Elissa Ony
Research Assistant - Summer 2023

Methodology:

Genome Sequencing:

Pure NB4-1Y culture stocks were maintained on Microbank beads (Pro-Lab Diagnostics, Inc., Richmond Hill, ON, Canada) and stored at - 80 °C. A single bead was streaked onto a nutrient agar plate and incubated at 30 °C until isolated colonies grew. Single isolated colonies from the nutrient agar plate were used to prepare inoculum cultures in autoclaved 50 mL glass tubes containing 10 mL yeast extract-malt extract medium (YEME). A sterile control was also prepared. Starter cultures were incubated in a Barnstead MaxQ 4000 Orbital Incubator Shaker set to 150 rpm at 30 °C for 96 hrs. **Cell pellets were harvested by removing 10 mL aliquots of inoculum and centrifuging at > 8000 xg for 10 min. Cell pellets were large, so they were split into three separate aliquots after resuspending them and before DNA extraction.**

To determine the most effective DNA extraction method for NB4-1Y, mechanical, chemical, and enzymatic methods were investigated. Four DNA extraction kits were tested that varied by extraction method. Two enzymatic DNA extraction kits were tested, EZNA Bacterial DNA kit and the Monarch HMW DNA Extraction Kit. For the Monarch kit both the High Molecular Weight DNA Extraction from Bacteria protocol by itself and with a bead beating step incorporated were performed. Invitrogen TRIzol Reagent Protocol for cells grown in suspension was followed as a potential chemical extraction method. The DNeasy Powersoil Pro Kit used bead beating as a physical extraction method. Various bead beating times (0.25 min, 0.5 min, 0.75 min, 1 min, 5 min, and 10 min) were tested to determine how long to bead beat to obtain the highest DNA concentration with the least amount of DNA shearing.

All DNA samples were quantified using Qubit Fluorometer high sensitivity and broad range kits. All samples were then amplified using end point PCR with 27F and 1492R 16S primers and visualized using gel electrophoresis. Sanger Sequencing was performed to confirm the extracted DNA samples were in fact NB4-1Y and not contaminated. PCR amplification and cycle sequencing were performed following the Big Dye Direct Cycle Sequencing Kit Protocol. For JVH1, did end point PCR with 27F and 1492R and then followed BigDye Terminator V 1.1 Kit protocol instead of BigDye Direct Cycle Sequencing Kit. Purification of the sequencing products

was completed with ethanol/EDTA precipitation outlined in the Big Dye Terminator v3.1 Cycle Sequencing Kit User Guide. Protocol was followed according to the starting volume of 10 mL. To prepare purified samples for Sanger sequencing samples were resuspended with 10 mL of Hi-Di Formamide and transferred to a MicroAmp™ Optical 96-Well Reaction Plate according to the SeqStudio Genetic Analyzer Instrument and Software User Guide.

To complete NB4-1Y's genome, long stranded high molecular weight DNA was sequenced using Nanopore MinION sequencing technology. DNA samples were extracted for Nanopore sequencing following **DNeasy Powersoil Pro Kit protocol with a bead beating time of 45 seconds** and quantified using Qubit Fluorometer Broad Range kit. Gel electrophoresis was performed using a 0.7 % agarose gel ran at 70 volts for 60 min to determine if the DNA extracted was high molecular weight DNA. Pippin prep was used for size selection of DNA to retain all strands between 6000-85000 base pairs long and discard any shorter pieces. Pippin Blue protocol was followed for the preparation and loading of a 0.75 % gel. Size selection for JVH1 was done following MagBio HighPrep protocol for getting rid of < 5kb. Used 3.1 x beads by mixing 20 mL of sample with 62 mL of 35 % beads made by mixing 24.5 uL beads with 45.5 uL of elution buffer. Eluted in 10 mL of elution buffer. **Native Barcoding Kit 24 V14 Protocol** was followed to prepare sample for Nanopore sequencing. For DNA repair and end-prep and native barcode ligation all mixing steps used P20 pipet tips cut into wide bore pipet tips with sanitized scissors to avoid damaging long stranded DNA. For adaptor ligation and clean up, long fragment buffer was used to enrich DNA fragments 3 kb or longer. When loading the spotON flow cell 14uL of DNA library was used instead of 12uL to get the most reads possible. Sequencing results were analyzed and the complete version of the NB4-1Y genome was published on the National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/nuccore/CP132196.1/).