse number, cost and space for experiments and to accelerate research collaborations in TMDU. The MKBS provides three services, deposit, supply of mice and their information, exclusively for TMDU researchers. We retain mouse lines by the embryo cryopreservation and share the information of these within the on-campus network. In the three years from the starting up of the MKBS, we gained the information of 179 lines and the 118 deposits. Since the mouse usage is confined to our campus, researchers feel easier to deposit their mice to the MKBS than to the other worldwide services, making a chance of new research collaboration. We also promote cooperation with other institutes, Jackson laboratory and RIKEN Center for Developmental Biology, to provide useful reporter and cre lines. We currently do not provide mice to outside researchers. However, some users wish to provide their own mice to their outside collaborators and thus we are now constructing the rules for it. We appreciate any suggestion to improve our system.

P-45  Hematological analysis of Japan Mouse Clinic in RIKEN BRC
○Mao Ozaki, Eiji Oka, Tadashi Ohshima, Ryoko Yanagisawa, Tomoko Kagami, Shigeharu Wakana

The Japan Mouse Clinic (JMC) has performed comprehensive phenotyping of the mouse lines deposited in RIKEN BRC based on standardized operating protocols (SOPs). We have built the phenotypic pipeline consisting of a fundamental and in depth screens. In hematological test, it enables to measure a blood count of 24 parameters. So it is able to evaluate a blood disorder such as anemia, an infectious, inflammation. The hematological phenotypic data of 141 mouse lines were released to pheno-pub HP (http://phenopub.brc.riken.jp/).

In 2011, the JMC participated in the International Mouse Phenotyping Consortium (IMPC). The IMPC have also required the primary phenotyping included the hematological test. The phenotypic data are released over 2000 mice lines based on the IMPReSS (IMPC SOP) including the parameters and the meta-data (http://www.mousephenotype.org/).

In order to obtain a correct data of blood, the blood collecting method is important factor. We have found that there was a change to parameter of blood counts by re-collecting blood in the same day. The data of re-collected blood has increased the count of neutrophils and has degreased the count of red blood cell and lymphocytes. The frequency of hemoglobin and hematocrit had also degreased. The blood count data of the mouse peripheral blood fluctuate according to few factors. It is necessary to consider the situation at blood collection for the interpretation of the blood data result.
P-46 Use status of mouse resources at RIKEN BRC by research community

○Atsushi Yoshiki, Fumio Ike, Noriko Hiraia, Hatsumi Nakata, Shinya Ayabe, Toshiaki Nakashiba, Keiji Mochida, Atsuo Ogura, Yuichi Obata

RIKEN BioResource Center, Tsukuba, Japan

The mouse has contributed as a model animal of the human widely in life sciences. With support of the research community, RIKEN BRC has participated as the core facility of mouse resources in the NBRP by the MEXT to collect, preserve, conduct quality control and distribute useful mouse strains developed by the Japanese scientists. Over 7,600 mouse resources have been archived so far. In FY2014, 602 researchers used our mouse resources. The user registration of researchers has reached 5,236 around the world as of Dec 2015. Approximately 80% of user organizations where we distributed our mice belonged to medical, pharmaceutical and health-related research fields. The most frequently requested strains included fluorescent reporters such as CAG-EGFP, GFP-LC3 and Nanog-GFP, knockout mice of Nrf2, Irf7, and Rbp-J and various Cre mice. These strains were frequently used in the research fields of the neuroscience, immune and allergy, developmental and regenerative biology, various diseases, cancer, autophagy, etc. Our users have so far published 638 papers in high-impact journals (Mean Impact Factor: 9.1) and 31 patents. These users’ research outcomes have been listed on the BRC and NBRP web sites. We will make every effort to meet demands of research community further to collect useful mouse resources including rapidly increasing genome-edited mice, conduct strict quality control and distribute mice as quickly as possible.

P-47 Laboratory Animal Resource Bank at NIBIOHN- Contribution to innovative drug discovery

○Minako Koura, Akiko Kawai, Ruriko Tanabe, Mitsuho Sasaki, Yoko Noguchi, Junichiro Matsuda, Osamu Suzuki

National Institutes of Biomedical Innovation, Health and Nutrition

As of April, 2015, National Institute of Biomedical Innovation (NIBIO) and National Institute of Health and Nutrition (NIHN) were merged and renamed National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN). As a variety of disease models in small laboratory animals significantly contribute to innovative drug discovery, we established the Laboratory Animal Resource Bank at NIBIO in 2006. At NIBIOHN, we also continue to develop our bank to promote drug discovery ultimately for the health of the nation. Since 2006, we have been conducting the collection, maintenance, preservation, supply and database construction of disease model animals, especially mice. At present, more than 200 unique mouse strains are deposited to our bank and their embryos/sperm are safely cryopreserved. Total number of use of our bank including mouse distribution and safe deposit service reached more than 3,000 since 2006. We will give an overview of our 10 years activities. Information on our bank is available from the following URLs: http://animal.nibiohn.go.jp/ https://alldbs.nibiohn.go.jp/ https://mbrdb.nibiohn.go.jp/cgi-bin/index.cgi http://sagace.nibio.go.jp/index.html http://www.shigen.nig.ac.jp/mouse/jmsr/
P-48  The 3rd Term of the National BioResource Project-Rat in Japan

Takehito Kaneko, Hiroaki Taketsuru, Birger Voigt, Miyuu Tanaka, Yuki Neoda, Kazumi Hagiwara, Zong-hu Cui, Kosuke Hattori, Mayo Yamazaki, Satoshi Nakanishi, Ken-ichi Yamasaki, Takashi Kuramoto
Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan

National BioResource Project-Rat (NBRP-Rat) is now operating in its 14th year and contributes as the largest rat repository to various fields of biomedical research. The major goal of NBRP-Rat comprises the collection of rat strains, the cryopreservation of embryos and sperm, and the worldwide supply of these rat strains. By January 2016, 790 rat strains are deposited to the NBRP-Rat, of which 90 strains are kept as live animals, 428 have already been preserved as embryos and 328 are stored as frozen spermatozoa. Rat strain information, Phenome data, Phylogenetic tree, SSLP and SNPs, Reproductive technologies, Whole genome sequencing of F344/Stm rat strain, GFP rats, Functional polymorphisms, BAC browser, Rat mutant map, RI strain information, and ENU mutant archives, are now available from our NBRP-Rat website at http://www.anim.med.kyoto-u.ac.jp/nbr. Recent progressing technologies for genetically modified rats (GMR); ENU mutagenesis, rat ES cells, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas can provide thousands of useful rat models for functional genomics and human diseases.

P-49  Influence of blood property by the difference in the euthanasia method in Suncus murinus

Motohito Goto, Riichi Takahashi
Central Institute for Experimental Animals, Kawasaki, Japan

In animal experiment, Serum biochemistry values (SBV) is one important factors of experimental data along with improvement of the performance of the measuring instrument. Influence of SBV by the difference in the euthanasia method in mouse and rat was reported in the previous report. We established closed colony strains [High emetic response (Jic:SUN-Her)] by selection breeding of emetic response using veratrine sulfate. We have been worked on production and supply of Jic:SUN as an experimental resources. In this report, we investigated an influential possibility to the blood property by the difference in the euthanasia methods in suncus murinus using blood chemical analyses apparatus. SBV were measured by rodent mode by VETSCAN VS2 with VSDP rotor. It can measure 13 items with plasma 100ul. Euthanasia method using in this study was mixed anesthesia (medetomidine/midazolam/butorphanol) and cervical dislocation. SBV of suncus murinus was significantly different in ALP, ALT and BUN by euthanasia method. It was similar to a result of blood biochemistry of mouse reported before. It was indicated that blood biochemistry property is affected by euthanasia methods to be different in suncus murinus like mouse and rat. This result compared the SBV of the non-starin subspecific suncus murinus reported before. ALP, AMY, TP were low, and ALT, CRE were high. These results suggest that difference in the Blood biochemical test of suncus murinus are not only euthanasia method but subspecies and measuring instrument.
A novel combination of alfaxalone with medetomidine and butorphanol for inducing anesthesia in mice

Riku Yamada, Shota Higuchi, Asami Hashimoto, Kenjirou Miyoshi, Kazuto Yamashita, Takeo Ohsugi
School of Veterinary Medicine, Rakuno-Gakuen University, Ebetsu, Japan

Alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione) is an injectable neurosteroid anesthetic agent that is widely used to induce anesthesia in dogs and cats. With the aim of improving the safety and quality of anesthetic induction, several recent studies have tested various combinations of alfaxalone with sedatives and opioids in dogs. This study was performed to investigate the effects of alfaxalone combined with medetomidine and butorphanol to induce anesthesia in laboratory mice. Subcutaneous administration of the combination of 0.3 mg/kg of medetomidine, 5.0 mg/kg of butorphanol, and 60 mg/kg of alfaxalone (M/B/A60) achieved surgical anesthesia within 5 min of administration and anesthesia was maintained for 75 min. By contrast, surgical anesthesia was achieved within 10 min of administration and was maintained for 45 min with the widely used combination of 0.3 mg/kg of medetomidine, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol. Administration of atipamezole rapidly reversed anesthesia induced by M/B/A60 in mice. These results suggest that subcutaneous administration of M/B/A60, a novel alfaxalone-based combination, is suitable for inducing surgical anesthesia in laboratory mice.

Effects of Different Anesthesia Methods on Hematological Parameters in the CBDL Rats

Misao Terada, Hiroe Kon, Toshio Akimoto, Motoo Shinoda

Laboratory Animal Research Center, Dokkyo Medical University, Tochigi, Japan, Division of Laboratory Animal Science, Nippon Medical School, Tokyo, Japan

The common bile duct-ligated (CBDL) rat, which used as a model of human cirrhosis, rapidly develops secondary biliary cirrhosis. We previously reported literature about the effects of different anesthesia on hematological parameters in rats and mice. However, in this CBDL rat, a detailed examination of blood property in anesthesia has not been made. In the present study, we examined the effects of different anesthesia on hematological parameters in the CBDL rats. We divided Wistar male CBDL rats into four groups, namely 1) medetomidine + midazolam + butorphanol tartrate group (MMB), 2) isoflurane inhalation group (ISO), 3) pentobarbital + butorphanol tartrate group (BP) and 4) alfaxalone + medetomidine + butorphanol tartrate group (AMB). Blood samples were collected via the postcava for complete blood count and analysis of serum chemistry with Vet Scan HM2, Vet Scan VS2 and Piccolo Xpress. Serum chemistries of ISO group indicated relatively low value. On the other hand, there were difference between each group in AST, TBIL and GGT. Present study indicates that there are some differences of hematological parameters between each anesthesia methods, and these results suggest the necessity of considering selection of the anesthesia methods.
P-52  Assessment of neonatal anesthesia in rat

○Atsushi Tsukamoto, Yui Konishi

Laboratory of Laboratory Animal Science, Azabu University, Kanagawa, Japan

It is said that anesthesia in neonates have high-anesthetic risk. Although hypothermic anesthesia is recommended in rat up to age of 7-day, neonatal anesthesia for latter period has not been clarified. Present study investigated the feature of various anesthetic protocols in neonatal rat. Ten-day Sprague dawley rats were anesthetized with 4 anesthesia, including combination of ketamine and xylazine (K/X), medetomidine, midazolam, and butorphanol (M/M/B), isoflurane, and sevoflurane. Anesthetic score was assessed with noxious stimuli. Induction, anesthetic, and recovery times were recorded. For the safety assessment, vital signs and mortality rate were evaluated. K/X at doses of 60/6 and 80/8 mg/kg caused death in whole rats, indicating inappropriate as neonatal anesthesia. Although M/M/B at standard dose (0.15/2/2.5 mg/kg) did not provide surgical anesthetic depth, M/M/B at high dose (0.3/4/5 mg/kg) showed enough anesthetic depth with relatively stable vital signs. Isoflurane required long induction period before reaching surgical anesthetic depth. In addition, isoflurane mediated remarkable respiratory depression, resulted in 25% mortality rate. Sevoflurane provided consistent surgical anesthetic depth with rapid induction. Although respiratory depression was observed, every rat survived after anesthesia. Among the anesthetic protocols investigated in the present study, sevoflurane and high dose M/M/B are recommended as neonatal anesthesia. Compared to matured rat, the chemosensitivity of both anesthesia in neonates was lower.

P-53  Comparison of in vitro alternative assays and in vivo test for eye irritation of contact lenses

○Jung-Hee Yoon¹, Jun-Won Yun¹, Quan Hailian², Eun-Young Cho¹, Miri Lee³, Seung-Hyeok Seok², Kyung-Min Lim³

¹Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea, ²Department of Microbiology and Immunology and Institute of Endemic Disease, Seoul National University College of Medicine, Seoul, Korea, ³College of Pharmacy, Ewha Womans University, Seoul, Korea

In vivo rabbit eye irritation test for safety evaluation is necessary for the development of many kinds of contact lenses as medical device. However, with the increasing awareness on the animal welfare, the development of in vitro alternative tests has been recommended. In this study, we analyzed statistical correlation between in vivo rabbit eye irritation test and three in vitro alternative eye irritation tests [bovine corneal opacity and permeability test (BCOP, OECD TG 437) and two 3D reconstructed human cornea epithelium models (EpiOcular, OECD TG492 and MCTT HCE)] for contact lenses. For this, four types of commercial contact lenses (soft, disposable, hard, and color) and 2 reference lenses (dye-eluting and benzalkonium (BAK)-coated) were tested. All tested lenses were found to be non-irritant except for BAK-coated lens. More importantly, BCOP, EpiOcular, and MCTT HCE exhibited a significant correlation with in vivo rabbit eye irritation test. In conclusion, we suggest that these in vitro eye irritation tests can be introduced as alternative methods for the evaluation of contact lenses and can finally contribute to a reduction in the use of animals.
P-54  Comparison of ketamine and alfaxalone as anesthesia introduction for marmoset MRI

○Chihoko Yamada\textsuperscript{1,2}, Takashi Inoue\textsuperscript{3}, Tomoko Ishibuchi\textsuperscript{1}, Norio Okahara\textsuperscript{1}, Fumiko Seki\textsuperscript{1}, Yuji Komaki\textsuperscript{1}

\textsuperscript{1}CIEA, Japan, \textsuperscript{2}JAC Co., Ltd, Japan

Ketamine (KE) is restricted by designation of anesthesia, but KE may increase time for awaking animals and vomit as the side effect. We weighed KE and Alfaxalone (ALF) to consider use of ALF as alternative to KE. We divided 35 marmosets into 2 groups. Both were given 0.1 mg/kg of atropine sulfate intramuscularly before administration. GroupA was received 30 mg/kg of KE with intramuscular injection, whereas GroupB was received 12 mg/kg of ALF. Intratracheal intubation with 6Fr tube with Xylocaine was performed. Anesthesia was maintained using artificial respirator with isoflurane. In A, body movement restraint happened just after dosage, and onset of anesthesia effect was around 1-2 min. The time of awakening was 14-77 min, and vomiting was seen with 3 subjects at awakening. In B, body movement restraint happened after dosage in 3-5 min. Awakening time were within 13-51 min after anesthesia end. In B, awakening time was significantly earlier than A, and vomiting was unseen. Besides, at time of introduction and awakening, involuntary movements of limbs were seen in most individuals. Excessive secretion of saliva was seen, but improved by increasing Xylocaine. Comparison KE between ALF showed anesthesia introduction and maintenance did not show any problem with either group. Vomiting was not observed for only subjects who performed ALF at awakening, in addition to short and smooth awakening time. These demonstrated ALF can be used in substitution for KE for MRI.

P-55  Usefulness of monitoring body temperature by telemetry in toxicity evaluation of the drug

○Kazunori Ozawa\textsuperscript{1}, Kouki Kato\textsuperscript{2}, Kazuo Ushida\textsuperscript{1}, Hazuki Higuchi\textsuperscript{1}, Yuko Yamamoto\textsuperscript{1}, Kiyoaki Katahira\textsuperscript{1}

\textsuperscript{1}Translational Research Center, Fukushima Medical University, Fukushima, Japan, \textsuperscript{2}Center for Laboratory Animal Science, National Defense Medical College, Saitama, Japan

For early detection of drug toxicity, 7-days toxicity test was conducted in the rats with simultaneous monitoring of body temperature (BT) by telemetry, followed by the analysis of various organs by histopathology and gene expression profiling to create the database. We report an interesting BT features observed during the test of known drugs.

The transmitter was implanted to the male, 5-8 weeks old, SD rats, and their BT was monitored by the telemetry system. One week after the surgery, they were orally treated with toxic dose of the terfenadine, amlodipine, lenalidomide, thalidomide, imipramine, ibuprofen, metformin, aspirin, celecoxib or acetaminophen for 7 days with monitoring of BT, body weight (BW) and food consumption (FC).

All drugs except lenalidomide and celecoxib caused BW decrease or BW gain suppression with FC reduction. BT was scarcely affected by lenalidomide, metformin, aspirin and celecoxib. Terfenadine caused transient BT decease and gradual decline of mean BT. Amlodipine and thalidomide caused only transient BT decrease at 1st dosing. Profound hypothermia was observed at each dosing of imipramine and acetaminophen. By ibuprofen, circadian change was obscure, mean BT declined and transient decrease appeared on 6-7th dosing.

These results suggest telemetry monitoring of BT is useful for the toxicity assessment.
P-56  Differential diagnosis of tumor origin in tumorigenicity test with in situ hybridization

○Jae-Bum Ahn1,2, Euna Kwon3, Hanna Kim2, Jun-Won Yun2, Woo Ho Kim3, Byeong-Cheol Kang1,2,4

1Graduate School of Translational Medicine, SNU College of Medicine, Seoul, Korea, 2SNU Hospital, Seoul, Korea, 3Department of Pathology and Cancer Research Institute, SNU College of Medicine, Seoul, Korea, 4Institute of GreenBio Science Technology, SNU, Pyeongchang, Korea

Tumorigenicity test is needed vitally in the development of stem cell therapeutics. In this test, it is important to confirm the origin clearly when tumor occurs. BALB/c-nu mice were transplanted (s.c.) with stem cell-contained therapeutics (3X10^5, 9X10^5, 2.7X10^6), A375 (2.7X10^6, positive), or MRC-5 (2.7X10^6, negative). All mice were observed for 26 weeks after transplantation. In high dose group, 1 male was found dead at 21 weeks, and 1 male and 1 female were sacrificed at 26 weeks. Malignant cell infiltration is microscopically observed in liver of 2 males (lymphoma) and 1 female (leukemia) although tumor was not observed macroscopically. To determine the origin of these cells, we performed chromogenic in situ hybridization (CISH) using species-specific probes: PPIA gene mRNA (human), Mm-B1-repeat consensus sequence (mouse), and dapB (bacteria). In skin of positive controls, tumor cells were detected by human-specific probes, whereas all stromal cells were detected by mouse-specific probes. And, malignant cells in high dose group were stained by only mouse-specific probe. As a result, malignant cells were confirmed to be originated from mice, suggesting that CISH is useful to confirm the tumor origin in the safety evaluation of stem cell therapeutics.

P-57  Study of room temperature storage method using the muscle tissue-derived somatic cell nuclei

○Akari Obashi1,2,3,4, Mizuki Kajimoto1, Rika Azuma2, Moriyoshi Kubo1, Noriyuki Nonoue3, Takuya Orisugi1, Mizuki Sugimoto1, Yoshihiko Hosoi1,2,4, Masayuki Anzai4


Objective: Currently, the tissues except for the germ cell are required to use effectively and establish the various storage methods as research resource. In this study, we performed to storage of the somatic cell nuclei of the muscle and subcutaneous tissues at room temperature. Methods: The experiments used the thigh muscle of adult BDF1 mice and the abdominal subcutaneous tissues derived from Papio anubis died in Asa Zoological Park Hiroshima. These somatic cell nuclei were collected by modified MIP method from muscle tissues. Then, room temperature storage of somatic cell nuclei were stored for 7 days at three approaches (in the experiment group A: precipitate containing the somatic cell nuclei caused by centrifugation treatment, B: the group A was resuspended in TE buffer, C: the group B solution was centrifugation). These were observed changes the number of somatic cell nuclei by using fluorescence microscope with time. In addition we performed the nuclear transfer in order to confirm the functionality of nuclei. Results: The somatic cell nuclei collected from the tissues could store at room temperature for 7 days. Furthermore, these were observed ability of reconstructed pronuclear oocytes. In conclusion, we suggested that it is possible to storage of the somatic cell nuclei at room temperature.
**P-58  Study of myocyte nuclei derived from tissues treated with vacuum drying system**

Takuya Orisugi¹, Mizuki Kajimoto¹, Rika Azuma², Kazutoshi Takami¹, Moriyoshi Kubo⁴, Noriyuki Nonoue⁴, Tatsuya Inoue¹, Akari Obashi¹, Mizuki Sugimoto¹, Minoru Miyashita³, Yoshihiko Hosoi¹,², Masayuki Anzai⁵


Currently, we are considering the myocyte nuclei recovered from animal tissues stored for a long time. In addition, it is required to verify the impossibility of somatic cell nuclei re-covered from significant damage and exposed to extreme environment in order to perform the storage of genetic resources. In this study, we examined function of somatic cell nuclei recovered from muscle tissues treated vacuum drying system. This study used mature age BDF1 mice, the *Elephas maximus* which finished life span in Osaka Municipal Tennoji Zoological Gardens and the *Procavia capensis* which died in Asa Zoological Park Hiroshima. These tissues were treated with vacuum drying and stored at -30 C. And all the tissue preserved for 0-16 months. The myocyte nuclei recovered from these tissues by modified MIP methods. Then, these myocyte nuclei confirmed that formation of the pronuclear by somatic cell nuclear transfer. It was possible to recognized ability to form of pronuclear structure (mice:71%, *Elephas maximus*:76% and *Procavia capensis*:96%). In this result, it shows the myocyte nuclei derived from tissues treated with vacuum drying have normal function of nuclear. Thus, these muscle tissues are expected to store as research resource.

**P-59  Comparison of cryopreservation devices and solutions in the vitrification of rabbit embryos**

Fumikazu Matsuhisa¹, Toshiaki Akiyoshi¹, Manabu Niimi², Masatoshi Morimoto¹, Jianglin Fan², Shuji Kitajima¹

¹Analytical Research Center for Experimental Sciences, Saga University, Saga, Japan, ²Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

The cryopreservation of embryos plays a pivotal role in the maintenance of biological resources. Although several methods were reported in the cryopreservation of rabbit embryos, there are few studies to weigh the methods. Therefore, we compared cryopreservation devices and solutions to establish an effective cryopreservation method of rabbit morulae. We examined three devices, straw, cell sleeper and cryotop, and two solutions, EDS (ethylene glycol, DMSO and sucrose) and EFS (ethylene glycol, Ficoll and sucrose). The survival rate after vitrified-thawed in all vitrified groups were more than 90%. Although the blastocyst formation rate after vitrified-thawed in Cryotop-EFS group was comparable rate with non-vitrified group, the rate in the other vitrified-thawed groups below that in non-vitrified group. In addition, in Cryotop-EFS group, all recipient rabbits of embryo transfer were pregnant and delivered offspring. In vitrified-thawed embryos using straw, the delay of blastocyst formation was observed. We speculate that the time of freezing and thawing associated with the volume of cryopreservation solution affects the rate of blastocyst formation. Additional studies are now needed to clarify the most appropriate conditions for embryo transfer after vitrified-thawed.
P-60  Study of isoflurane anesthesia on gray short-tailed opossums (Monodelphis domestica)

○Yumiko Kirihara1, Mayumi Takechi1, Kaoru Kurosaki1, Noriyuki Ikeda1, Naoyo Kajitani1, Yuta Kobayashi1, Yoji Saito3, Hiroki Otani4

1Department of Experimental Animals, Interdisciplinary Center for Science Research, Shimane University, Izumo, Shimane, Japan, 2Department of Fundamental Nursing, Faculty of Medicine, Shimane University, Izumo, Japan, 3Department of Anesthesiology, Faculty of Medicine, Shimane University, Izumo, Japan, 4Department of Developmental Biology, Faculty of Medicine, Shimane University, Izumo, Japan

Objective: Gray short-tailed opossums (opossums) have become important laboratory animals due to their unique gestation and pregnancy. However, an appropriate anesthetic method for opossums is not yet clear. Therefore, we examined conditions to find an appropriate inhalational anesthesia using isoflurane (ISO) for them. Methods: Two percent of ISO was used for 60 minutes (min). The flow rate was fixed at 500 ml/min. In Group 1 (G.1), air was used as carrier gas. In G.2 and G.3, 200 ml/min of pure oxygen (O2) plus air was used for carrier gas. In G.3, we injected 0.1 mg/kg of medetomidine (MED) 5 min before ISO and reduced ISO from 2% to 1.5% at 10 min. G.4 was a non-anesthetic group. We measured a starting time for anesthesia using anesthetic scores. Results and Discussion: G.1 experienced respiratory arrest. G.2 indicated unsatisfactory starting time for anesthesia. G.3 showed satisfactory starting time for anesthesia and anesthetic duration. These results indicate that the injection of MED before ISO with O2 plus air as carrier gas is a useful inhalational anesthesia for opossums.

P-61  Hematological examinations in marmosets using automated blood cell analyzers

○Yoshitaka Hirasawa1, Takashi Inoue2, Norio Okahara2, Yasunori Oda1, Koji Otabe1, Takahiro Matsuo1, Shin-ichi Sato1, Toshiyasu Hombo1

1Ina Research Inc., 2Central Institute for Experimental Animals, 3Sysmex Corporation

We evaluated the viability of 2 automated blood cell analyzers, the ADVIA120 (Siemens Healthcare Diagnostics K.K.) and XT-2000iV (Sysmex Corporation), for use in hematological examinations in marmosets. ADVIA120: Since no appropriate setting could be found for differential white blood cells, analysis of these parameters was considered infeasible. For analysis of CBC, the Dog setting was selected. Good results were obtained in intra-assay precision and dilution linearity, and stability of the blood samples under refrigeration for 24 hours was confirmed. XT-2000iV: Using the Monkey setting, good results were obtained in intra-assay precision and dilution linearity, and stability of the blood samples under refrigeration for 24 hours was confirmed, except for platelet parameters (PDW, MPV, P-LCR and PCT). Correlation between these systems: A tendency to increase in RET was noted in the XT-2000iV data, which was likely to be the effect of Heinz bodies. However, since manual adjustment of the analytical area is feasible in the XT-2000iV, if a threshold with good collation is selected, the difference in the measured values of these systems can be resolved. Based on these results, analyses of CBC and Retic are feasible using the ADVIA120 in the Dog setting, and all parameters are generally feasible using the XT-2000iV in the Monkey setting in hematological examinations in marmosets.
Breeding mice and their various changes with aging: Toward clarifying the mechanisms of senescence

Noboru Ogiso¹, Kaori Muguruma¹, Satomi Takano¹, Kohei Tomita¹,², Kazumichi Yamagichi¹,², Naomi Matsui¹, Mitsuo Maruyama³
¹National Center for Geriatrics and Gerontology, Obu-city, Aichi, Japan, ²KAC Corporation, Kyoto, Japan, ³National Center for Geriatrics and Gerontology, Obu-city, Aichi, Japan

Our facility have kept many aged animals used for gerontology and geriatric researches. In case of long-term breeding animals, variations have been often found in symptoms and times of onset of age-related disease, and life expectancy in these animals depending on environmental factors (such as food, water or management). In the present study, we evaluate various characteristics as senescence indicators in aged mice kept in our facility. Four weeks-old male and female mice (C57BL/6N) were obtained from Japan SLC, and were kept over their lifetime. Physiological (measurement of body weight, food consumption and survival rates), behavioral (the rotarod test) and morphological (autopsy and histological examination) analyses were performed. Additionally, we investigate the intestinal flora of mice. Body weight peaks at 18-19 months-old in male mice and at 14-16 months-old in female mice. There is no significant sex difference in food intake and survival rates. Mice with age-related weight gain tend to get a low score on the rotarod test. Disorders of genital organs in male mice or splenic tumor were often found in dead animals at autopsy. Some alteration of the intestinal flora occurs at around 12 months-old. Our results show that some characteristics found in aged mice may be useful to score their senescence.

The effects of regular and proper handling on long-term breeding of rats

Kohei Tomita¹, Kazumichi Yamaguchi¹, Satomi Takano², Kaori Muguruma², Noboru Ogiso²
¹KAC, Kyoto, Japan, ²Laboratory of Experimental Animals, National Center for Geriatrics and Gerontology National Institute for Longevity Sciences, Aichi, Japan

It is well known that laboratory animals can be easily influenced by their breeding environment. Therefore researchers need to focus on that in case they keep animals for a long period as animal models of aging. In the present study, we examined the effects of breeding environment made by human, such as handling, cage change and restraint, on long-term breeding of rats. <Materials & Methods> Sixteen to 35 male rats (F344/NSlc, 4 weeks-old) were obtained from Japan SLC every three months. Rats were routinely handled using appropriate technique, and were allowed free access to a commercial standard diet (Labo MR Stock; Nosan Corporation, Yokohama, Japan) and Reverse Osmosis (RO) water. Body weight, daily food and water consumption were measured, and survival rates were also determined. Additionally autopsy was performed on all dead animals. <Results & Discussion> Body weight peaks at 13 months-old and then declines. There is no significant change in food (15.5 ± 1.4 g/day) or water (20.2 ± 3.7 ml/day) intake with aging. The average life expectancy of rats handled routinely tends to increase compared to rats without sufficient handling. Lung congestion, cecal enlargement and fundal gastritis were often found in dead rats at autopsy. Our results show that good handling may have a positive influence on animal health and be an effective way to prolong its lifespan.
Age related Changes in Heart Rate Variability and cardiac β1-adrenergic receptor in Nonhuman Primate

Boran Osman¹, Hiroshi Koie², Sachi Okabayashi¹, Kiichi Kanayama², Yasuhiro Yasutomi¹,
Naohide Ageyama¹
¹Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition,
²Nihon University Collage of Bioresource Science, ³Corporation for Production and Research of Laboratory Primates

Heart rate variability (HRV) is a useful marker of sympathetic and parasympathetic influences on the modulation of heart rate. Importantly, a great body of evidence correlate the changes in β1-adrenergic protein level with heart failure pathogenesis. The purpose of the present study was to gain further insight into age-related changes of HRV associated with cardiovascular disease of autonomic nerve activity, and to examine age-related changes in the expression of cardiac β1-adrenergic receptor in different ages of Cynomolgus monkeys. The autonomic function during normal daily activities was examined by using 24-hour Holter electrocardiogram. Proteins from three different ages of monkeys were extracted from left ventricles. Then the histopathological and western blotting studies were also performed. The results indicated that parasympathetic nerve activity decreases, whereas sympathetic nerve activity and myocardial damage increase with age. The activation of the sympathetic nervous system that occurs is associated with down-regulation of β1-adrenergic receptor. The down regulation of β1 adrenergic receptor may play an essential role in aging-associated cardiac fibrosis.

Intertrial effects on behavioral phenotypes in IMPC phenotyping pipeline

Ikuko Yamada, Tomoko Kushida, Misho Kashimura, Tomohiro Suzuki, Hideki Kaneda,
Kimio Kobayashi, Ikuo Miura, Tamio Fueuse, Shigeharu Wakana
RIKEN BioResource Center, Tsukuba, Japan

International Mouse Phenotyping Consortium (IMPC) is an international project that aims to carry out generating and systematically phenotyping 20,000 knockout mouse strains. Japan Mouse Clinic has been performing systematic phenotype analysis of knock-out mice using the globally common phenotyping pipeline with standardized procedures as a member of the IMPC. The behavioral phenotype is influenced by laboratory instruments and environment of mouse husbandry and so on. Hence, we have conducted experiments on the basis of the Standard Operating Procedure in order to minimize variability of the data among experimental batches. In our study, intertrial effects of control data were analyzed in open-field test (OF) and acoustic startle/Pre-pulse inhibition test (AS/PPI). The control data were collected from reference strain (B6/NTac) and wild type mice of each KO strains. As a result, there was no significant difference among the batches in distance travelled and percentage center time in OF. However, difference among batches was seen in the startle response in AS/PPI. In almost of the parameters in the behavioral tests, no significant difference was detected among the batches. However, there were also some parameters which have differences among batches. In order to minimized these data variation and perform more accurate analysis, it is necessary to conduct more detailed study and identify the factors that affect experimental data.
P-66  Histological analysis of deficient mice in galactosyltransferases responsible for LacCer synthesis

○Yoshihara Toru¹², Toshihisa Hatta³, Hiroyuki Satake², Koichi Furukawa⁴⁵, Masahide Asano¹²


We have previously reported that β-1,4-galactosyltransferase-5 (β4GalT-5) is an enzyme responsible for the biosynthesis of lactosylceramide (LacCer) and further revealed that β4GalT-5 is an important factor in early developmental stage (Nishie et al., 2010). In addition, a recent study has reported that β4GalT-6, another family gene in β4GalTs, also acts as LacCer synthase (Tokuda et al., 2013). In the previous meeting of JALAS, we have reported that 1) LacCer synthesis was reduced approximately half in each brain of brain-specific β4GalT-5-deficient mice and β4GalT-6 null mice, 2) LacCer and its downstream products (ganglioside) completely disappeared in gene deficient mice lacking both β4GalT-5 and β4GalT-6 (DKO mice) and 3) DKO mice showed a severe movement disability and growth retardation and they became lethal around weaning stage. Here we report further histological and in vitro examination based on electron microscopy observation and neurosphere assay for DKO mice.

P-67  Simultaneous measurement of electroencephalogram, motor activity, and body temperature in rats

○Takumi Okajima¹, Yujiro Taguchi², Sayaka Kohtoh², Michio Tuchiyama¹, Takeshi Iidaka¹

¹NISSEI BILIS Co., Ltd., ²KISSEI COTMEC Co., Ltd.

[Purpose] The purpose of the study was to evaluate the simultaneous measurement system of electroencephalogram (EEG), motor activity, and body temperature in rats treated with suvorexant, zolpidem, diazepam, or chlorpromazine.

[Method] Male Wistar rats were used. EEG (frontal cortex and hippocampus), motor activity, and body temperature were simultaneously measured using an electroencephalograph and a nano tag, a small device implanted into the body, under unanesthetized and unrestrained condition during a dark phase. The sleep cycle was categorized into five stages by the sleep analysis program, and sleep stage occupancy and sleep latencies until the appearance of a sleep stage were calculated.

[Result] A decrease in stage awake and motor activity and an increase in stage sleep were observed in all treatment groups. In diazepam and chlorpromazine group, body temperature dropped as well. Sleep latencies until slow-wave light sleep stage and slow-wave deep sleep stage decreased in suvorexant group whereas that until fast-wave sleep stage was prolonged in diazepam and chlorpromazine group; sleep latencies until slow-wave deep sleep stage was prolonged in chlorpromazine group as well.

[Conclusion] The simultaneous measurement of EEG, motor activity, and body temperature makes it possible to determine the characteristic effects of various medicines; nano tag could be used for the development of a new experimental method.
P-68  An efficient reproductive method for Irs2/- mice with C57BL/6Jcl genetic background

○Haruo Hashimoto1, Tomoo Eto1, Tsutomu Kamisako1, Toshimasa Yamauchi2, Naoto Kubota2, Koujiro Ueki2, Kyoji Hioki1, Munen Saio1, Takashi Kadowaki2, Mamoru Ito1

1Centaral Institute for Experimental Animals, Kawasaki, Japan, 2Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo

Efficient reproduction using natural mating and reproduction technology [in vitro fertilization (IVF) and embryo transfer (ET)] was investigated in IRS2 deficient mice with C57BL/6Jcl genetic background (Irs2/- mice) as a typical type 2 diabetes model. From the results using various combinations of Irs2/- and Irs2/+/ mice, the combination of female Irs2/+/ × male Irs2/- was found to be more efficient than other combinations. In applications of reproduction technology using IVF and ET, the combination of female Irs2/+/ × male Irs2/- involves the possibility of Irs2/- production by repeats using female Irs2/+/ mice. However, reproductive continuity using this combination is difficult because of dependence on human technique and the cost of ET. Therefore, we concluded that Irs2/- mice should be produced by embryo transfer using Irs2/- mice from a colony consisting of female Irs2/+/ × male Irs2/-.

P-69  Practical rapid back-crossing to NOG strain using immature female mice

○Takahiro Kagawa1, Motohito Gotou1, Naoshi Koide2, Michiko Mandai2, Riichi Takahasi1

1Animal Resources and Technical Research Center, Central Institute for Experimental Animals, Kanagawa, Japan, 2Laboratory for Retinal Regeneration, Riken Center for Developmental Biology, Kobe, Japan

A severe immunodeficient NOG mouse is the important tools for stem cell research and regenerative medicine. If we could establish NOG background animal model for human disease, The clinical human cell/organization is as transplantation evaluation model of cell therapy. The speedy back-crossing system is required. In this study of preceding example, Pde6brd1-2J(RD1)and B6.CXB-Pde6brd10(RD10) that has retinal degeneration disease caused by a mutation in phosphodiesterase 6B gene was used. We try to establish practical rapid back-crossing to NOG strain using immature female mice(4w).In each generations, mated female could driver naturally and keep nursing until weaning without any cannibalism. The averages number of pups per 1 female was 9.7, it was similar with NOG mouse. The replacement rate confirm by microsatellite marker at 5th generation. And then advanced the backcross,we could reache100% replacement of RD1 by 6th generation(419 days), and RD10 by 7th generation(324 days).These result suggested that back-cross breeding using immature NOG mouse was effective to establish congenic NOG mouse strain.
P-70  Comparison on reproductive efficiency between two different production colonies of TK-NOG mouse

○Kayo Tomiyama1,2, Masaki Sekiguchi1,3, Tomoyuki Ogura1, Riich Takahashi1, Kyoji Hioki1, Hiroshi Suemizu1

1Central Institute for Experimental Animals, Kawasaki, Japan, 2JAC Inc, Tokyo, Japan, 3Advantec Co., Ltd, Osaka, Japan

[Purpose] TK-NOG [NOG-Tg(ALB-UL23)7-2/ShiJic] developed from severe immunodeficient NOG® mouse can receive Xeno-Hepatocytes. Its usefulness as a [humanized mouse model] leads to an expansion of demand. In the 61st General Assembly, we reported the date of general feature in planned production of TK-NOG mouse. In this report, we compared the reproductive efficiency between two different production colonies, the isolator group and the barrier room group.[Materials and Methods] The foundation/expansion colonies in the vinyl isolator group, we prepared one group of 15 male and 60 female. The goal of production is to obtain 60 animals (15 TK-NOG Tg males) every other week. For the barrier room groups, we prepared one group of 20 male and 140 female (7 groups of 20). The goal of production is to obtain 80 animals (20 TK-NOG Tg males) every week. Transgene screening was carried out by the PCR.[Results and discussion] No difference was observed in reproductive efficiency between two production colonies in the isolator and the barrier room. Therefore it was confirmed that planned production of TK-mouse can be carried out in barrier breeding room, which is more efficient for mass production.

P-71  Application of new reproduction method using the BMY in C57BL / 6 strain mice

○Tsunekata Ito, Junko Ozaki, Naoki Fukuda, Mayumi Suto, Daisuke Tanaka

Laboratory Animal Center, Institute for Promotion of Medical Science Research, Yamagata University Faculty of Medicine, Yamagata, Japan

BMY (Breeding Method in Yamagata Univ.) is a new reproduction method in mice. This method can be used to breed mice with only a single administration of PMSG (Pregnant Mare Serum Gonadotropin). The use of BMY method, there is no need to selecting proestrus female mice, it is possible to efficiently reproduction and production than mice in natural mating. However, achievements ever is the result of using ICR strain mice.In this experiment, BMY method it was examined whether or not applicable to the C57BL / 6 strain mice. BMY (1.2IU / 10gBW) is compared to the natural mating group, it showed good results in survival litter size and production index and pregnancy rate. Especially production index was more than five times the value of the natural mating group. From this result, BMY method to C57BL / 6 strain mice is to be sufficiently applicable was found. BMY method In addition to applicable to the planned large-scale reproduction in mice, applicable to planned mating donors for cesarean it can be expected.
P-72 Artificial insemination as a reproductive technology in cynomolgus monkeys

Nobuhiro Shimozawa1, Yoshimi Otsu2, Mutsumi Togo2, Akio Hiyaoka2, Sachi Okabayashi2, Yasuhiro Yasutomi1

1Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition, Tsukuba, Japan, 2The Corporation for Production and Research of Laboratory Primates, Tsukuba, Japan

In the breeding systems at Tsukuba Primate Research Center, Japan, one female cynomolgus monkey is typically housed together with one male. We studied artificial insemination (AI) in cynomolgus monkeys, because its use is important when monkeys will not copulate or cannot be housed as mating couples. In the ovulation phase, as estimated by measuring serum estradiol and progesterone levels, sperm suspension was injected into the female reproductive tract (vaginal canal, cervix, or uterine cavity) via a syringe or catheter. Serum levels of the two hormones were measured with an automated immunoassay analyzer. We achieved pregnancy in an individual by AI. In addition, other individuals are being followed up now. We also report on sperm counts and the sperm infusion locations used in AI.

P-73 Reduction of administration volume of ultra-superovulation reagent in mice

Yukie Haruguchi1,2, Kiyoko Yamashita1,2, Tomoko Kondo1,2, Yumi Takeshita1,2, Yuko Nakamuta1,2, Tomoko Umeno1,2, Ai Miyagawa1,2, Mari Iwamoto1, Fumi Takahashi1, Wataru Sakamoto1, Yoshiko Nakagawa1, Yuki Yamamoto1, Shuuji Tsuchiyama1, Toru Takeo1, Naomi Nakagata1

1Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan, 2Kyudo Co., Ltd., Tosu, Japan

Recently, we developed a novel superovulation technique for C57BL/6J mice at 4 weeks old (Takeo and Nakagata, PLoS ONE, 2015). As per the superovulation method, we administered an aliquot of 3.75IU eCG (0.1 mL) and inhibin antiserum (IAS, 0.1 mL) to prepubescent female mice. However, the administration of a high volume of reagent (0.2 mL: 0.1 mL eCG and 0.1 mL IAS) may cause the female mice stress due to their small body size. In this study, we examined the effect of reducing the volume of eCG and IAS on the number of ovulated oocytes in C57BL/6J mice. A mixture of IAS (0.05 or 0.1 mL) and eCG (1.875IU, 3.75 IU or 7.5IU) were administered to C57BL/6J female mice. 48 hours after the injection, 7.5IU hCG was injected into the mice. 17 hours later, oocytes were collected from the mice and then used for in vitro fertilization (IVF). The 2-cell embryos obtained thereby were cultured to the blastocyst stage. Results showed that half of the usual volume of IAS (0.05 mL) and 1.875IU eCG (0.05 mL) exerted an effect equivalent to that exerted using the usual volume of IAS (0.1 mL) and 3.75IU eCG (0.1 mL) in female mice at four weeks old.
P-74  Superovulation using inhibin antiserum and equine chorionic gonadotropin in C57BL/6J female mice

○Yumi Takeshita1,2, Kiyoko Yamashita1,2, Yukie Haruguchi1,2, Tomoko Kondo1,2, Yuko Nakamuta1,2, Tomoko Umeno1,2, Ai Miyagawa1,2, Mari Iwamoto1, Fumi Takahashi1, Wataru Sakamoto1, Yoshiko Nakagawa1, Yuki Yamamoto1, Shuji Tsuchiyama1, Toru Takeo1, Naomi Nakagata1

1Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan., 2Kyudo Co., Ltd., Tosu, Japan

Previously, many papers reported that an injection of inhibin antiserum (IAS) as an alternative to eCG induced superovulation in mice. However, the effect of IAS on the C57BL/6J strain, which is the most widely used inbred strain for the production of genetically engineered mice, has not been investigated. In addition, the combined effect of IAS and eCG (IASe) in superovulation has not been examined. In this study, we examined the effect of IASe on the number of ovulated oocytes in female C57BL/6J mice at 4 weeks old. Furthermore, we evaluated the quality of oocytes obtained through superovulation using IASe via in vitro fertilization (IVF) and embryo transfer. The number of ovulated oocytes obtained via the administration of IASe increased around three-fold in comparison to that obtained via the administration of IAS or eCG alone. Oocytes derived from superovulation using IASe developed normally into 2-cell embryos via IVF. The 2-cell embryos produced via IVF subsequently developed normally into live pups following embryo transfer. In summary, a novel superovulation technique using IASe is extremely useful for producing a great number of oocytes and offspring from genetically engineered mice.

P-75  Efficient production of cryopreserved mouse oocytes by ultra-superovulation

○Ayumi Mukunoki, Toru Takeo, Naomi Nakagata

Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Japan

Genetically engineered mouse is a valuable tool to investigate gene functions and human disease. Previously, we developed a technique for oocyte cryopreservation and in vitro fertilization (IVF) using the oocytes. Recently, we achieved to produce more than 100 oocytes from single female of C57BL/6J mice at 4 weeks old by coadministered inhibin antiserum (IAS) and equine chorionic gonadotropin (eCG) termed as ultra-superovulation (Takeo and Nakagata, PLoS ONE, 2015). Combined the techniques of oocyte cryopreservation and ultra-superovulation will enhance the efficiency of animal production of genetically engineered mice. In this study, we examined efficiency to produce cryopreserved oocytes by IAS and eCG (IASe) and evaluated the fertilizing and developmental ability of the oocytes. In the IVF, we evaluated the efficacy of N-acetyl-L-cysteine in fertilization medium. As a result, IASe ovulated 4 times higher number of oocytes than eCG and increased the efficiency to produce cryopreserved oocytes. NAC enhanced the fertilization rate of vitrified-warmed oocytes. Obtained two-cell embryos from the oocytes normally developed to blastocysts in vitro. In conclusion, ultra-superovulation method can produce 4 times higher number of cryopreserved oocytes compared with superovulation using eCG and fertilize the oocyte by IVF using NAC.
P-76  Induction of superovulation by administration of inhibin antiserum and equine chorionic gonadotropin

○Toru Takeo, Ayumi Mukunoki, Naomi Nakagata

Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan

Superovulation is a reproductive technique to artificially promote follicle development and induce ovulation by administering hormones. Injection of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) has been used for superovulation in mice. Recently, we developed a novel technique of superovulation using coadministration of inhibin antiserum (IAS) and eCG which produced more than 100 oocytes from single female C57BL/6 mouse at 4 weeks old (Takeo and Nakagata, PLoS ONE, 2015). In this study, we examined the efficacy of embryo production using matured female mice by natural mating or in vitro fertilization (IVF) after coadministration of IAS and eCG (IASe). The percentage of mated female increased by treatment of eCG or IASe compared with saline (control). All mice were ovulated by eCG and IASe. The number of ovulated oocytes increased in the order of control < eCG < IASe. IVF indicates high fertilization rates in all groups. On the other hand, fertilization rate of natural mating fluctuated in IASe. In summary, superovulation using IASe is applicable to a very efficient production of genetically engineered mice and development of reproductive technology alternative to eCG.

P-77 Effective production of embryos by superovulation with AIS and by IVF protocol for frozen sperm


'RIKEN BioResource Center, Ibaraki, Japan, 2'Tokyo University of Agriculture and Technology, Tokyo, Japan

We have developed a high-yield superovulation method in several mouse strains by synchronizing the estrous cycle followed by injection of anti-inhibin serum (AIS) (Hasegawa et al. 2015, Wang et al. 2001). Here we examined the applicability of this superovulation method combined with an optimized IVF protocol for frozen sperm to some strains resistant to ART. Females were treated with 0.1 ml of AIS on day 3 and 4 following synchronization of estrous cycle by injections of progesterone on day 1 and 2. In 2 strains of A background, the total numbers of morphologically normal oocytes increased about 4 times (59 vs 14) and 3 times (43 vs 14) against the standard method with 5-7.5 IU eCG injection. In 2 strains of B10 background, the numbers of normal oocytes increased about 3 times (51 vs 18) and 2 times (38 vs 18), respectively. The fertilization rates were also increased from 6% to 92% and from 8% to 85%, respectively, when HTF supplemented 1-1.25 mM reduced glutathione for oocytes and PVA-HTF supplemented 0.4 mM methyl-β-cyclodextrin for sperm were used as preincubation media. Thus, the efficiency of embryo production was dramatically improved even in the strains considered resistant to reproductive technologies.
P-78  Effect of in vitro maturation medium with β-NMN on the early development of the immature oocyte

Mizuki Sugimoto¹, Tatsuya Inoue¹, Manami Nishimura², Yoshihiro Noda³, Rika Azuma⁴, Akari Obashi¹, Takuya Orisugi¹, Masatake Nakaya¹, Takao Nakagawa³, Yoshihiwo Hosoi¹,⁴, Masayuki Anzai⁶


Objective: Prolonged operation of in vitro maturation (IVM) impairs metabolic pathway by oxidative stress. In recent data, NAM treatment were improved spindle formation or oxidative phosphorylation. We examined effect of IVM medium with β-NMN on in vitro matured oocyte derived cytoplasmic function and developmental competence.

Methods: GV-stage oocytes were collected from the ovaries of C57BL/6J mouse. IVM performed using mTaM medium with 1-10mM β-NMN for 16 hours. Then in vitro matured oocytes were used to measure intracytoplasmic reactive oxygen species (ROS) and to perform in vitro fertilization (IVF). Furthermore, we performed immunocytochemical staining to observe morphology of spindle at MII-stage.

Results: The fluorescent brightness measurements of ROS were significantly low (β-NMN non addition: 436.40 pixel; 1mM : 375.30 pixel ; 2mM : 368.57 pixel). IVF rate of β-NMN non addition was 76%, then it didn’t show declining in among 1mM (72%) and 2mM (83%). Furthermore, rate of development to blastocyst stage were increased (β-NMN non addition: 28% ; 1mM : 39% ; 2mM : 39%). Also, spindle elongation in 2mM was improved as compared with β-NMN non addition.

P-79  Influence of the fertility rate on repeated ovum pick-up in common marmoset

○Yuko Yamada¹, Yoko Kurotaki¹, Junko Okahara¹, Chia-Ying LEE¹, Tomoko Ishibuchi¹, Mitsuyoshi Togashi¹, miku Yamasaki¹,², Yoshihisa Sawada¹,², Erika Sasaki¹,³

¹Central Institute for Experimental Animals, ²JAC Inc., Tokyo, Japan, ³Keio University

One marmoset is used multiple times for ovum pick-up (OPU). It has been found that the number of oocytes decreases as OPU is repeated. In this study, we analyzed fertility rate to examine to what extent the quality of the marmoset oocyte is reduced by repeated OPU. We observed the number of successfully fertilized ova obtained from marmoset during repeated OPU (1-8 times) from September 2015 to December 2015. The mean fertility rate at OPU #1, 2 and 3 was stable at 55.7%, 69.2% and 57.5%, respectively. However, as the oocyte collection number increased to 4, 5, 6, 7 and 8 times, the fertility rate decreased remarkably to 27.9%, 27.9%, 53.8%, 16.7%, and 41.1%, respectively. A significant difference was found between the two groups of 1-3 collections (n=15) and 4-8 collections (n=17) (p<0.05). It is clear from this analysis that repeated collection of oocyte from some marmosets significantly decreased the fertility rate. However, some individuals continued to show a high fertility rate despite repeated oocyte collection. In future research, we will examine for differences between the individuals which show decrease fertility rates and those which do not, with the goal of maintaining a stable fertility rate during multiple oocyte collection.
P-80 Ovulation detection by monitoring urinary progesterone for embryo collection in common marmosets

 ○Chia-Ying Lee¹, Masae Tanaka¹, Takashi Inoue¹, Junko Okahara¹, Yuko Yamada¹, Yoko Kurotaki¹, Miho Nagasawa¹, Takefumi Kikusui², Erika Sasaki¹,³
¹Central Institute for Experimental Animals, ²Laboratory of Companion Animal Research, Azabu University, Sagamihara, Japan, ³Keio Advanced Research Centers, Tokyo, Japan

In common marmosets (Callithrix jacchus), monitoring the progesterone level in blood is the standard method by which the ovulation date is determined. This method helps to precisely track the estrus cycle; however, it requires repeated blood sampling from the animals. As an alternative and non-invasive method, we developed urinary progesterone monitoring for ovulation detection in common marmosets. In the present study, the ovulation date was determined based on the urinary progesterone, and then we attempted to collect embryos. Four adult female marmosets paired with males were used periodically for embryo collection, and their blood was routinely sampled to monitor the progesterone. After changing from blood progesterone monitoring to urine progesterone monitoring, embryo collection was attempted 5-6 times in each animal. As a result, embryos were obtained 4-5 times from each animal; embryo collection success rates were similar between the two progesterone monitoring methods. Further, stress response was evaluated in these animals using a urinary cortisol. The urinary cortisol levels of these animals were decreased 90 days after switching to the method. These results suggest that urinary progesterone monitoring is useful for ovulation detection and decreases stress in marmosets.

P-81 Haploid rat ES cell lines derived from androgenetic and parthenogenetic blastocysts

 ○Hiromasa Hara¹, Teppei Goto¹, Akiko Takizawa², Melinda Dwinell², Shinichi Hochi³, Hiromitsu Nakauchi⁴, Masumi Hirabayashi¹
¹National Institute for Physiological Sciences, ²Medical College of Wisconsin, ³Shinshu University, ⁴University of Tokyo

Pluripotent and germline-competent ES cell lines have been established from haploid blastocysts in rodents (Development 2014). Haploid mouse ES cell lines can contribute more efficiently to generation of mutants with multiple knockouts or large deletions when compared to diploid ES cell lines (Int J Mol Sci 2015). To establish haploid rat ES cell lines in the present study, we prepared 702 androgenetic haploids by removing female pronucleus from Slc:SD x WDB-Rosa26ΔloxP(ROSA26)Nips (RGD ID: 8552371) knockin rat zygotes, and 138 parthenogenetic haploids by activating CAG/Venus transgenic rat-derived oocytes with 5 μM ionomycin + 10 μg/ml cycloheximide. These haploid zygotes were cultured in vivo for 4 days, resulting in the harvest of 26 androgenetic blastocysts (4%) and 2 parthenogenetic blastocysts (2%). These blastocysts were further cultured in 2iF medium, resulting in the establishment of 7 (27%) and 2 (100%) ES cell lines, respectively. FACS was applied to purify the haploid ES cell population after staining with 50 μM verapamil + 10 μg/ml Hoechst33342. Among the established ES cell lines, 1 (14%) and 2 (100%) lines contained haploid cell population, respectively. The haploid ES cell lines are being maintained with >30% proportion of 1n cells and investigated for karyotype and pluripotency.
P-82 Effect of DNA repair ability of muscle tissue nuclei by somatic cell nuclear transfer

○Rika Azuma1, Mizuki Kajimoto2, Akari Obashi2, Takuya Orisugi2, Mizuki Sugimoto2, Yoshikazu Hosoi1,2,3, Masayuki Anzai3

Currently, it has enabled to produce reconstructed embryos and offspring by nuclear transfer technology using of somatic cell nuclei. We also confirmed that somatic cell nuclei recovered from muscle tissue formed the reconstructed oocytes by nuclear transfer. In this study, we examined the effects on DNA repair ability of muscle tissue-derived somatic cell nuclei for incorporation in ooplasm. The experiments used the tissues collected from thigh muscle of adult BDF1 or B6C3F1 mice. Subsequently, these tissues were frozen and stored at -30 C for 0 to 60 months. The somatic cell nuclei from muscle tissue introduced to a mixture of the NIM and 0.05% trypsin EDTA and crushed using BioMasher III (Nippi). The recovered somatic cell nuclei were injected enucleated oocytes by nuclear transfer. And reconstructed oocytes examined localization of Histone γH2A.X in pronuclei. Moreover, in addition of 1mM BrdU into activation media was observed cell cycle by fluorescence microscopy. Results of nuclear transfer, the development of reconstructed pronuclear oocytes after activation treatment was about 70%. In addition, Histone γH2A.X was localized in pronuclei of reconstructed oocytes. Furthermore, reconstructed oocytes were transitioned from M-G1 to S phase. Moreover, this has been found to proceed faster than the control group (injected with cumulus cell nuclei were detected for 6 to 7 hours).

P-83 Multi-gene knockouts by the CRISPR/Cas9 system in mouse ES cells

○Hiroshi Kiyonari1,2, Takaya Abe2, Yui Yamashita1,2, Yoshiko Mukumoto2, Atsumi Denda2, Mari Kaneko2, Yasuhide Furuta1,2
1Animal Resource Development Unit, RIKEN CLST, Kobe, Japan, 2Genetic Engineering Team, RIKEN CLST, Kobe, Japan

The CRISPR/Cas9 system has been actively used as an emerging powerful tool for genome editing in various model organisms (Cong et al., 2013 & Wang et al., 2013). Here we report the use of this system to establish an efficient approach for analyzing embryonic lethal, multi-gene mutants in mouse embryos. We have employed the D10A mutant nickase version of Cas9 (Cas9n) to minimize the off-target effect (Ran et al., 2013). The strategy comprises: 1) evaluation of sgRNAs, 2) accommodation of multiple sgRNAs in a unique vector containing puromycin and Cas9 or Cas9n cassettes, 3) screening of mutant ESCs by PCR and sequencing, 4) production of high-contribution chimera embryos, and 5) analyses of mutant phenotypes in F0 chimera embryos. This approach provides a stable source of mutants, and thus allows for efficient genetic analyses of embryonic lethal multi-gene mutations. In the current study, to examine the role of Fgf signaling in the developing eye, multi-gene mutants lacking Fgf3, Fgf9 and Fgf15 expressed in the developing retina have been generated. These initial studies have efficiently yielded triple mutant ES clones by using both Cas9 and Cas9n, and preliminary phenotypic analyses of the F0 mutant embryos will be reported.
P-84 Variation introduction efficiency to a mouse by using the CRISPAR/Cas9 system
○Masato Tanaka1, Miki Nisio1,2, Yuuki Gotou1,2, Akira Suzuki1,2

1Development Engineering Laboratory, Medical Institute of Bioregulation, Kyusyu University, Fukuoka, Japan, 2Division of Cancer Genetics, Medical Institute of Bioregulation, Kyusyu University, Fukuoka, Japan

[purpose] The genetically modification technology become advanced, and it becomes a required technique in performing the gene functional analysis of the individual. Particularly, by the spread of genome editing technologies using the CRISPR/Cas9 system, the knockout mouse became easy. Therefore I examined the most suitable rate condition to let it was more stable and introduce variation into a high rate.

[method] (1) density of CRISPR/Cas9 plasmid pX330-cetn1 (addgene) (5 ng/μl, 10 ng/μl) (2) injection needle (580 μm, 780 μm) efficiency and compared 2cell incidence, the birth rate and the variation introduction efficiency. Single stranded DNA oligomer (140mer) density (5 ng/μl, 20 ng/μl, 40 ng/μl) in addition to CRISPR/Cas9 plasmid density (5 ng/μl), (2) changed culture time (8h, 10h) after the IVF and compared 2cell incidence, the birth rate and the knockin efficiency after microinjection to examine knockin efficiency of 40 bases by the homologous recombination next.

[result] By the comparison of the variation introduction efficiency, it was the density of 5 ng/μl, and variation introduction efficiency was good, and the birth rate and variation introduction efficiency improved it by changing the injection needle to 780 μm inside diameter. By the comparison of the knock in efficiency of 40 bases, culture time after the IVF watched improvement again in 10h.

P-85 Genome Editing with the St1-CRISPR/Cas9 system in Mouse Zygote
○Kanako Kato, Natsumi Iki, Yoko Daitoku, Yoko Tanimoto, Yoshikazu Hoshino, Satoru Takahashi, Ken-ichi Yagami, Seiya Mizuno, Fumihiro Sugiyama

Laboratory Animal Resource Center, University of Tsukuba, Ibaraki, Japan

The Streptococcus pyogenes CRISPR/Cas9 (Sp-CRISPR) system is mainly used in genome editing. Although DNA cleavage activity of the Sp-CRISPR is robust, the PAM sequence (NGG) is a requirement for Sp-Cas9 binding to the target genome site. This limitation is an obstacle in genome editing of the point mutation and epitope tag Knock-in. To achieve more flexible genome editing, we tried to perform genome editing with Streptococcus thermophiles CRISPR1/Cas9 (St1-CRISPR) and Neisseria meningitides CRISPR/Cas9 (Nm-CRISPR) in mouse zygote. Esvelt K.M. et. al reported that the PAM sequence of St1- and Nm-Cas9 is NNAGAA and NNNNGATT, respectively. The Sp-, St1- and Nm-CRISPR expression vectors were constructed with the Addgene #42230, #48669, and #48670 plasmid. We first target Tyr gene by these 3 CRISPRs. The Tyr null mutant shows albino phenotype. The cleavage activities of them were confirmed by EGxxFP assay. The cleavage activity of St1-CRISPR was weaker than that of Sp-CRISPR. Addition, Nm-CRISPR had very low cleavage activity. The St1-CRISPR-Tyr plasmid vectors were microinjected into 323 C57BL/6j zygotes. We obtained 64 founders but no albino mouse was found. Genomic analyses revealed that 4 founders carried heterozygous frame-shift mutations. As expected, offspring from these founder intercross showed albino phenotype. This result indicates that St1-CRISPR can be used for genome editing in mouse zygote.
P-86 Generation of Knockout or Knockin mice using CRISPR/Cas9 gene editing

○Eri Nakamura¹, Norihiro Tada¹,²


Recently, CRISPR/Cas9 system have been used to generate knockout mice by generating DSB and followed by NHEJ-mediated repair, resulting in mutant mice carrying indel mutation. This procedure is a much shorter time than can be performed by using gene-targeted ES cells. In this study, we show generation efficiency of mutant mice with targeted mutation in seven target genes by CRISPR/Cas9 system. Cas9 mRNA and sgRNA for four target genes (A~D) were injected into the cytoplasm of oocytes at the pronuclei stage, respectively. Plasmid of Cas9 mRNA and sgRNA for three target genes (E~G) were coinjected into oocytes with spermatozoa, respectively. The 2-cell stage embryos developed from injected oocytes were transferred into oviducts of pseudopregnant mice at 0.5 dpc. After birth, genomic DNA was extracted from the tail tips of weaned pups and subjected to PCR. The purified PCR products were analyzed by T7E1 assay and TA cloning/sequencing for genome modification. We found that 15 (100%), 3 (60%), 7 (100%) and 12 (75%) pups were mutated for A, B, C and D target genes, and in A target gene 9 out of 15 pups were homozygous mutant. On the other hand, E, F and G target genes, 1 (6%), 3 (25%) and 1 (100%) pups were inserted of donor genes, respectively. These results indicate that the CRISPR/Cas9-mediated genome editing makes possible highly effective method for the generation of knockout/knockin mice and the one-step generation of homozygous mutants for indel mutation.

P-87 ssODN-mediated knock-in with CRISPR-Cas9 for large genomic regions in zygotes

○Yayoi Kunihiro¹, Kazuto Yoshimi², Takehito Kaneko³, Tomoji Mashimo¹

¹Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University, ²Mouse Genomics Resource Laboratory, National Institute of Genetics, ³Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

The CRISPR-Cas system is a powerful tool for generating genetically modified animals; however, targeted knock-in (KI) via homologous recombination remains difficult in zygotes. In this study, we show efficient gene KI in rats by combining CRISPR-Cas with single-stranded oligodeoxynucleotides (ssODNs) (Yoshimi et al. Nat Commun 2016). First, a 1-kb ssODN co-injected with guide RNA (gRNA) and Cas9 mRNA produce GFP-KI at the rat Thy1 locus. Then, two gRNAs with two 80-bp ssODNs direct efficient integration of a 5.5-kb CAG-GFP vector into the Rosa26 locus via ssODN-mediated end joining. This protocol also achieves KI of a 200-kb BAC containing the human SIRPA locus, concomitantly knocking out the rat Sirpa gene. Finally, three gRNAs and two ssODNs replace 58-kb of the rat Cyp2d cluster with a 6.2-kb human CYP2D6 gene. These ssODN-mediated KI protocols can be applied to any target site with any donor vector without the need to construct homology arms, thus simplifying genome engineering in living organisms.
P-88  Production of CRISPR/Cas9-mediated gene edited rats with cytoplasmic injection

○Yoshihiro Ooguchi, Takao Tanaka, Yuki Toshinari, Sho Motomura, Makoto Kitaura
Genetically Engineering Animal Laboratories, KAC Co., Ltd. Siga, Japan

[Introduction] CRISPR/Cas9 system is widely used to generate KO and KI animals. Cas9 mRNA, guide RNA and ssODN are injected mainly into pronuclei. In this study, we conducted two experiments to verify whether we could produce gene edited rats by injecting RNAs and ssODN into cytoplasm. Tyrosinase gene was selected as target gene since the phenotypes (coat color) of KO and KI rats are obvious at a glance. [Methods] Study #1; Production of albino rats by knocking out of Tyrosinase gene. Embryos were collected from the females (Jcl:Wistar, albino) mated with males (DA/Slc, colored). Cas9 mRNA and guide RNA were injected into cytoplasm. Study #2; Production of colored rats from albino embryos. Embryos were collected from the females mated with males (both F344/Jcl, albino). RNAs and ssODN which has colored type sequence of Tyrosinase gene were injected into cytoplasm. In both studies, The embryos were transferred into pseudopregnant rats. We checked the rates of genome editing by coat color of the founders. [Results] Study #1; RNAs were injected into 77 embryos. 58 embryos were transferred into pseudopregnant rats, resulting in birth of 29 pups. All pups had albino phenotype. Study #2; RNAs and ssODN were injected into 57 embryos. 56 embryos were transferred into pseudopregnant rats, resulting in birth of 29 pups. All pups had albino phenotype. 2 pups out of 26 had colored phenotype. We demonstrated that cytoplasmic injection could be applicable to production of KI rats as well as KO rats.

P-89  Non-human primate model of severe combined immunodeficiency by highly efficient genome editing

○Kenya Sato1, Kumita Wakako1, Henry Rachel2, Sakuma Tetsushi1, Ito Ryoji1, Nozu Ryoko1, Inoue Takashi1, Okahara Norio1, Okahara Junko1, Hanazawa Kisaburo1, Weinstein Edward2, Yamamoto Takashi1, Okano Hideyuki1, Sasaki Erika1,5  
1The Department of Applied Developmental Biology, Central Institute for Experimental Animals, Kanagawa, Japan, 2SAGE Labs, Missouri, USA, 3Hiroshima University, Hiroshima, Japan, 4Juntendo University Nerima Hospital, Tokyo, Japan, 5Keio University, Tokyo, Japan

Non-human primate (NHP) experimental animals are important for understanding of human diseases, physiology and genetics, due to their high level of similarity to humans. The common marmoset (Callithrix jacchus) is a species often used in biomedical research because of its prolificacy and ease of handling. On the other hand, the recent development of innovative genome editing technologies such as ZFN, TALEN, CRISPR/Cas9 can resolve this issue, and it is now possible to generate target-gene KO or KI animals without the use of ESCs. The interleukin receptor 2 gamma (IL2RG) gene is one of the genes responsible for X-linked severe combined immunodeficiency (X-SCID) in patients and the immunodeficient mouse.

In our results, 7 of 21 neonates showed somatic mutagenesis in the IL2RG using ZFN and TALEN, moreover, 4 marmosets which dead case were observed lack of thymus. On the other hand, we have succeeded long term breeding in three IL2RG KO marmosets.

Here we report our attempt to generate immunodeficient marmosets and results of immunological analyses in survived IL2RG KO marmosets.
P-90 Investigation of the marmoset gene modification efficiency with CRISPR/Cas9

©Wakako Kumita¹, Ken-ya Sato¹, Erika Sasaki¹,²,³

¹Central Institute for Experimental Animals, Kawasaki, Japan, ²Keio University, Tokyo, Japan, ³RIKEN, Wako, Japan

Many methods are used to produce knock-in (KI) and knock-out (KO) animals. In mice, embryonic stem (ES) cells are used to produce chimeric animals to obtain KI. Although ES cells have been established in the common marmoset (Callithrix jacchus), they cannot be used to generate chimera. In many species, this situation limits the production of target gene KO or KI animals. Recent studies suggest that genome-editing technologies can be applied to produce animals with modified target genes. Our laboratory has already obtained KO marmosets using Zinc-finger nuclease and TALENs; furthermore, we are investigating the efficacy of CRISPR/Cas9. This study investigated the optimisation and improvement of CRISPR/Cas9 for marmoset embryos. This investigation is an important basal study for generating KO/KI marmosets. CRISPR/Cas9 that targeted the marmoset c-kit gene was injected into marmoset embryos. The optimum concentration of mRNA solution, including single-guide RNA/humanised Cas9 mRNA, was determined, and a suitable humanised Cas9 for marmoset embryos was found. These results should not only permit highly efficient KO production but would also be useful for producing KI marmosets.

P-91 Production of genetically engineered animals by electroporation

©Takehito Kaneko¹, Tomoji Mashimo³

¹Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan, ³Institute of Experimental Animal Sciences, Faculty of Medicine, Osaka University, Osaka, Japan

Genetically engineered animals can be produced more simply and rapidly by the development of genome editing techniques. However, the microinjection of nucleic acids into embryos still requires a high skill level. We have been developed simple production method of genetically engineered mouse and rat by using electroporation (TAKE method). Cas9 mRNA, gRNA and ssODN could be introduced into intact pronuclear-stage embryos by electroporation. High proportion of knockout and knock-in mouse and rat could be obtained using this method. This study indicated that the TAKE method provides a simple and effective method to produce genetically engineered animals.
P-92  Efficient method for mRNA delivery into cryopreserved mouse embryos by electroporation

Kenta Nakano1,2, Rieko Takanashi1, Yukiko Shimizu1, Tetsuya Arai1, Tadashi Okamura1

1Research Institute National Center for Global Health and Medicine, 2Kitasato Graduate School of Veterinary Medicine

CRISPR / Cas9 system has been widely used as a new genome editing technology, due to its convenience and high cutting efficiency. This system was possible to produce more easily genetically modified(GM) mice compared to conventional method using ES cells. Recently, a new method which produces GM mice by electroporation was developed and was able to produce more easily GM mice than microinjection method. In this study, we investigated optimal electric conditions of mRNA delivery into cryopreserved mouse embryos. Cryopreserved embryos were obtained from of BDF1 strain. By electroporator (NEPA21), we investigated voltage and poring pulse width and calculated introduction efficiency of mCherry mRNA encoding a fluorescent protein and survival rate. In two-cell embryos, fluorescence intensity of mCherry increased in proportion to the voltage when poring pulse width was fixed and mCherry mRNA was electroporated with voltage 100V-250V. However survival rate decreased in proportion to the voltage. Next, we investigated poring pulse width when voltage was fixed. Fluorescence intensity of mCherry increased in proportion to the poring pulse width when mCherry mRNA was electroporated with poring pulse width 1.0ms~4.0ms. In conclusion, electroporation is useful as a gene introduction method into cryopreserved mouse embryos.

P-93  Generation of kidney-deficient model rats by knocking-out Sall1 gene

Teppei Goto1, Hiromasa Hara1, Shinichi Hochi2, Hiromitsu Nakauchi3, Masumi Hirabayashi1

1National Institute for Physiological Sciences, Aichi, Japan, 2Shishu University, Nagano, Japan, 3University of Tokyo, Tokyo, Japan

Regeneration of human kidneys in animal model would contribute to provide enough number of functional donor kidneys in transplantation therapy. This study was conducted to generate kidney-deficient model rats by knocking-out the transcriptional factor Sall1 gene essential for nephrogenesis. Targeting vector was designed to replace 2nd and 3rd exons encoding DNA-binding domain of Sall1 locus with tdTomato. The vector was introduced into WDB/Nips embryonic stem cells (ESCs) by electroporation, and the targeted ESCs were selected by G418 and southern blot analysis. Two lines of ESCs were identified as targeted from 60 G418-resistant colonies. To generate chimeric rats, Crlj:WI blastocysts injected with the targeted ESCs were allowed to develop to full-term. Five out of 7 male chimeras were found to be germline-competent, and heterozygous Sall1-knockout (KO) G1 rats were used to produce homozygous and heterozygous KO rats at G2 generation. The tdTomato fluorescence was located in kidney, uterus, ovary and brain of E21.5 heterozygous KO rats. Homozygous KO rats were found to lack both kidneys at E21.5, and died at postnatal day-1 due to their inability of suckling. Since we successfully generated kidney-deficient rats by knocking-out the Sall1 gene, kidney regeneration study is attempting with blastocyst complementation using pluripotent stem cells.
P-94  Transplantation of pancreatic progenitor cells into mouse fetus for organ complementation

○Shinsuke Seki1,2, Tomoyuki Yamaguchi1, Hiromitsu Nakauchi1

1Division of Stem Cell Therapy, The institute of Medical Science, The University of Tokyo, Tokyo, Japan,
2Center for Bioscience Education and Research, Akita University, Akita, Japan

Our ultimate goal is to generate functional and transplantable human organs from pluripotent cells in vivo using a xenogenic environment. As a proof of principal, rat pancreas was successfully generated in mouse by interspecific blastocyst complementation. However, human pluripotent cells injected animal embryos cannot be transferred into animal uterus because of ethical concerns in Japan. Therefore, we are trying to generate organ by transplantation of tissue progenitor cells into fetus. In this study, we tried to transplant pancreatic progenitor cells into pancreas of mouse fetuses. Immature pancreases were collected from mouse fetuses (B6 GFP, E13.5-E15.5), and the dissociated cells were transplanted into mouse fetuses (E12.5). Because pancreas in mouse fetus was not visible through uterus, it was difficult to transplant cells into pancreas by in utero transplantation. When exo utero transplantation was conducted, GFP positive cells were confirmed in few neonates delivered by caesarian section. When we tried to transplant cells into pancreas through liver, bile duct and pancreatic duct, GFP positive cells were confirmed in pancreas of spawned neonates. We will examine whether pancreas is produced in apenicric mouse (Pdx1 knockout) by transplantation of pancreatic progenitor cells into the fetus for the future study.

P-95  Towards a INSR gene knock down transgenic model of type II diabetes in a common marmoset

○Tsukasa Takahashi1,2, Kenta Kobayashi3, Erika Sasaki1,2

1Central Institute for Experimental Animals, Kanagawa,Japan, 2Keio University School of Medicine, Tokyo, Japan, 3National Institute for Physiological Sciences, Aichi, Japan

The common marmoset is usefulness as an experimental animal because they are small body size and also has high reproduction rate. Furthermore, transgenic marmosets have successfully produced since 2009. Currently, loss-of gene function mutants are able to create various human disease models. In particular, gene knockdown technology with tet regulation that can avoid embryonic lethality allows producing a model that cannot be produced by gene knockout. In this study, we are attempting to generate a target gene knock down marmoset using the vector coding the insulin receptor (INSR) - specific shRNA under tet regulator and ubiquitous EGFP expression as integration marker. In this vector the Dox treatment leads the siRNA and ablation of INSR protein results in onset of type II diabetes. Six shRNA were designed from marmoset INSR cDNA sequence and Western blot analysis were performed to evaluate RNAi efficiency of these shRNA. The highest efficient shRNA lentiviral vector was injected into marmoset embryos produced in vitro or in vivo and the GFP expressing embryos were transferred to surrogate mothers non-surgically. Four transgenic offspring were successfully derived. Although three offspring were dead after birth, one was survived and reached sexual maturity. Expression of GFP and knockdown of INSR were confirmed in fibroblast which we collected from offspring.
P-96 Genetic analysis of a new dominant white spotting mutant

Tomoki Maegawa, Hiroto Katoh, Miyako Suzuki, Misato Kobayashi, Masahide Takahashi, Fumihiko Horio, Tamio Ohno

Division of Experimental Animals, Graduate School of Medicine, Nagoya University, Nagoya, Japan, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, Department of Pathology, Graduate School of Medicine, Nagoya University, Nagoya, Japan

A female mouse showing white spotting appeared spontaneously in the C3.NSY-Chr11R1 strain. The white spotting phenotype showed autosomal dominant inheritance, and we tentatively named the mutation Dws (dominant white spotting). We backcrossed mutant mice to C3H/HeN and generated C3H/HeN-Dws/+ mice. Linkage analysis involving C57BL/6J and C3H/HeN-Dws/+ mice showed that all white spotting mice produced (64 in total) were heterozygous for the D1Mit132 (77.15Mb) and D1Mit215 (78.21Mb) markers. During gestation, homozygotes for these 2 markers displayed exencephaly and tail abnormalities between E14.5 and E17.5; homozygosity was eventually embryonic lethal. These features are similar to mutants of the Pax3 gene that is located in the vicinity of D1Mit132 and D1Mit215. Dws is therefore likely to be a mutation of the Pax3 gene.

P-97 Paracrine Wnt/β-catenin signaling plays a role in proliferation of undifferentiated spermatogonial

Hinako M Takase, Roel Nusse

Department of Experimental Animal Model for Human Disease, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan, Department of Developmental Biology, Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA

Spermatogonial stem cells (SSCs) fuel the production of male germ cells but the mechanisms behind SSC self-renewal, proliferation and differentiation are still poorly understood. Using the Wnt target gene Axin2 and genetic lineage tracing experiments, we found that undifferentiated spermatogonia, comprising SSCs and transit amplifying progenitor cells, respond to Wnt/β-catenin signals. Genetic elimination of β-catenin shows that Wnt/β-catenin signaling promotes the proliferation of these cells. Signaling is likely initiated by Wnt6 which is uniquely expressed by neighboring Sertoli cells, the only somatic cells in the seminiferous tubule that support germ cells and act as a niche for SSCs. Therefore, unlike other stem cell systems where Wnt/β-catenin signaling is implicated in self-renewal, the Wnt pathway in the testis specifically contributes to the proliferation of SSCs and progenitor cells.
**P-98** Functional analysis of Meis1 in skin tumor using ChIP sequencing assay

○Yasuhiro Yoshizawa\(^1,2\), Kazuhiro Okumura\(^1\), Megumi Saito\(^1,2\), Yoshimasa Aoto\(^3\), Eriko Isogai\(^1\), Yasubumi Sakakibara\(^1\), Yuichi Wakabayashi\(^1\)

\(^1\)Division of Experimental Animal Research, Chiba Cancer Center Research Institute, \(^2\)Graduate School of Medicine, Chiba University, \(^3\)Department of Biosciences and Informatics, Bioinformatics Laboratory, Keio University

Meis1, a transcription factor, has been known as responsible for lymphocytic leukemia and its upregulation has been confirmed in various tumors. Our phenotypic and skin tumorigenesis analysis of skin specific conditional Meis1 knockout mice previously showed that Meis1 plays roles in stem cell maintenance and skin tumorigenesis. Moreover, it was confirmed that Meis1 was over expressed with skin tumor progression. All of above indicates that Meis1 plays key roles in tumor survival, however its mechanisms are still obscure. In this study, we performed ChIP sequencing assay using the Meis1 antibody for skin epidermis (Ep), skin papilloma (Pa) and carcinoma (Ca) to investigate Meis1 alternative regulation due to tumor progression. Sequencing data were analyzed by using MACS (Zhang et al.). Peak finding was performed under setting no or with control. By analysis under no control, 856, 733 and 127 peaks were found in Ep, Pa and Ca respectively. On the other hand, under setting Ep as control, only 346 and 25 positive peaks, 19 and 28 negative peaks were found in Pa and Ca respectively. Therefore, most of peaks found in Ep under no control were thought to exist in Ca. These results indicated that Meis1 in Ca remains transactivation found in Ep and strongly activates specific genes.

**P-99** Mc1r gene deletion found in a novel yellow color mutant mouse

○Shuji Takabayashi, Hideki Katoh

_Laboratory Animal Facilities & Services, Education & Research Center for Preeminent Medical Photonics, Hamamatsu University School of Medicine_

We found a novel color mutation in B6 congenic mice and called the Ham yellow. The Ham yellow mutant is characterized by a light yellowish brown coat color. An allelism test was performed for yellow mutant and agouti yellow (\(A'\)). An allelism test indicated our mutant gene to be not a new allele at the agouti locus. Genetic analyses revealed that the Ham yellow is an autosomal recessive. We used whole exome-targeted next-generation sequencing and Sanger sequencing to determine the responsible gene for yellow mutant. As a result, we found that Ham yellow mice have approximately 2.8 Kb deletion mutations in melanocortin-1 receptor (\(Mc1r\)) locus. The MC1R is the G protein-coupled receptor protein for melanocyte-stimulating hormone (MSH). It is known that the \(Mc1r\) locus controls the distribution and quantity of eumelanins and pheomelans and coat color variants in animals. At the \(Mc1r\) locus of mice, four dominant mutant alleles and three recessive mutant alleles have been reported. Here, we report novel recessive mutation in the \(Mc1r\) gene and the proposed gene symbol for the Ham yellow is \(Mc1r\)\(^{\text{e-Ham}}\).
Identification of the responsible gene for Stmm1a by congenic mapping and gene expression analysis

Kazuhiro Okumura1,2, Megumi Saito2, Yasuhiro Yoshizawa2, Eriko Isogai2, Ikuo Miura3, Shigeharu Wakana3, Ryo Kominami2, Yuichi Wakabayashi2

1Division of Oncogenomics, Chiba Cancer Center Research Institute, Chiba, Chiba, Japan, 2Division of Experimental Animal Research, Chiba Cancer Center Research Institute, Chiba, Chiba, Japan, 3Technology and Development Team for Mouse Phenotype Analysis, BRC, RIKEN, Tsukuba, Ibaraki, Japan

In recent study, we found that MSM/Ms shows dominant resistance to chemically induced skin tumor development when crossed with the susceptible FVB/N strain. To identify genetic determinants of skin tumor susceptibility, we generated a large series of F1 backcross mice, and subjected the animals to DMBA/TPA induced skin carcinogenesis. We previously identified strong genetic loci Skin tumor modifier of MSM 1 (Stmm1) on chromosome 7 for early stage papilloma. Therefore, we generated multiple congenic strains segregating chromosomes 7, and have confirmed Stmm1a including D7Mit351-149 lies within the genetic distance of about 1.1 cM (physical distance; 2 Mb) on proximal chromosome 7. In order to identify of the responsible gene for Stmm1a, we performed RNA-seq (FPKM) analysis using normal skin tissues of MSM mice and also FVB mice. As a result, we detected the difference of FPKM values between MSM and FVB in several candidate genes. Now, we are trying to congenic mice and bioinformatics analysis will facilitate the following gene identification step.

Identification of a novel Col2a1 mutant mouse line by ENU mutagenesis

makoto kimura1, Satoki Ichimura1, Hiroshi Masuya2, Tomohiro Suzuki2, Shigeharu Wakana2, Tatsuya Furuichi1

1Co-Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Japan, 2RIKEN Bioresource Center, Tsukuba, Japan

In humans, mutations in the COL2A1 gene encoding the α1(II) chain of type II collagen, create many clinical phenotypes collectively termed type II collagenopathies. However, the mechanisms generating this diversity remain to be determined. Here we identified a novel Col2a1 mutant mouse line by screening a large-scale N-ethyl-N-nitrosourea mutant mouse library. This mutant possessed a p.Tyr1391Ser missense mutation in the C-propeptide coding region, and this mutation was located in positions corresponding to the human COL2A1 mutation responsible for platyspondylic lethal skeletal dysplasia, Torrance type (PLSD-T). As expected, p.Tyr1391Ser homozygotes exhibited lethal skeletal dysplasias resembling PLSD-T, including extremely short limbs and severe dysplasia of the spine and pelvis. The secretion of the mutant proteins into the extracellular space was disrupted, accompanied by an abnormally expanded endoplasmic reticulum (ER) and the up-regulation of ER stress-related genes in chondrocytes. Chondrocyte apoptosis was severely induced in the growth plate of the homozygotes. These findings strongly suggest that ER stress-mediated apoptosis caused by the accumulated mutant proteins in ER contributes to skeletal dysplasia in Col2a1 mutant mice and PLSD-T patients.
P-102 SNP genotyping by PCR using multicolor fluorescent primers with locked nucleic acids

Osamu Suzuki, Minako Koura, Kozue Uchio-Yamada, Mitsuho Sasaki, Junichiro Matsuda

Laboratory of Animal Models for Human Diseases, National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Japan

[Aim] Zygosity checks of SNP alleles in mouse genomes are often laborious. In this study, we tried to establish a simple method for zygosity checks of SNP alleles by one-tube PCR with multicolor fluorescent primers containing locked nucleic acids (LNA) and differential fluorescence detection. [Methods] We used Slc11a1 gene for SNP genotyping. Hepatic DNA from C57BL/6NCr (Slc11a1<sup>s</sup>/Slc11a1<sup>s</sup>) and DBA/2 (Slc11a1<sup>r</sup>/Slc11a1<sup>r</sup>) mice and a mixture of both (Slc11a1<sup>s</sup>/Slc11a1<sup>r</sup>) were used. Two kinds of anti-sense primers annealing SNP positions at 3'-ends of the primers, and a common sense primer were prepared. Primers for Slc11a1<sup>s</sup> and Slc11a1<sup>r</sup>, and common were labeled with different fluorescent dyes at 5'-end (Cy3, Alexa647 and Alexa488, respectively). Anti-sense primers with LNA at 3'-ends were also prepared. PCR were conducted with mouse genomic DNA and three primers. After agarose electrophoresis, PCR products from each allele were checked by a fluorescent imager (FX pro, Bio-rad) in Cy3, Cy5, and Cy2 modes for detecting, Slc11a1<sup>s</sup>, Slc11a1<sup>r</sup> and both alleles, respectively. [Results and Discussion] Zygositites were successfully identified when the anti-sense primers with LNA were used, confirming that the beneficial effect of LNA for PCR specificity. Despite a high cost, our simple method using primers with fluorescence and LNA would be useful for routine SNP genotyping.

P-103 Generation of CRISPR/Cas9-mediated bicistronic knock-in Ins1-cre driver mice

Yoshikazu Hasegawa<sup>1</sup>, Yoshikazu Hoshino<sup>1,2</sup>, Ibrahim Abdelaziz E<sup>1</sup>, Kanako Kato<sup>1</sup>, Yoko Daitoku<sup>1</sup>, Yoko Tanimoto<sup>1</sup>, Yoshihisa Ikeda<sup>1,3</sup>, Hisashi Oishi<sup>1</sup>, Satoru Takahashi<sup>1</sup>, Ken-ichi Yagami<sup>1</sup>, Hiroyoshi Iseki<sup>1</sup>, Seiya Mizuno<sup>1</sup>, Fumihiro Sugiyama<sup>1</sup>

<sup>1</sup>Laboratory Animal Resource Center, University of Tsukuba, Ibaraki, Japan, <sup>2</sup>Hoshino Laboratory Animals, Inc., Ibaraki, Japan, <sup>3</sup>Charles River Laboratories Japan, Inc., Ibaraki, Japan, <sup>4</sup>Experimental Animal Division, RIKEN BioResource Center, Ibaraki, Japan

In the present study, we generated novel cre driver mice for gene manipulation in pancreatic β cells. Using the CRISPR/Cas9 system, stop codon sequences of Ins1 were targeted for insertion of cre, including 2A sequences. A founder of C57BL/6J-Ins1<sup>ins(2A-cre)</sup>Utr strain was produced from an oocyte injected with pX330 containing the sequences encoding gRNA and Cas9 and a DNA donor plasmid carrying 2A-cre. C57BL/6J-Ins1<sup>ins(2A-cre)</sup>Utr F1 mice were histologically characterized for cre-loxP recombination in the embryonic and adult stages; cre-loxP recombination was observed in all pancreatic islets examined in which almost all insulin-positive cells showed tdsRed fluorescence, suggesting β cell-specific recombination. Furthermore, there were no significant differences in results of glucose tolerance test between genotypes (homo/wild). Taken together, these observations indicated that C57BL/6J-Ins1<sup>ins(2A-cre)</sup>Utr is useful for studies of glucose metabolism and the strategy of bicistronic cre knock-in using the CRISPR/Cas9 system could be useful for production of cre driver mice.
P-104  Assessment for the mouse exome enrichment system by whole exome sequencing

○Hayato Kotaki\(^1\), Ryutaro Fukumura\(^1\), Liu Tracy\(^2\), Yoichi Gondo\(^1\)

\(^1\)RIKEN BRC, \(^2\)Agilent Technologies

Whole Exome Sequencing (WES) has been essential for the positional cloning, QTL analyses and many others with the NGS technologies. We have examined conventional (V1) and newly designed (V2) versions of SureSelect Mouse All exon kit (Agilent Technologies) which target 46.9Mb (mm9) and 48.5Mb (mm10) of mouse exome. We constructed the 1 and 2 barcoded libraries using the same genomic DNA in DBA/2JC57BL/6JF1 background with the V1 and t V2 kits, respectively. The three libraries were mixed in equal molar ratio and subjected to ~one lane run of HiSEQ2000, which gave rise to 66, 54 and 55 million paired-end 100bp-reads from V1, V2-1 and V2-2, respectively. The average coverage of the targeted exome was > 60 folds in all the three libraries. The ratio of target regions with >20-fold coverage to the whole exome was 97.84% (V1), 96.95% (V2-1) and 96.69% (V2-2). All the target exons were read at least once by V2 whereas 37 target exons were not covered at all by V1. With respect to the enrichment for the target sequences with the high GC contents, we selected 8730 target sequences of 120-bp bin with >70% GC content covering a total of 1,047,598 bp and found 45.49% (V1), 97.83% (V2-1) and 97.64% (V2-2) of the high GC sequences were read >20 coverage. From the V2 library data, we also succeeded in finding 101 SNPs from the target regions that were not read by V1 at all. Thus, V2 libraries gave more uniform enrichment of all the target exome regions by expanding the recovery efficiencies to the high-GC sequences.

P-105  SLA-1 allele expression detected by a SLA-specific monoclonal antibody

○Shino Ohshima\(^1\), Asuka Miyamoto\(^1\), Atsuko Shigenari\(^1\), Rihito Kinami\(^1\), Masaki Takasu\(^2\), Tatsuya Matsubara\(^2\), Hitoshi Kitagawa\(^2\), Takashi Shiina\(^1\), Asako Ando\(^1\), Yoshie Kametani\(^1\)

\(^1\)Dept. of Molecular Life Science, Tokai Univ. Sch. of Med. Isehara, Japan, \(^2\)Dept. of Veterinary Medicine, Fac.of Applied Biol. Sci. Gifu Univ. Gifu, Japan

The regulation of the expression of major histocompatibility complex (MHC) is important as it is closely related to various hereditary diseases. In order to examine the pig MHC (SLA) protein expression along with mRNA, we prepared an anti-SLA epitope specific monoclonal antibody (mAb) and characterized the reactivity. SLA-1\(^*\)0401/\(\beta2M\) transfected A20 cells were immunized and the conventional spleen cells/P3X fusion was performed. Specific antibody-secreting hybridoma was selected using SLA-1\(^*\)0401/\(\beta2M\) transfected HEK293 cells and PBMCs from haplotype-defined Microminipigs, common marmoset and human. Array Scan and FACS analyses were used for screening. The clone X2F6 reacted with swine PBMCs in a haplotype specific manner. X2F6 was predicted to react with Y102, L103, L109 (YLL set ) but not with D102, V103, F109 (DVF set ) of SLA-1. As Hp-16.0 carry only one locus (SLA-1\(^*\)0401) with reactive YLL set, we used the Hp-16.0 PBMC to analyze the SLA-1 allele-specific expression profile. After the stimulation of PBMC with TSST-1, SLA-1 mRNA level was increased after 24 hrs and decreased thereafter, but the surface expression of SLA-1 was maintained up to 72 hrs. These results suggest that the SLA-1\(^*\)0401 expression can be well-monitored by the combination of mAb and SLA-defined Microminipigs.
P-106  High-throughput knockout mouse production using Cas9 endonuclease

○Shinya Ayabe¹, Kenichi Nakashima², Mizuho Iwama¹, Maiko Ijuin¹, Koji Nakade²,
Takehide Murata², Atsushi Yoshiki¹, Yuichi Obata²

¹Experimental Animal Division, RIKEN BioResource Center, Ibaraki, Japan, ²Gene Engineering Division,
RIKEN BioResource Center, Ibaraki, Japan

A growing number of knockout mice, fluorescent reporter mice, and Cre and tTA driver mice have been
deposited to RIKEN BioResource Center (BRC). The quality of the genetics and the health of each strain
is ensured, which is crucial for comparing the results of different experiments. The International Mouse
Phenotyping Consortium (IMPC) is currently composed of 18 research institutions including RIKEN BRC.
IMPC is generating a knockout mouse strain for every protein-coding gene and characterising their phenotypes
in a broad-based phenotyping pipeline. In order to meet growing need for more immediate delivery of mouse
lines and phenotyping data, pilot studies for the knockout mouse production have been started using Cas9
endonuclease. Our approach that combines paired gRNAs and wild-type Cas9 or D10A nickase would be highly
beneficial to produce knockout mice. While the time and cost for generating knockout lines was reduced with
traditional gene targeting in embryonic stem (ES) cells, quality control of the alleles obtained through CRISPR/
Cas9 will be essential and complex. Issues faced by the mouse repositories including genetic quality control of
the mouse stains using engineered endonuclease technologies are the concerns that deserve broader discussion.