BIOF 520: Culture-independent pathogen detection for public health and patient care

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CLINICIAN WORKFLOW

The clinician will collect the biological specimen from the patient, depending on what symptoms are being exhibited (1). For example, if the patient is exhibiting gastrointestinal symptoms, a gut or fecal sample may be taken, whereas with respiratory symptoms, sputum or buccal sample may be taken. Along with the sample, metadata about the patient (e.g. age, sex, location, etc.) is also recorded for public health purposes.

LABORATORY WORKFLOW

With each different type of biological specimen, the appropriate corresponding sample preparation and processing protocol will be used. Following standard library construction protocols, a syndromic PCR panel (2) will be conducted to test for common pathogens such as *E. coli,* salmonella, etc. If the test results are positive, then the pathogen has been identified, and the workflow can proceed to public health surveillance. If the results are negative, metagenomic sequencing will be completed. To enrich prokaryotic DNA, the protocol Depletion of Abundant Sequences by Hybridization (DASH) (3) will be used to deplete the eukaryotic human DNA from the microbiome and pathogen DNA. In this protocol, human DNA is targeted for cleavage through the use of the recombinant Cas9 protein with a library of guide RNAs, where the cleaved DNA sequences no longer have the required adapters, consequently not amplified and sequenced. DASH is an ideal method as it can be adapted for any sample type while increasing yield without additional cost (4).

BIOINFORMATICS CLINICAL CARE WORKFLOW

For the clinical care workflow (rapid turn-around time of 1 day), long read sequencing with the MinION Mk1B sequencer, chosen for its price ($1000), speed, ease of use, and portability, will be used. To ensure assembly of only pathogens and not microbiome, the long reads will be filtered using BioBloomTools (BBT) (5), a state-of-the-art tool used to filter out contaminants from sequencing data. The bloom filters (BF), a fast, accurate and memory-efficient probabilistic data structure, will be constructed from sequences obtained by the NIH Human Microbiome Project (6). The reads that match those in the BF will be *filtered out*. If time-sensitive, a multi-index BF can be used instead (7). In this case, the majority of typical microbiome sequences will be filtered out, but some may remain so a metagenomic-capable assembler should be employed. The remaining long reads will be assembled using Flye (8) if time-sensitive, otherwise using Canu (9), based on the results of a comparative study of long read metagenomic assemblers (10).

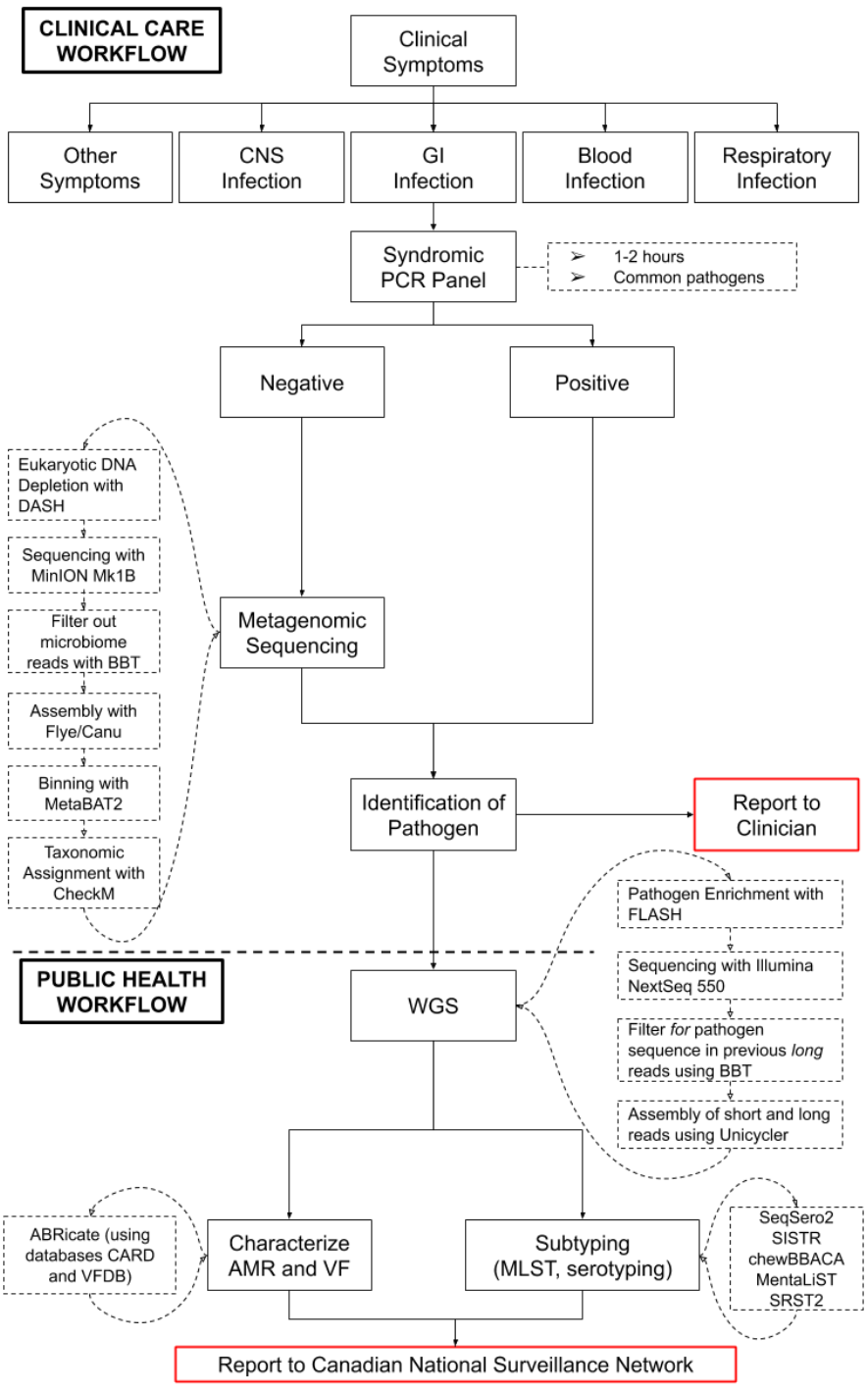
First, the reads will be aligned to the assemblies using BWA (11) as abundance input for binning. Using MetaBAT2 (12), chosen for its adaptive binning algorithm thereby bypassing any parameter fine-tuning, the resulting contigs will be binned based on tetranucleotide frequencies and abundance scores. Then, CheckM (13), chosen for its binning and quality control capabilities, will then be used to bin based on taxonomy and operational taxonomic unit (OTU), while assessing the contamination and completeness of each assembled metagenome. Abundance can be quantified with RPKM by aligning the reads to each bin using BWA. Optionally, for better results, only assemblies with high completeness and low contamination can be used. At this point, since human DNA and microbiome DNA had been filtered out before assembly, the pathogen will be identified. This sequence can be queried against our pathogen database to identify closely related sequences. This ends the workflow for clinical care, and the identified pathogen can be reported to the clinician to facilitate patient treatment. This data can be uploaded to the Canadian Genomics Cloud (which in turn, is integrated with electronic health records).

BIOINFORMATICS PUBLIC HEALTH WORKFLOW

For the public health workflow (rapid turn-around of 3 days), whole genome sequencing (WGS) will be performed. Since the pathogen (and its sequence) has been identified at this point (for designing guide RNAs), the enrichment protocol Finding Low Abundance Sequences by Hybridization (FLASH) (14) can be used to enrich pathogen DNA after DNA extraction. Since FLASH only works for genomic DNA and cDNA, a reverse transcription step must be added beforehand if the pathogen is an RNA virus. FLASH works very similarly to DASH but before library prep,

where the Cas9 targets are cleaved and available for adapter ligation and sequencing. FLASH is an optimal method for pathogen DNA enrichment as it requires low hands-on time and has a low cost per sample (4). In this case, sequencing will be carried out on the Illumina NextSeq 550, a $400,000 flexible benchtop sequencer with a run-time of 12-30 hours, resulting in paired-end 150 base-pair short reads. Next, the raw long reads from the metagenomic sequencing will be filtered with BBT, using a BF with the identified pathogen sequence. Contrary to before, the reads that match those in the BF will be *filtered for* and kept for assembly. Then, a hybrid assembly, combining the high accuracy of short reads with the long range information of long reads, will be executed. The short and long reads will be assembled by Unicycler (15), a hybrid assembler designed for small (bacterial, viral, and organellar) genomes. Finally, the assembled pathogen sequence will be deposited to our existing pathogen database, expanding it further.

The resulting assembly will be screened for antimicrobial resistance genes and virulence factors using ABRicate (https://github.com/tseemann/abricate) and the following databases: the Comprehensive Antimicrobial Resistance Database (CARD) (16) and Virulence Factors Database (VFDB) (17). Next, *in silico* subtyping will be conducted to identify the isolate. If the identified pathogen is salmonella, serotyping can be conducted with SeqSero2 (18) and SISTR (19) to identify surface antigens. Multi-locus Sequence Typing (MLST) for strain identification can be achieved with chewBBACA (20) using the genome assembly, and with MentaLiST (21) and SRST2 (22) using short reads. The combined subtyping, serotyping, MLST profiles can be compiled into a report for the Canadian national surveillance networks. Using the clinical individual data from the Canadian Genomics Cloud and these MLST profiles, health authorities can conduct transmission tracking and outbreak detection using generated transmission maps. This entire workflow is summarized in **Figure 1**.



2019-nCoV

Overall, this workflow should work relatively well for the 2019 novel coronavirus as it is an RNA virus. This is accounted for in the workflow by adding a reverse transcription step before library prep for WGS since most of the protocols selected only work on genomic DNA and cDNA sequences.

Figure 1. The complete workflow for culture-independent pathogen detection for public health and patient care. *Adapted from Jimmy Liu.*

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