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MASSEY UNIVERSITY
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Institute of Fundamental Sciences

A comparison of next-generation sequencing protocols for microbial profiling

A thesis

submitted in partial fulfillment

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By

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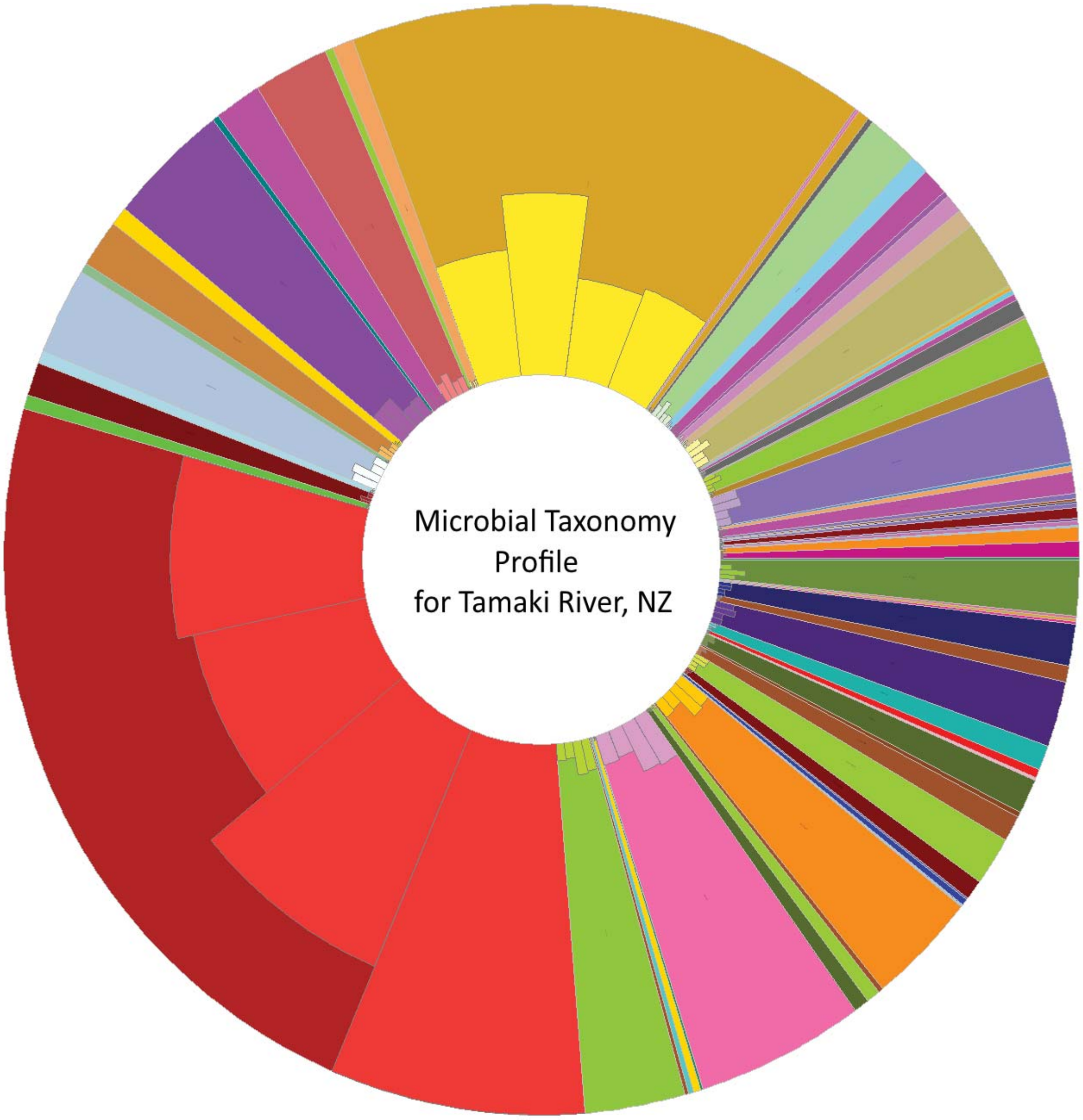

One of the responsibilities faced by the Environmental Genome Project is to provide the science base upon which society can make better informed risk management decisions.

-Samuel Wilson-

Abstract

The introduction of massive parallel sequencing has revolutionized analyses of microbial communities. Illumina and other Whole Genome Shotgun Sequencing (WGS) sequencing protocols have promised improved opportunities for investigation of microbial communities. In the present work, we compared and contrasted the findings from different NGS library preparation protocols (Illumina Nextera, Nextera-XT, NEXTFlex PCR-free and Ion-Xpress-400bp) and two sequencing platforms (MiSeq and Ion-Torrent). Short reads were analysed using the rapid database matching software PAUDA and visualization software MEGAN5, which provides a conservative approach for taxonomic identification and functional analyses. In analyses of a Tamaki River water sample, biological inferences were made and compared across platforms and protocols. For even a relatively small number of reads generated on the MiSeq sequencing platform important pathogens were identified in the water sample. Far greater phylogenetic resolution was obtained with WGS sequencing protocols than has been reported in similar studies that have used 16S rDNA Illumina sequencing protocols. TruSeq and Nextera-XT sequencing protocols produced similar results. The latter protocol offered cheaper, and faster results from less DNA starting material. Proteobacteria (alpha, beta and gamma), Actinobacteria and Bacteroidetes were identified as major microbial elements in the Tamaki River sample. Our findings support the emerging view that short read sequence data and enzymatic library prep protocols provide a cost effective tool for evaluating, cataloguing and monitoring microbial species and communities. This is an approach that complements, and provides additional insight to microbial culture “water testing” protocols routinely used for analysing aquatic environments.

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List of Acronyms

%	Percent
°C	Degrees Celsius
µl	Microlitre(s)
µM	Micromolar
100 PE	2 x 100 base pair paired-end read
150 PE	2 x 150 base pair paired-end read
250 PE	2 x 250 base pair paired-end read
300 PE	2 x 300 base pair paired-end read
A	Adenine
A260	Nanodrop absorbance at 260 nanometres
A280	Nanodrop absorbance at 280 nanometres
AFLP	Amplified Fragment Length Polymorphism
ATL	A-Tailing Mix
ATM	Amplicon Tagment Mix
ATP	Adenosine Triphosphate
BAM	Binary Alignment Matrix
BGI	Beijing Genomics Limited
BIPES	Illumina Multiplexed Paired-end Sequencing Adapter
BLAST	Basic Local Alignment Search Tool
bp	Base pair(s)
C	Cytosine
CCD	Charge-coupled Device
cDNA	Complementary Deoxyribonucleic Acid
contig	Continuous Sequence
CTA	A-Tailing Control

CTE	End-Repair Control
CTL	Ligation Control
ddNTP	Dideoxy Nucleotide Triphosphate
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
dNTP	Deoxy Nucleotide Triphosphate
ds	Double Stranded
EB	Elution Buffer
eDNA	Environmental Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetra-Acetic Acid
emPCR	Emulsion Polymerase Chain Reaction
ERP	End-Repair mix
EtBr	Ethidium Bromide
E-value	A parameter that describes the number of expected matches when searching a sequence database of a particular size and composition
FC	Flowcell
fq	Fastq File Format
g	Gram(s)
G	Guanine
Gb	Gigabytes
gDNA	Genomic DNA
HiFi	High fidelity enyzme
HMW	High Molecular Weight
HT1	Hybridization Buffer
Inc.	Incorporated
Indel	Small Insertion or deletion
ISFET	Ion Sensitive Field Effect Transistor

KEGG	Kyoto Encyclopedia of Genes and Genomes
LCA	Lowest Common Ancestor
LIG	Ligation Mix
Log^{10}	Logarithm to the base 10
M	Molar
Mb	Megabytes
MDA	Multiple Displacement Amplification
MEGAN	Metagenome Analyzer
MGS	Massey Genome Service
min	Minute(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
MPSS	Massive Parallel Signature Sequencing
mRNA	Messenger Ribonucleic Acid
mtDNA	Mitochondrial Deoxyribonucleic Acid
ng	Nanogram(s)
NGS	Next-generation Sequencing
No	Number
NPM	Nextera PCR Master Mix
NPS	Non-point Source
nt	Nucleotide
NT	Neutralize Tagment Buffer
NZGL	New Zealand Genomics Limited
OTU	Operational Taxonomic Unit
PAUDA	Protein Alignment Using a DNA Aligner

PCoA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
pDNA	Pseudo DNA
PE	Paired-end
PGM	Personal Genome Machine
PhiX	Bacteriophage PhiX174
PMM	PCR Master Mix
pmol	Picomole(s)
PPC	PCR Primer Cocktail
PP_i	Pyrophosphate
Q_{10}	Phred Quality Score 1 error in 10
Q_{20}	Phred Quality Score 1 error in 100
Q_{30}	Phred Quality Score 1 error in 1000
QC	Quality Control
qPCR	Quantitative Polymerase Chain Reaction
Q-score	Phred Quality Score
RNA	Ribonucleic Acid
rpm	Revolutions per Minute
rRNA	Ribosomal Ribonucleic Acid
RSB	Resuspension Buffer
RTA	Real-Time Analysis
s	Second(s)
SAM	Sequence Alignment Map
SBS	Sequencing by Synthesis
SCIMM	Sequence Clustering with Interpolated Markov Models
SEED	Database infrastructure for comparative genomics in MEGAN5 software

SMRT	Single Molecule Real Time
spp.	Species
SPRI	Solid Phase Reversible Immobilization
ss	Single Stranded
STL	Stop Ligation Buffer
T	Thymine
TAE	Tris-Acetate EDTA buffer
TAP	Taxonomic Assignment Pipeline
Taq	*Thermus aquaticus*
TB	Tuberculosis
TD	Tagmentation Buffer
TE	Tris EDTA Buffer
V	Volts
WGS	Whole Genome Shotgun Sequencing

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