

The McGill Centre for Microbiome Research Year 2 Narrative Report

Reporting Period: April 1st, 2020 – March 31st, 2021

Lead Investigator: Dr. Irah King and Dr. Ken Dewar

The two MI4 supported platforms are now coordinated within the McGill Centre for Microbiome Research. Specific milestones and deliverables for each platform are marked as [Gnoto] or [Genome]. Progress towards Annual Milestones and Deliverables of both platforms:

	Annual Milestones and Deliverables (from proposal)	Description of Progress
1	[Gnoto][Genome] 0.25 FTE Platform Coordinator for platform-based studies will be identified	Ms. Cynthia Faubert, with expertise in germ-free and gnotobiotic animal facility management, experimental design support and animal care, was hired on Sept. 1, 2020, as a 1.0 FTE platform manager and Microbiome Centre Program Manager. Cynthia is central to the success of the Centre and our platform.
2	[Gnoto] Training of Animal Health Technician for equipment sterilization and maintenance and handling/breeding of axenic mice will be completed	In coordination with the Animal Resources Division at the MUHC-RI, Ms. Faubert installed and initiated use of the gnotobiotic animal research platform. She is also performing all related procedures and techniques of the platform.
3	[Gnoto] Establish axenic breeding colonies in the platform	This deliverable has been delayed, but axenic mice have been ordered for this purpose and breeding will begin this month (July).
4	[Gnoto] Research milestone: Successfully house mice using isocage system under germ-free conditions for two months without detectable microbial contamination	We are currently housing germ-free mice that have maintained sterile status for 6 weeks, and a weekly testing protocol in place. Testing of gnotobiotic animals with a minimal microbiota (< 10 bacterial species) is planned for August 2021.
5	[Gnoto][Genome] Institute a Quality Control Program and related SOPs.	With close collaboration from both platforms, we have developed an efficient and cost-effective in-house microbial testing program for axenic mice.
6	[Genome] Implement robust methods for the purification of low and high molecular weight DNA.	We have benchmarked a series of commonly used DNA extraction kits to assess extraction efficiency, reproducibility and biodiversity representation. We have now optimized protocols for low-throughput studies (24 samples or less) and begun testing automated 96-well plate protocols for high-throughput studies. High molecular weight DNA extraction protocol development is ongoing,

		with preliminary attempts achieving >20 kb suitable for ONT sequencing.
7	[Genome] Expand and publicize bacterial strain availability (local biobank) for microbiology applications.	The development of our anaerobic culturing facility has allowed us to expand our collection of bacterial strains. Currently, we have cultured and banked 112 fecal or skin bacteria isolates from human, canine, and murine origins. We currently house these strains in the Cousineau lab as a temporary measure. Longer term, we are in discussion with the AMR group to synergize our biobanking efforts.
8	[Genome] Perform benchmark study comparing 16S-PCR (two-steps), optimize 16S-PCR (one-step) and full genome sequencing for the genomics/bioinformatics applications.	<p>16S-PCR amplicon sequencing:</p> <p>Working with the Genome Centre, primers have been designed and stocked, and benchmarking experiments have been performed and analyzed. Encouragingly, both PCR strategies are highly reproducible, allowing the Genome Centre to offer both strategies as services. Cost models have been put in place, and the service is now being used by clients.</p> <p>Bacterial Genome sequencing:</p> <p>Laboratory protocols for DNA extractions of small batches are in now place in the Cousineau lab, and protocols for large batch DNA extractions are progressing at the Genome Centre. While we optimize for the recovery of microbial diversity in microbiomes, these protocols also can serve for pure culture bacterial strains. In order to improve bacterial genome sequencing (which we also expect to become a valuable resource for the antimicrobial resistance research community), we have tested and now implemented a cost reduction protocol at the Genome Centre. Whereas a single bacterial genome previously cost ~\$120 (sample processing, library making, sequencing), we can now offer it at ~\$50/genome when processed in batches of 48 or more.</p>
9	[Genome] Complete Genome Reconstruction for bioinformatics applications.	The development of the automated pipeline has been paused due to redistributions of bioinformaticians to other platform (web portal) and Genome Centre (Covid) priorities. We continue to offer it ad hoc as projects arise and a publication using the pipeline is expected to be submitted in July 2021 (Paul H. Roy et al., CHUL).



10	[Genome][Gnoto] Web Portal	The inception of the McGill Centre for Microbiome Research and the AMR Centre has led us to step back from our original plans and develop a larger solution that will meet the needs of all the groups. The portal will now be anchored in the McGill Centre for Microbiome Research and thus be tied to its social media presence and community communications. The branches of the portal more specific to the [Gnoto] and [Genome] platforms will be integrated within the larger portal and will concentrate on protocols, results, and pricing.
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Plans and updated Milestones for the next reporting period: (July 1st, 2021 – June 30, 2022)

- [Gnoto] Determine per diem rates for axenic CL1 animal studies
- [Gnoto] Establish an online database for storage of information related to technical services rendered, reagent inventory and maintenance of platform operations
- [Gnoto][Genome] Establish a scientific advisory committee that reviews platform operations (e.g. space efficiency, SOPs, budget, etc.) on an annual basis
- [Gnoto] Expand QC Program for performing CL2 studies
- [Gnoto] Research milestone: Successfully maintain a line of mice monocolonized with a defined bacterial species for 3 months
- [Genome] Implement cost models for microbiology fee-for-service activities (DNA extractions, direct colony PCR, bacterial load estimation, gnotobiotic mouse sterility testing)
- [Genome] Benchmark studies of sample collection and storage conditions.
- [Genome] Full microbiome sequencing cost reduction.
- [Genome] Automated processing of 16S sequencing data into ASV tables (amplicon sequence variants and their counts).
- [Genome][Gnoto] Web portal development and implementation.

List of Personnel involved in the Centre and Description of Roles:

- Dr. Irah King: director of the McGill Centre for Microbiome Research, director of the Gnotobiotic Animal Research Platform
- Dr. Corinne Maurice: assistant director of the McGill Centre for Microbiome Research
- Dr. Ken Dewar: director of the Microbial Genomics platform
- Dr. Benoît Cousineau: associate director of the Microbial Genomics platform
- Dr. Jesse Shapiro: associate director of the Microbial Genomics platform
- Ms. Caroline Monat: microbiology lab technician of the Microbial Genomics platform
- Dr. Emmanuel Gonzalez, Romain Gregoire, Francoise Lefebvre, Xiaomeng Jiang: bioinformatics and web site development
- Mr. Antoine Paccard: project manager for the Microbial Genomics platform
- Pierre Lepage, Patrick Willet, and Janick St-Cyr: microbial genomics protocol development and cost reductions
- Ms. Cynthia Faubert: program manager of the McGill Centre for Microbiome Research

Challenges and Lessons Learned:

- [Gnoto] In addition to dealing with the extended COVID-related delays, it has been a significant challenge to install and order equipment repairs for the gnotobiotic platform. While all involved parties have done their best to be responsive and helpful, the RI chain of communication with commercial vendors is difficult to navigate. As such, we have learned that we must lead the effort in transparent communication to not only ensure the success of our research platform but also synergize with other RI platforms to help increase their success!
- [Gnoto] We have also become acutely aware that the specialized equipment/technology used for the Gnotobiotic Animal Research Platform makes us vulnerable to delays and study experimental failure. To overcome this obstacle, we will develop contingency plans and pursue backup equipment when possible.
- [Gnoto][Genome] The web portal underwent a conceptual reorganization, but we feel it will now better explain and coordinate the key parts of our collaborative group. This is key to allowing us to minimize duplicated efforts and maximize the fullest extent of research support to our community.