# DOWNLOAD PDF DISCOVERY OF BACTERIAL SRNAS BY HIGH-THROUGHPUT SEQUENCING JANE M. LIU AND ANDREW CAMILLI

## Chapter 1 : CiNii Books - High-throughput next generation sequencing : methods and applications

*sRNA-Seq is an unbiased method that allows for the discovery of small noncoding RNAs in bacterial transcriptomes through direct cloning and massively parallel sequencing by synthesis. Small bacterial transcripts are enriched from a total RNA preparation and modified with 5â€² and 3â€² linkers that.*

Single-molecule DNA sequencing provides minimal sequence bias across diverse genomic content. The local GC content and observed mean sequencing coverage were tabulated using a bp sliding window. Windows were then aggregated into GC-content bins ranging from 0 to 1 with a step size of 0. Distilled water Invitrogen, Carlsbad, CA. Heatblock equipped with block milled for 1. PolyA Tailing Reaction 1. Aluminum block milled for 0. Determining the Success of Tailing Reaction 1. Short Tail Correction 7 1. A bead-based size-selection step after the shearing step removes salts and small nucleic acids that would be tailed, but are not sufficiently long to yield meaningful sequence information. A quality control step ensures that samples are sheared to the appropriate size. Alternative approaches for detecting much longer variants using assembly-based methods are under development. The water should cover the visible parts of the microTube when it is in the microTube holder i. Turn on the S2 unit by depressing the red switch located at the upper right corner of the instrument. After the instrument is on, open the software. Place an unfilled Covaris microTube into the preparation station holder. Place the tip along the interior wall of the tube. Slowly discharge the fluid into the tube, moving the pipette tip up along the interior wall as the tube fills. Be careful not to introduce a bubble into the bottom of the tube. Slide the tube into the microTube holder while keeping the tube vertical. Make sure the tube is centered in the holder. Carefully insert the holder into the machine. Take care not to introduce bubbles into the bottom of the tube during this process. Click on Return to Main Panel. Click Start and Start again when the second screen appears. After shearing is complete, remove the tube from the S2 holder and place it into the preparation station. Remove the snap cap with the tool supplied with the preparation station. Use a p pipette to transfer the sheared DNA to a new, clean 1. A brief centrifugation may be used to collect any DNA remaining in the microTube. When the shearing is completed, click the OFF button under DEGAS, empty the water tank, turn off the chiller, close the software, and power down the instrument. Pipette up and down ten times to mix. Carefully aspirate the supernatant keeping the tube s on the magnet. Do not disturb the beads adhering to the side of the tube. Keep the tubes on the magnet and carefully aspirate the supernatant see Note 7. Repeat steps 7 and 8. Take care not to over dry the pellets as they will be difficult to resuspend. Pipette the entire volume of each tube up and down 20 times so that the beads are completely resuspended. Place the tube back on the magnet. This supernatant contains the sheared, size-selected DNA see Note 8. Repeat steps 13 and 14, this time adding the supernatant to the first elute. Non-gradient gels are not recommended. Remove the comb, rinse the wells with water two to three times and remove the plastic strip at the bottom of the gel. Complete the assembly of the gel unit. Cover with para-film and invert to mix. Running buffer may be stored at room temperature. Add running buffer to the center reservoir of the gel apparatus. Check for leaks and reassemble if necessary. Determine the average size of your sample by comparing the size of the middle of the sample smear to the size standards see Note  Calculate the pmoles of ends in the sample using the following formula: Put that volume of DNA into a 0. Prepare a sample master mix by adding 4. Mix thoroughly by pipetting the entire mix up and down several times see Note  Prepare a control master mix by adding 4. Mix thoroughly by pipetting up and down ten times see Note  Collect the contents of the tubes into the bottom by briefly centrifuging. Determining the Success of the Tailing Reaction 1. Instructions here will be limited to how to prepare samples for loading and how to interpret results. The sample itself is difficult to visualize. The band corresponding to the TR oligo spike is visible in the control lanes and monitors the tail length of the sample. However, if sample is not limiting, we recommend repeating the PolyA tailing reaction on another sheared DNA aliquot using twice the amount of input DNA. Short Tail Correction 1. After snap cooling the tubes, the following reagents are added. For the 3

pmole sample reactions, add 3. Mix by pipetting up and down thoroughly ten times. Migration pattern of tailed TR oligonucleotide in control reactions with optimal dA tail lengths. PolyA tailed samples do not migrate normally in the gel. The reagents to be added to the tubes are outlined below. Sample Quantification and Sequencing Sample quantification and sample loading are typically performed by the operator of the HeliscopeTM Single Molecule Sequencer, and will therefore be outlined only briefly here. For the assay, a 1: After washing and blocking, the plates are incubated with an HRP horseradish peroxidase conjugate. The streptavidinâ€"biotin complexes that are formed are washed to remove excess HRP. The HeliScopeTM Sequencer obtains sequence from one or two 25 channel flow cells, making it possible to sequence 50 bacterial genomes per run. The samples hybridize to the oligo dT primers on the flow cell surface and are locked into place by a procedure that ensures that sequencing-bysynthesis starts immediately after the first nonA base on the DNA samples. The HeliScopeTM analysis engine on the instrument creates. After the run is completed,. The HeliSphereTM data analysis pipeline is an open-source software written in the Python programming language. It is available for download at: Download both the HeliSphereTM package and the examples. Running the Resequencing Pipeline The resequencing pipline uses the raw sequence input file. The current version of the resequencing pipeline analyzes data from a single channel. Helicos Single-Molecule Sequencing of Bacterial Genomes 15 In order to run the pipeline, you must specify certain analysis parameters. A detailed description of all analysis parameters and how to apply them under more complex situations e. Default values for most analysis parameters are appropriate for running the pipeline on a single bacterial genome in a single channel. The following example outlines what must be specified for each analysis, how to set up the corresponding run configuration file, and how to launch the analysis. Determine the input file directory and the. Determine the flow cell and channel to be processed. Chose an output directory. A reference fasta file has to be chosen and placed in the reference data directory. If an indexDPgenomic database does not exist for this reference it needs to be created with preprocessDB and placed in the same directory. In the example, the reference is human. For accurate mutation detection using SNPSniffer, consideration should be given only to mutations for which the allele with maximum p-Value is 1e or less. The above information is incorporated into a config file for the run. For example, this file is named run. To incorporate the parameters used in the example, the pipeline should be launched with the following command: Resequencing Summary Report The resequencing pipeline generates various reports. The Resequencing summary report reseq. The table generated contains the following information: To accurately detect indels of longer lengths up to 4 during bacterial genome resequencing, filtering out reads shorter than 25 is recommended. This score takes into account length of the read, the number of matched nucleotides, and penalties for misalignments using a scoring scheme unique to the resequencing pipeline.

## Chapter 2 : Jane M. Liu's Publications

*sRNA-Seq is an unbiased method that allows for the discovery of small noncoding RNAs in bacterial transcriptomes through direct cloning and massively parallel sequencing by synthesis.*

Advanced Search Abstract Direct cloning and parallel sequencing, an extremely powerful method for microRNA miRNA discovery, has not yet been applied to bacterial transcriptomes. Our results provide information, at unprecedented depth, on the complexity of the sRNA component of a bacterial transcriptome. From sequence reads, all 20 known V. In addition, characterization of a subset of the newly identified transcripts led to the identification of a novel sRNA regulator of carbon metabolism. While several experimental and bioinformatic approaches have proven useful in identifying sRNAs in diverse species, it is widely accepted that these approaches have yielded only a partial catalogue of these transcripts 4 , 5. Moreover, these computational screens have almost uniformly been limited to intergenic regions IGRs of the genome. This method allows the identification of RNAs that are expressed in an organism under a given set of conditions regardless of whether they are encoded as distinct genetic elements or are generated via post-transcriptional processing. In recent years, direct cloning, in combination with parallel sequencing technology, has enabled researchers to ascertain the microRNA miRNA component of several eukaryote transcriptomes, such as those of Caenorhabditis elegans and Arabidopsis thaliana 9 , These studies collectively demonstrate that previous transcriptome analyses have been incomplete and suggest that parallel sequencing is necessary for comprehensive identification of transcribed regions of the genome. Massively parallel sequencing technology, or deep sequencing, has only recently been used to explore the transcriptomes of bacteria In the lone example, only those transcripts that interacted with the sRNA-chaperone Hfq were sequenced. A major reason for this lag in prokaryotic transcriptome analysis through deep sequencing is the lack of a robust and readily adaptable method of removing highly abundant housekeeping RNAs, such as the tRNAs and rRNAs. Several commercial kits exist for the depletion of 16S and 23S rRNA from bacterial RNA preparations through either hybridization-based physical removal or ribonuclease activity. A method was recently reported in which PCR products from direct cloning of Drosophila melanogaster small RNAs were screened prior to sequencing using a filter hybridization technique This method, however, requires that each sequence be individually evaluated which may not be practical in massive-scale sequencing experiments. We therefore reasoned that a robust and unbiased method for the removal of bacterial tRNAs and 5S rRNA was needed to allow for more in depth analyses of prokaryotic transcriptomes, particularly the sRNA component. To investigate the sRNA component of bacterial transcriptomes in an unbiased manner, we developed a method to directly clone and analyze whole populations of short bacterial transcripts, 14â€” nt in length, by parallel pyrosequencing Because both the RNA species targeted for depletion and the size range of RNA to be sequenced are user-defined, sRNA-Seq represents a comprehensive cloning protocol that is versatile and readily applicable to the cloning of small RNAs of any size range from any organism. Several sRNAs have been identified in V. For characterization of IGR7, V. Arabinose was added at 0. Antibiotics were added at the following concentrations: This was followed by a second gel purification; 1 nmol of the Oligo Mix was added to the extracted, linkered-RNA prior to ethanol precipitation. The Oligo Mix consisted of an equal molar mixture of 29 oligos complementary to either V. The depletion reaction was repeated for one additional cycle followed by another gel purification. The reaction was terminated by phenol: The extracted material was amplified by PCR for 20 cycles with the following primers: The cDNA libraries were purified on a native gel. For each independent sample, the small and large fractions were combined in a 1: Analysis of sequencing data The starting materials for the bioinformatics pipeline Supplementary Figure 1B were a set of four files in FASTA format, containing the results of the sequencing runs for the four different sRNA-Seq samples. These files are provided herein as Supplementary Table 2. They contain a total of reads. Removal of linker sequences and collapse of identical reads Each read was examined in turn. Of the total reads, met this criterion. There

were a total of unique sequences. The genome of V. The genome of S. Several miscellaneous sequences from other species, found to represent minor fractions of the contaminant pool. The highest-scoring hit first hit in the output list was accepted as the source of the sequence. By setting an E-value cutoff of 0. Genomic characterization of V. Most sequences overlapped only a single genomic feature or else lay entirely in intergenic regions IGR. However, to deal systematically with cases of multiple overlaps, the following procedure was applied to all sequences. Each nucleotide along the length of the sequence was tabulated as being in one of the following nine classes: Because some sets of overlapping sequences belonged to more than one of these nine classes, the class with the greatest tabulated count was accepted as the class of the sequence. For IGR sequences, note was taken of the closest upstream and downstream features and their distances. To accomplish this, the following steps were taken. First, each list of sequences was sorted by total number of reads, with the most frequent sequence at the top of the list. This process continued down the list until all sequences had been examined. At the end of this process, the original sequences had been merged into 16 transcripts. First, we removed any transcript that was observed in only a single sample out of the four independent samples. This reduced the size of the list to transcripts. This further reduced the length of the list to candidates. Blots were prehybridized in Ultrahyb or Ultrahyb-oligo buffer Ambion according to instructions. Control reactions were performed for each run and included RNA samples not treated with reverse transcriptase or samples lacking template DNA. In all cases, no band was observed in these controls. All primers used for this analysis are listed in Supplementary Table 4. Growth of strains was determined by measured OD using a Bio-Tek microplate reader. The OD was adjusted to 0. All growth experiments were performed in triplicate. At least three independent samples were tested for each condition and each template sample was tested in duplicate. In all cases, controls lacking reverse transcriptase were included and afforded results below the baseline of detection. Total RNA was isolated from exponential or early-stationary phase V. The linkered-RNAs were then annealed to a pool of oligodeoxynucleotides, each 29 nt, that were complimentary to V. The resulting sequences trimmed of linkers had the length distribution shown in Figure 1 A. Based on analysis of all possible n-mers, of the unique 15 nt reads that we assigned as being V. We also observed many small transcripts that mapped to the yeast genome Table 1. We hypothesize that the media that the bacteria were grown in was the source of these contaminating sequences. Additional unidentified sequences may be the result of trace DNA contaminating materials used during the cloning process. We then took all the reads that mapped to the V. Based on this method of grouping reads together, the sequences that mapped to the V. Breakdown of all sequences from pyrosequencing.

## Chapter 3 : Publications Authored by Andrew Camilli | PubFacts

*Liu J.M., Camilli A. () Discovery of Bacterial sRNAs by High-Throughput Sequencing. In: Kwon Y., Ricke S. (eds) High-Throughput Next Generation Sequencing. Methods in Molecular Biology (Methods and Protocols), vol*

Faculty can login to make corrections and additions. A cocktail of three virulent bacteriophages prevents Vibrio cholerae infection in animal models. A comparative study of ChIP-seq sequencing library preparation methods. Transposon-sequencing analysis unveils novel genes involved in the generation of persister cells in uropathogenic Escherichia coli. ManLMN is a glucose transporter and central metabolic regulator in Streptococcus pneumoniae. Global Tn-seq analysis of carbohydrate utilization and vertebrate infectivity of Borrelia burgdorferi. Vibrio cholerae phosphatases required for the utilization of nucleotides and extracellular DNA as phosphate sources. A globally distributed mobile genetic element inhibits natural transformation of Vibrio cholerae. Carbon catabolite repression by seryl phosphorylated HPr is essential to Streptococcus pneumoniae in carbohydrate-rich environments. Genes contributing to Staphylococcus aureus fitness in abscess- and infection-related ecologies. Evolutionary consequences of intra-patient phage predation on microbial populations. Multiplex genome editing by natural transformation. Curr Protoc Mol Biol. Vibrio cholerae-induced inflammation in the neonatal mouse cholera model. Identification of in vivo regulators of the Vibrio cholerae xds gene using a high-throughput genetic selection. Identification of a membrane-bound transcriptional regulator that links chitin and natural competence in Vibrio cholerae. Gene fitness landscapes of Vibrio cholerae at important stages of its life cycle. The core promoter of the capsule operon of Streptococcus pneumoniae is necessary for colonization and invasive disease. A core microbiome associated with the peritoneal tumors of pseudomyxoma peritonei. Orphanet J Rare Dis. Understanding barriers to Borrelia burgdorferi dissemination during infection using massively parallel sequencing. Characterization of undermethylated sites in Vibrio cholerae. Genotyping yeast strains by next-generation sequencing. Homopolymer tail-mediated ligation PCR: Toll-like receptor TLR 2 mediates inflammatory responses to oligomerized RrgA pneumococcal pilus type 1 protein. Identification of essential genes of the periodontal pathogen Porphyromonas gingivalis. Phase variable O antigen biosynthetic genes control expression of the major protective antigen and bacteriophage receptor in Vibrio cholerae O1. A fine scale phenotype-genotype virulence map of a bacterial pathogen. Control of virulence by small RNAs in Streptococcus pneumoniae. Mlp24 McpX of Vibrio cholerae implicated in pathogenicity functions as a chemoreceptor for multiple amino acids. Export requirements of pneumolysin in Streptococcus pneumoniae. Immunization of mice with vibrio cholerae outer-membrane vesicles protects against hyperinfectious challenge and blocks transmission. Extracellular nucleases and extracellular DNA play important roles in Vibrio cholerae biofilm formation. A genome-wide approach to discovery of small RNAs involved in regulation of virulence in Vibrio cholerae. Streptococcus pneumoniae is desiccation tolerant and infectious upon rehydration. Evidence of a dominant lineage of Vibrio cholerae-specific lytic bacteriophages shed by cholera patients over a year period in Dhaka, Bangladesh. Discovery of bacterial sRNAs by high-throughput sequencing. Genome-wide fitness and genetic interactions determined by Tn-seq, a high-throughput massively parallel sequencing method for microorganisms. PhoB regulates both environmental and virulence gene expression in Vibrio cholerae. Mucosal immunization with Vibrio cholerae outer membrane vesicles provides maternal protection mediated by antilipopolysaccharide antibodies that inhibit bacterial motility. Growth in a biofilm induces a hyperinfectious phenotype in Vibrio cholerae. A broadening world of bacterial small RNAs. A novel regulatory protein involved in motility of Vibrio cholerae. High prevalence of spirochetosis in cholera patients, Bangladesh. Glycogen contributes to the environmental persistence and transmission of Vibrio cholerae. An alternative polyamine biosynthetic pathway is widespread in bacteria and essential for biofilm formation in Vibrio cholerae. Pneumolysin localizes to the cell wall of Streptococcus pneumoniae. Characterization of Vibrio cholerae outer membrane vesicles as a candidate vaccine for cholera. Transmission

of Vibrio cholerae is antagonized by lytic phage and entry into the aquatic environment. Isolation of Streptococcus pneumoniae biofilm mutants and their characterization during nasopharyngeal colonization. Immunization with Vibrio cholerae outer membrane vesicles induces protective immunity in mice. A new Vibrio cholerae sRNA modulates colonization and affects release of outer membrane vesicles. The vibrio cholerae hybrid sensor kinase VieS contributes to motility and biofilm regulation by altering the cyclic diguanylate level. Roles of the sortases of Streptococcus pneumoniae in assembly of the RlrA pilus. The structural basis of cyclic diguanylate signal transduction by PilZ domains. Complexity of rice-water stool from patients with Vibrio cholerae plays a role in the transmission of infectious diarrhea.

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## Chapter 4 : High-Throughput Next Generation Sequencing: Methods and Applications - PDF Free Downloa

*Jane Liu studies the role of functional RNAs (such as catalytic and regulatory RNAs) in controlling the dynamic aspects of gene expression in response to a changing environment. She seeks to apply this knowledge to the development of novel tools for chemistry and biology.*

News Publications Page, K. Engineering riboswitches in vivo using dual genetic selection and fluorescence-activated cell sorting. MtlR negatively regulates mannitol utilization by Vibrio cholerae. Searching for synthetic antimicrobial peptides: An experiment for organic chemistry students. Journal of Chemical Education. A cis-regulatory antisense RNA represses translation in Vibrio cholerae through extensive complementarity and proximity to the target locus. RNA Biology , 12, Journal of Bacteriology , , Discovery of sRNAs by high-throughput sequencing. Methods in Molecular Biology. A broadening world of bacterial small RNAs. Current Opinions in Microbiology , 13, Modulation of RNA metal binding by flanking bases: Journal of Organic Chemistry , 74, Nucleic Acids Research , 37, e Discovery of a mRNA mitochondrial localization element in Saccharomyces cerevisiae by nonhomologous random recombination and in vivo selection. Nucleic Acids Research , 35, Functional dissection of sRNA translational regulators using nonhomologous random recombination and in vivo selection. Directed evolution of protein enzymes using nonhomologous random recombination. Simultaneous and stereoselective formation of planar and axial chiralities in enantiopure sulfinyl iron diene complexes. Organic Letters , 5,

## Chapter 5 : Camilli, Andrew | Profiles RNS

*Discovery of Bacterial sRNAs by High-Throughput Sequencing Jane M. Liu and Andrew Camilli. 6. Identification of Virus Encoding MicroRNAs Using FLX Sequencing.*

## Chapter 6 : - NLM Catalog Result

*High-Throughput Next Generation Sequencing Methods and Applications Edited by Young Min Kwon Department of Poultry Science, and Cell and Molecular Biology Program.*

## Chapter 7 : Andrew Camilli | Profiles RNS

*Jane M Liu's 11 research works with citations and reads, including: Supplementary File 1. Jane M Liu has expertise in Biology and Agricultural Science.*

## Chapter 8 : High-Throughput Next Generation Sequencing : Steven C. Ricke :

*[et al.] -- Transcriptome profiling using single-molecule direct RNA sequencing / Fatih Ozsolak and Patrice M. Milos -- Discovery of bacterial sRNAs by high-throughput sequencing / Jane M. Liu and Andrew Camilli -- Identification of virus encoding microRNAs using FLX sequencing platform / Byung-Whi Kong -- Ribosomal RNA depletion for.*

## Chapter 9 : Publications â€“ Jane M. Liu

*Experimental discovery of sRNAs in Vibrio cholerae by direct cloning, 5S/tRNA depletion and parallel sequencing (Article begins on next page) The Harvard community has made this article openly available.*