**Methods**

RNA extraction and cDNA synthesis from Thao…

Total RNA was extracted with TRIzol reagent. The swab was transferred to a 2ml centrifuge tube containing 500 ul of Ramel MH broth and 1 mL RNAprotect bacterial reagent. The sample was incubated at room temperature for 5 minutes and centrifuged at maximum speed for 5 minutes to pellet the cells. The supernatant was discarded and 1ml TRIzol added to the cell pellet. The TRIzol mixture was transferred to 2ml lysing matrix tube (MP cat# 6911-050) and homogenized for 20 seconds with a homogenizer. 200 ul of Chloroform was added to the tube, and the homogenate was centrifuged at high speed for 10 minutes to separate into a clear aqueous upper layer containing RNA, an interphase, and a red lower organic layer containing DNA and proteins. RNA from the aqueous layer was precipitated with 500 ul of isopropanol and purified using Qiagen’s RNeasy protocol (QIAGEN cat #74104). Dnase treatment was then performed using Ambion’s Dnase treatment kit (Ambion cat# AM2238). RNA was converted to cDNA using the Life Technologies High-capacity cDNA reverse transcription kit ((Life Technologies cat# 4368814)).

From Jennifer Anderson…

Total cDNA samples were purified and concentrated using the QIAquick PCR Purification kit (QIAGEN, Valencia, CA). Libraries were prepared for sequencing using the KAPA HyperPlus Library Preparation Kit (KAPA Biosystems, Inc, Wilmington, MA) as described below. Depending on the quantity and quality of cDNA available for each sample, 1-50 ng of starting material was enzymatically fragmented for 2 min at 37 ºC. The ends of each fragment were enzymatically repaired, with 5’ phosphorylation and 3’ a-tailing. Illumina® (San Diego, CA) TruSeq universal adapters (IDT, Coralville, IA) were duplexed in solution by annealing together at a concentration of 3 µM and ligated to the end-repaired fragments. Ligation reaction products were cleaned up using Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN). Libraries were amplified using iTru5 and iTru7 primers (Glenn et al, 2016), to incorporate dual 8 bp indexes. PCR products were cleaned up using size selection beads as described above. The final cDNA libraries were sized using the TapeStation (Agilent Technologies, Santa Clara, CA) and quantitated by qPCR using a KAPA library quantitation kit (KAPA Biosystems). The libraries were pooled in equimolar ratios, diluted to 10 pM, and sequenced on a MiSeq instrument using the V3 150 cycle reagent kit (Illumina®). Fastq files from read 1 and 2 were assembled… add analysis from Harold

**Results**

A total of 16 cDNA samples were sequenced using 4 MiSeq V3 flow cells (Illumina®). Total sequencing reads per sample ranged from 3,867,530 – 7,552,309 (avg 5,316,860).

References

Glenn T. C., R. Nilsen, T. J. Kieran, J. w. Finger, T. W. Pierson, K. E. Bentley, S. Hoffberg, S. Louha, F. J. Garcia-De-Leon, M. A. del Rio Protilla, K. Reed, J. L. Anderson, J. K. Meece, S. Aggery, R. Rekaya, M. Alabady, M. Belanger, K. Winker, B. C. Faircloth. 2016. Adapterama I: Universal stubs and primers for thousands of dual-indexed Illumina libraries (iTru & iNext). bioRxiv doi: https://doi.org/10.1101/049114.