Preparation and Computational Analysis of Bisulphite Sequencing in Germfree Mice

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Abstract- *Over the years, several researchers have been interested in the natural intestinal flora. In the 1920's, James Reyniers' pioneering work created the first sterile guinea pig. Comparing the physiology of sterile bacteria and traditional farm animals offers useful knowledge on how host biology affects the gut flora. We now know that the intestinal flora can control the immune system, proliferation of epithelial cells, angiogenesis of the intestines, development of hormones, energy absorption and conduct. Moreover, recent studies indicate that obesity is associated with changes in the intestinal flora and the use of sterile mice has shown the direct involvement of the microbial flora in disease development.*

Keywords- gut microbiota, germ free mice, Isolator for housing germ-free mice.

I. INTRODUCTION

There coexists rich and complex microbial species in humans and mice. These microorganisms are primarily present in the human gastrointestinal tract, including bacteria, ancient fungi , and viruses, and are collectively referred to as intestinal flora. Study into symbiotic flora in nearly 30 years can be identified. There is increasing evidence that microbiomas are related to cell biology's physiology and pathology, and thus have an impact on health and disease. The various microflora inherited from the mother line can change at birth because of our dietary patterns and environmental signs. The role of Microflora has long been identified in numerous physiological processes, including the immune system. In addition , changes in intestinal microbiota in reaction to main immune messages contribute to intestinal and distant organ disorders, such as inflammatory bowel disorder, autoimmune diseases, and different forms of cancer. Matching and growth of the human central nervous system (CNS) is controlled by internal and external influences. Most experiments involving sterile animals (FGs) or animals infected with broad-spectrum antibiotics indicate that particular microbiomas influence the physiology and neurochemistry of the central nervous system. GF mice with no applicable flora have comprehension, memory, understanding, and mental impairments. They display variations in essential neurotransmitters such as 5-HT, NMDA, and BDNF relative to conventional mice. Evidence on associations between gastrointestinal pathology and neuropsychiatric conditions in diseases such as anxiety , depression and autism have been documented in humans. In addition, it has been shown that the intestinal flora controls the growth and homeostasis of the central nervous system in the form of gastrointestinal, circulatory and nerve pathways. In this study, we will first address the latest results related to the relationship between the intestinal flora and the immune system, in particular basic endogenous and adaptive immunizations and signal transduction pathways. It further addresses the contribution of bacteria to the central nervous system and the pathogenesis of central nervous system disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS) and glioma.

1.1 Interplay and reciprocal regulation between microbiota and immune system

The human immune system has evolved to sustain the symbiotic relationship of the host to microbes, and its disruption in complex immune-microbial interactions has had a significant impact on human health. In this segment, we will address the association of resident microbes with main immune signals and their contribution to the growth of the central nervous system and neurological disorders.

1.2 Sequence Databases

RefSeq— For ancient taxonomics, bacteria, eukaryotics, and viruses, the National Center for Reference Biotechnology (NCBI RefSeq) database provides carefully designed, non-redundant genomic regions, transcripts, and protein sequences. The RefSeq database is derived from the available sequence data from the unwanted GenBank archive collection. The RefSeq sequence includes encoding regions, conserved domains, variants, etc., As well as updated metadata such as articles, names, icons, names, gene identifiers and database references. The RefSeq sequence comprises coding regions, conserved domains, variants, etc., as well as improved annotations such as articles, names, icons, aliases, gene ids, and database references. Using a combination of teamwork, automated analysis and manual control to construct sequences and annotations. On 6 November 2015, RefSeq 73 contains

54,766,170 proteins, 12,998,293 transcripts and 55,966 cells. Through scanning the nucleotide or protein databases, running BLAST searches on chosen databases, and installing FTP, you can access the RefSeq logs directly from the national central bank's website. RefSeq logs can also be accessed from other national central bank instruments, such as Gene, Genome, BioProject, dbSNP, ClinVar, and Map Viewer, via indirect connexions. RefSeq further facilitates programmable access through the Entrez utility.

Mice

As mentioned above, under normal special conditions free of pathogens or bacteria (GF) female mice in C57B16 / N emulsion have been kept in an experimental biomedicine laboratory at the University of Göteborg. Mice were put on a 12-hour light period and fed autoclaved food ad libitum (Labdiet, St. Louis, Missouri, USA). Mice were sacrificed in three separate stages : 1, 4 and 12 to 16 weeks, each phase having n = 5 animals. Owing to a cervical dislocation the mouse was killed, and the small intestine was removed to separate the IEC. Both animal procedures were approved by the Animal Ethics Committee in Gothenburg.

1.3 Transcriptional profiling by RNA sequencing

The TRIZOL method was used to extract purified IEC RNA small intestine. In a 50-75 mg mortar, briefly add 1 ml of TRIzol to the homogenised tissue, then shake, incubate for 5 minutes at room temperature, and add 200 µl of chloroform. After mixing, incubate at room temperature and centrifuge (12,000 g) for another 2-3 minutes at 4 ° C for 5 minutes. Mix the clear supernatant with 500 micron of isopropanol and then incubate at room temperature for 10 minutes. After extra centrifugation (1,2000 g) at 4 ° C for 10 minutes, discard the supernatant and wash the pellet with 1 ml of cold 75 percent EtOH, then shake and centrifuge (7,500 g, 4 ° C, 5 minutes). In RNase-free water the precipitate was dried up and dissolved. The RNA library was prepared using the TruSeq v4 kit (Illumina), as directed by the manufacturer. All samples were sequenced at the IKMB NGS base facility using the Illumina HiSeq 2000 sequencer (Illumina, San Diego, California), averaging 23 million pair reading pairs (2 x 125 bp). To align the readings, we use TopHat 2[39], and Bowtie 2[40]. Use TopHat 2 to allocate mouse-genome reads (build version 10 of MGI). The mean comparison rate for RNA-seq was 83.3 percent (73.3-89.9 percent, median = 85.7 percent), and the number of expressions was standardised by library size. The transcript's gene expression value had been measured using HTSeq. DEseq2 is used to identify genes which express themselves differently. The gene is assumed to be significantly different if the modified p-value (Benjamini-Hochberg (BH) Multiple Test Correction Method) is less than 0.05. The gene expression variations were visualised using the MA plot, This is a variation of the Bland-Altman plot and was used to represent usable genomic data across genomes. M is the logarithmic shift in gene expression (y-axis), and A is the normalised mean number (x-axis).

1.4 Co-expression network analysis

To create a network of gene co-expression, we created a compound of genes with different expression in order to compare particular pathogen-free (CONV-R) and GF conditions that evolve at the same time regularly. Using BioLayout Express 3D, the co-expression study was based on the expression values of these genes in the 30 samples. Using the cutoff correlation value of 0.8 can get a network of co-expression with 970 nodes (genes) and 34,437 endpoints. Using an organic system, the measured gene-gene pairs and the Spearman correlation coefficients are inserted visually into Cytoscape. Next, we map the multiple conditional changes of each condition (based on comparing each condition to the average of all conditions) in the network, respectively, to define particular conditions of topological differences in the co-expression network conditions. The following basic parameters are used for assigning genomes depending on the period and based microbial expressions: group 1, high expression in W1 (conditional gene expression value normalised to mean state value, Z score>+1), expression in W1 Low (Z-score status in W4 + W12/16<−1), independent of GF / CONV-R. Community 2, high W12 CONV-R expression but low W12 GF expression, In W1 and Regular in W4 CONV-R, low expression in W4 GF, low expression in W12 CONV-R, low expression in W12 GF, low expression in W1 + W4. Section 4, high W12 CONV-R expression but low W12 GF expression and low W1 + W4 expression. Community 5, High W12 GF expression, Low W12 CONV-R expression, Moderate W4 GF expression, Group 6 expressed high in W12 GF but low in W12 CONV-R, moderate in W4 GF, low in W4 CONV-R and regular in W1 GF + CONV-R.

1.5 Alignment and data processing for bisulfite sequencing

As BS-seq converts unmethylated cytosine (C) to thymine (T), the next steps in the study concentrate on estimating the number of conversions between C and T, and quantifying the percentage of methylation per base. For each cytosine in the genome, simply evaluate the conversion of C to T in the associated reads and then divide the C number by the sum of Ts and C. Whether it can be quantified reliably depends on quality control of prealignment, method of alignment, and quality control of postalignment. Because the quality of the basic calls is not constant and can vary between

sequences and within the same reading, it is important to check the quality of the base (which represents the confidence in the base call). As TC conversions, incorrect bases may be calculated incorrectly, and such errors should be avoided where possible. This simple quality control can be carried out using the programme for easy quality control (http:/www.bioinformatics.babraham.ac.uk / projects / fastqc/). Additionally adapters can be sequenced from time to time. If the adapters can not be correctly removed, they can either decrease the match rate or trigger an incorrect C-T conversion. We suggest that bases of low quality be cut at the end of the series and that adapters be removed to reduce C-T conversion errors and increase comparison efficiency.

1.6 CURRENT UNDERSTANDING OF THE GUT MICROBIOTA

Several large-scale studies, such as the Human Microbiome Project, have examined the microbiomes of different parts of the body, including the skin, as well as the oral cavity, vagina, and nasal cavity over the last decade. 2 Gastrointestinal tract is relatively easy to acquire and therefore remains a challenging area requiring sampling and definition. Most research currently concentrate on the intestinal flora, since the highest density and number of bacteria is found here. Most of the data comes from stool samples, although there are fewer mucosal biopsy samples. While obtaining a new stool sample is relatively straightforward, the information obtained from the stool sample does not reflect the entire bowel state. We know from some limited studies that the concentration and composition of bacteria are very different in the small intestine and their complex changes are greater than in the colon. 3 The colon is primarily due to the efficient breakdown of complex, indigestible carbohydrates, but the shape of the small intestine of the small intestine is determined by its ability to rapidly absorb and transform small carbohydrates and its ability to rapidly adapt to total nutrients. Although stool is not an optimal replacement for gastrointestinal tract, it offers a snapshot of diversity throughout the broad intestine. Most of the data also comes from research in North America and Europe, though research is uncommon in Asia , Africa or South America. And we have some biases against intestinal flora.

II. TRADITIONAL METHODS

DNA methylation plays a very important role in tissue and disease growth. The difficulty of the DNA methylation study, however, makes further research into the mechanisms of DNA methylation difficult in some diseases. Here we discuss some of the problems and difficulties in aligning the bisulfite series. Second, uncomplete conversion of acid sulfite may cause misalignment when re-annealed during the process of sulfite conversion. In addition, the alignment of bisulfite sequences is made more complex by sequencing errors, transformed C-to-T conversions and reference genome conversion. There are particular problems related to the alignment of BS-Seq reads as opposed to the alignment of the standard genome tags. [1]

The central nervous system (CNS) development is controlled by both internal and peripheral signals. Previous studies have shown that environmental influences, under physiological and pathological conditions, can influence neuronal activity. Amid the anatomical distinction, emerging research suggests that the gut microbe (the different microorganisms living in the human stomach) and brain interact in two ways. The interaction of the intestinal flora with the brain may have a significant effect on the underlying process of neurogenesis, central nervous system neurodegenerative diseases, and tumours. In this study , we examine the biological connexions between the gut-brain axis, and further analyse how neurological disorders control this connexion. Furthermore, we concentrate on new insights into improvements in the composition of the intestinal flora which can become a successful treatment for central nervous system diseases. [2]

Many data repositories and tools that are publicly accessible have been developed to support protein knowledge processing, data-driven hypotheses and biological exploration. To help researchers find the right protein-related IT tools quickly, in this chapter, we have provided a complete overview (including classification and description) of the basic bioinformatic protein databases. We will also address the challenges and opportunities of creating bioinformatic protein sources and databases of the next decade in order to facilitate data integration and analysis during the age of big data. [3]
Interaction between epigenetic processes and intestinal flora may play a significant role in the development of the intestines and homeostasis. Previous studies showed that the microbiota controls most of the transcription of the intestinal epithelium in the adult host, but the impact of microbes on DNA methylation and gene expression is not well known during early postpartum growth. Here we are attempting to investigate the impact of microorganisms during postnatal development on DNA methylation and gut epithelial cell transcription (IEC). [4]

The advent of sequencing technology has allowed us to research the microbes that make up human symbiosis and in the past these germs were not cultivated. Most of the early studies focused on the bacterial flora that makes up the gut microbe, so samples are readily available in both health and

disease. But little is known about "silent organisms" (fungi species, sometimes called flora). By living with bacteria in a symbiotic relationship we can sense that the germ has an adverse impact on the germ. Here we review the latest information acquired from the study of microbial and fungal interactions, these interactions include health and disease resistance, immunity and antimicrobial therapy impact. [5]
DNA methylation is one of the most significant epigenetic alterations to the eukaryotic genome. It has been shown to play a role in cell-type - specific gene expression regulation and thus also play a role in cell-type identity. The sulphite sequence is the gold standard for calculating target genomic methylation. Here we consider different techniques for sequence analysis of high-throughput bisulfite. Before and after comparison, we implement special short reading comparison technology and quality management methods to ensure accuracy of the results. We are also considering the next steps of the post-alignment review. We present various differential methylation methods and use actual and simulated bisulfite sequencing data sets to compare their results. We also address the methods of subdividing methyl to classify regulatory regions. We have presented annotation methods that can be used to subdivide and subdivide the regions returned by the methylation method for further classification. Finally, we review software packages that implemented strategies to efficiently process large sets of locally forked sequence data and discuss a web-based analysis workflow that requires no programming skills. The analysis strategies described in this review will guide investigators to understand best practices for sulfite sequencing at all levels. [6]

The intestinal nervous system (ENS) is a distinct branch of the autonomic nervous system. It is located along the entire gastrointestinal (GI) tract length and is a collection of interconnected networks of ganglions. Since the intestinal flora is close to the ENS, it will come as no surprise that their production and function is influenced by the intestinal flora. Such interactions, however, are complex and can be either overt or indirect. They usually include signal transduction triggered by host-derived microbe-derived components, metabolites, or intermediates, which in turn affect bowel nerve stimulation and gastrointestinal tract. Role Dao. Different micro-organisms and strains may have different effects on ENS function and neurochemistry. In this analysis, we will briefly summarise the role of the microbiome in the development of ENS, and further discuss the mechanism by which microbiomes can influence the activity and function of ENS. [7]

Bioinformatics is a modern science which has arisen in recent years. It is an interdisciplinary discipline, composed of biology, computer science , chemistry, statistics, mathematics and other research areas. It is a great challenge for researchers to identify this emerging area in a systematic and scientific fashion and to draw attention to its applications and services. One of these essential resources to which bioinformatics can be applied is cancer diagnosis, diagnosis and care for many beneficial reasons. This essay would provide a detailed description of bioinformatics, its concepts, goals, implementations, technology, the large amount of data generated in the biological field and how to arrange, interpret and store bioinformatics and address some of them. This can be done by a bioinformatics algorithm. Data and how to use bioinformatics to the detection and diagnosis of diseases such as cancer.[8]

Drosophila's gut (Drosophila) is used as a model for the study of host microorganisms interaction. There are some parallels between vertebrate intestines and fruit flies in the developmental, physiological, functional, and immune regions. These similarities encourage its effectiveness as a model framework for researching different bowel diseases. Wild-type flies found in nature have their own natural microbial colonies in the intestines and wild-type flies reared under closed laboratory conditions. This natural intestinal flora can communicate with fruit flies by oral germs. Several laboratories are growing sterile flies to solve the problem. Bacteria-free flies do not present any form of microorganisms with intestinal flora or colonisation. These non-poisonous flies can serve as a powerful model for exploring the role of specific microbial strains or the specific separation and interaction of a specific microbial genus in one or more binding states with the hostthereby exposing their complicated relationship with each other. The present work describes a method for studies of microbial interaction in the development of sterile flies. [9]

Emerging genome-wide hairpin sequencing technology (hairpin-BS-Seq) can simultaneously establish double-stranded DNA methylation patterns. Hairpin-BSSeq can establish methylation fidelity and increase mapping efficiency compared to conventional bisulfite sequencing (BS-Seq) technology. There is, however, no calculation method designed to calculate the BS-Seq hairpin results. Here we present the HBS package, which is a collection of command-line tools for preprocessing, mapping, methylation calls and BS-Seq genome hairpin summary results. Accepts paired hairpin-BS-Seq reads for universal alignment to restore the original sequence (before bisulfite conversion) and then find the original sequence for both DNAs in the reference genome. The chain demands a state of cytosine methylation. After applying to the hairpin-BS-Seq dataset we find that the HBS tool has less mapping time and better mapping efficiency compared with the new mapping tools. You can download

source scripts, user guides and trial data for free from HBS tool. [10]

Comprehension of the structure and function of the human intestinal flora has increased exponentially in the last 10-15 years. This is primarily because the modern "omic" technology has allowed a broad study of the microbial community's genetic and metabolic characteristics; In this way it shows its comparability with other body organs and offers a new opportunity for therapeutic intervention. It may also be more fitting to think of it as the immune system: a group of cells that work with the host to promote health, but also cause illness. This review provides the latest bowel disease information, especially those related to metabolic syndrome and obesity, liver disease, IBD, and colon cancer. By analysing the current and most important data relating to antibiotics, probiotics, prebiotics, polyphenols and microbial stool transplantation, the probability of controlling the intestinal microbe in these diseases has been assessed. [11]
Changes in intestinal flora observed in chronic gastrointestinal disorders have led to a growing interest in the role of intestinal bacteria in maintaining intestinal barrier function. The impact of common bacteria on intestinal barrier function and motor secretory function is still poorly understood, while acute changes in motor-motor secretory function in response to pathogens have been well defined. Mice without embryos represent a typical model for studying the impact of gut microbes on the host 's gastrointestinal physiology. Investigations by Lomasney et al. As long as secretory motor control in sterile mice is generally retained, it represents a significant step in this direction, making the model ideal for studying the impact of gut flora on host control. [12]

An significant aspect of working with sterile mice is ensuring the mice are bacteria-free. The easiest way to check infertility is to incubate a stool sample under aerobic and anaerobic conditions or under a light microscope to perform a stool smear test and perform a Gram stain and assessment. Both methods, however, have limitations: most bacteria can not be produced outside of the intestine, and little smear contamination can not be detected. Molecular techniques such as the amplification of 16S rRNA genes by PCR are also a reasonable choice (see Alternatives). [13]

Background: Sulfite sequencing is a common method of analysing the DNA methylation patterns in high resolution. Targeting the target region by PCR usually scans around 20-50 subcloned molecules of DNA for methylation status and molecular analysis of a single CpG site. Reviews: BISMA (Bisma Sequence DNA Methylation Analysis) software used to analyse large sulfite sequencing data performs data sequence extraction and enhances data collection, quality control, methylation status analysis and representation. It uses an advanced technique to identify cloned molecules and accurate CpG localization, and supports the first sequence analysis of repeats. [14]

Vertebrates are sterile in nature, But normally complex intestinal flora is acquired immediately after birth. Most of these species are not pathogenic for highly resistant hosts. Some are, in fact, helpful. They will provide vitamins for the host 's diet and fill the microbial site available to restrict the entry route and subsequent pathological conditions while interacting with pathogens. Hence mammalian health depends on the relationship between the host and the plant. This is apparent in inflammatory conditions such as inflammatory bowel disease, where abnormal microbiome responses can lead to pathology in the host. Studies in asexual (free of pathogenic bacteria) or intentionally colonised animals have shown that symbiotic species are necessary to establish a fully functioning immune system and affect several normal processes within the host. Here we explain the technical criteria for feeding and preservation of raw and axial animals, and illustrate the extreme complexity of changes in and out of the immune system when sterile bacteria settle on symbiotic bacteria. © The Elsevier Ltd. 2006. Reserved all rights.[15]
Rice (Oryza sativa) is a standard species of monocots, especially of the grass family. The small size of the genome, the diploid nature, the ability for change and the creation of genetic and molecular tools make it an observable organism for plant biologists. The estimated genome size is 430 Mb (Arumuganathan and Earle, 1991), and the complete sequence of the rice genome can be collected using current technologies. An international initiative and a sequence of rice (O. sativa spp) has been identified. Japanica var "Nipponbare" uses an artificial chromosome sequencing technique for bacterial artificial chromosome / P1. Rice's Genome Commentary uses mathematical and confessional measures to identify genes. Annotation approaches are being developed to increase the precision of annotations, such as targeted gene prediction programmes for rice. Resources for the use of rice genome sequences for part of the genome project are also being created, such as the expression project of the sequence being expressed, in order to improve the productivity of the rice genome project. [16]

III. METHODOLOGY

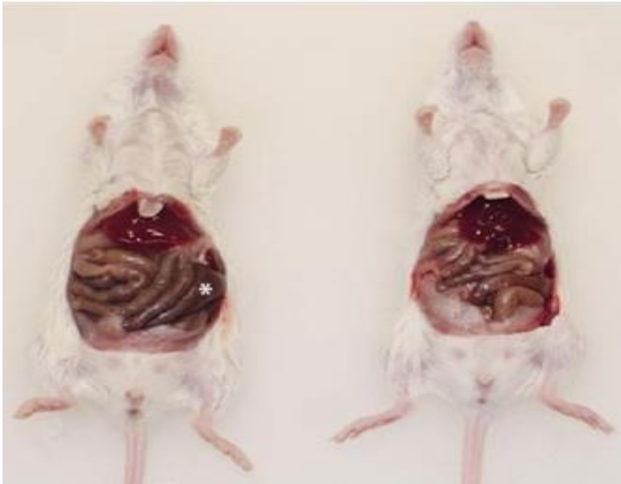

Figure 1: Photos of 12-week-old germ-free (left) and conventionally reared (right) Swiss Webster females. * Denotes the blind

Maintaining Germ Gree Mice Practical Considerations For Housing And Equipment Sterility

This must be raised under sterile conditions to keep mouse colonies sterile. They were originally made of stainless steel, which made them very heavy, costly, and rigid. Highly common today are versatile membrane insulators, and complete systems can be purchased from Class Biological Clean or Standard Protection. We agreed to purchase Class Biological Clean replacement parts and produce them ourselves (Figure 2A). Both food , water, and equipment need sanitization. We find that the safest approach is autoclaved steel cylinders (eg stainless steel cylinders, Classic Biological Clean) (Figure 2B). However, for some diets which contain special supplements and heat-sensitive medicines, radiation may be more suitable. Medicines susceptible to both treatments should be poured into a sterile test tube, sprayed with Clidox, and then injected into the isolator.

To optimise functions we build insulators and the Biological Clean Class. The company also offers a full solution, however, which may be more fitting for new users. In general, we give three types of isolators:

(1) Wide insulators with racking systems capable of accommodating 18 cages,
(2) Minor 24 in. Experimental three-cage insulators and a re-bypass isolator (see Re-bypass section below). In addition to food, water and equipment being imported, the mice are treated as in traditional facilities.

Figure 2 : (A) Isolator for housing germ-free mice. (B) Sterilizing cylinder.

NOTE: As each opening in the insulator increases, it is important to prepare your work thoroughly
A Sterility Hazard.
NOTE: Check the insulator regularly to find any holes. Pay particular attention to these
Wear dust-accumulation gloves and philtres.
NOTE: Hold all mouse strains in at least two insulators to allow one strain to expand Following potential interference with the insulation.
NOTE: Separate rearing insulators and experimental isolators, as the introduction of new equipment or diets is the greatest danger to sterility. Never allow something in the brood isolator other than autoclaved supplies and avoid spreading mice among the brood insulators.
Materials

1. Autoclavable Breeder Mouse Diet (Labdiets, cat. No. 5021)
2. Clidox (Agent for Sterilization)
3. Small trolley, or similar
4. Autoclave Accessories
5. Mylar tape (Bio-Clean class)
6. Blowers
7. Including Isolator
8. Clean Biological Animal. 2312180) No.
9. 1-in Stopers. Napkins
10. Isolator port
11. Tiny autoclavable sacks of paper
12. Shelving systems
13. Large tweezers
14. 12-in × 24-in. Sterilizing drum (Class Biological Clean; see Fig. 2B)
15. Sterilization darts (Steris)
16. 12-in. × 12-in. × 18-in. long transfer sleeve with two 1-in. nipples (Class
17. 3 M Scotch Brand Yellow Vinyl Tape
18. Mylar film
19. Bedding

20. valve water

ASSESSING GERM-FREE STATUS BY FECAL BACTERIA ANALYSIS

An significant part of working with sterile mice is the evidence that the mice are free of bacteria. The easiest way to check infertility is to incubate a stool sample under aerobic and anaerobic conditions or under a light microscope to perform a stool smear test and perform a Gram stain and assessment. Both methods, however, have limitations: most bacteria can not be produced outside of the intestine, and little smear contamination can not be detected. Molecular techniques such as the amplification of 16S rRNA genes by PCR are also a reasonable choice (see Alternatives).

NOTE: It is recommended to use multiple methods to assess the absence of bacteria, and should be cultured every two weeks, and PCR should be performed at least once a month.

Materials

A. Microcentrifuge tubes
B. Anaerobic jar
C. Fecal pellets
D. Anaerocult A system (Merck)
E. Brain Heart broth *or* Sabouraud broth (for culture of possible fungi contaminations; e.g., Sigma) *or* nutrient broth (e.g., Sigma)
F. 37°C incubator

Sample collection and processing

1) Collect three to four fecal pellets from six randomly selected cages per isolator into an autoclaved microcentrifuge tube.
2) Transfer two to three granules to two 14 ml culture tubes filled with 3 ml Brain Heart Infusion (BHI), Sabouraud, or nutrient broths.
3) Sample culture and analysis
4) Place one set of tubes in an anaerobic flask
5) Activate the Anaerocult A system with water and place it into the anaerobic jar without delay.
6) Incubate the anaerobic jar and the second set of tubes (aerobic) at 37 ° C for 48 h. Check for bacterial growth.

NOTE: For aerobic and anaerobic environments, remember to add negative and positive controls (stool from colonised mice). Usage of the anaerobic bacteria test to validate the anaerobic condition is also recommended.

ASSESSING GERM-FREE STATUS BY MOLECULAR METHODS

The technology is based on the following principle: bacterial genes 16S rRNA contain preserved regions contained in most bacterial organisms. An successful way of demonstrating infertility is to activate primers in these areas and amplify DNA isolated from stools.

Materials

Lysis buffer SL2
Ice
Nucleospin Soil Bead tube
Fecal pellets
Elution buffer SE
Precipitation buffer SL3
Binding buffer SB
NucleoSpin Inhibitor Removal Column (red ring)
2-ml collection tubes
NucleoSpin Soil Column (green ring)
Washing buffer SW1
Washing buffer SW2
Enhancer solution SX
NucleoSpin Soil kit for genomic DNA isolation (MACHEREY-NAGEL) including:
Accuprime DNA *Taq* polymerase (Invitrogen)
Buffer I (provided with *Taq* polymerase)
4°C incubator
Autoclaved microcentrifuge tubes
Reverse primer (338R; TGCTGCCTCCCGTAGGAGT)
Vortex mixer
Forward primer (8F; AGAGTTTGATCCTGGCTCAG)

−20°C freezer
FastPrep:24 beadbeater (MP Biomedicals)
1% agarose gel
Centrifuge
Molecular biology-grade water
PCR machine

REDERIVATION OF MICE

Historically, the method of choice for restoring new strains of germ-free mice was hysterectomy. The uterus is squeezed, separated, sterilised and transferred to a sterile isolator along with the supported stepmother shortly before the planned delivery.

NOTE: The agreement includes sterile mice to be able to act as adoptive mothers or as recipients of embryo transfer. Mice

can be bought from Taconic (Swiss Webster) or Charles River (C3H), without embryos.

NOTE: This procedure assumes you are familiar with basic surgery on small animals. Doctors should ensure that the surgery can be carried out only by trained and accredited staff to avoid damage and animal waste. The surgery specifics needed are not listed here, as they do not fall within the scope of this paper. See also Fossum (2012).

Materials

Tweezers
Sharp scissors
Radiation protection isolator with small storage tank (Classic Biological Clean)
Non-toxic mice [Swiss Webster from Taconic or Charles River (C3H)] autoclaved water
Sterilization solution (1% chlorine)
Cotton swabs (Q-tips)

1) Set up the Ties. The next morning search for the connectors.
2) Mount a by-pass insulator (dH2O cylinder head, scissors, tweezers, swabs)
3) Prepare a 1% chlorine solution in the E19 gestation and soak it in the insulator (place it in the port and fog through the nipple).
4) Fill the room temperature chlorine in the dip tank and remove the outer cap from the dip tank.
5) Attach the re-bypass insulator to the germ-free female isolator that has just given birth (less than three days ago).
6) Hysterectomy in a typical female at gestation E19.5 and pass through the uterus for 30 to 45 seconds in the chlorine.
7) put in the uterus into the isolator.

Critical Parameters

Extreme care must be taken at any stage when using neutered animals, as one error may have disastrous effects and contaminate the isolator or, in the worst case, all the equipment. To prevent unsterilized consumables, make sure the autoclave is balanced and never put non-autoclaved consumables into the set isolator. Do not position two reproductive insulators to prevent pollutants from spreading between isolates. Regularly inspect the insulating system for damage and infection. Make sure you have access to several devices, until you decide to build your own computer.

AUTHORS REVIEW

The author had written on several posts. He found a system for generating and testing sterile mice using functional equipment and house sterilisation, isolators for isolating sterile mice, sterile tubes, faecal bacterial analysis, and sterile molecular techniques. Design and build approaches which are hybrid.

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