NGS IN THE HEFLIN CENTER GENOMICS CORE

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DIRECTOR

INSTRUMENTATION IN THE CORE:

HiSeq2500-Shared with the Stem Cell Institute

Exome	RNA
miRNA	Metagenomic

2 MiSeq Sequencers

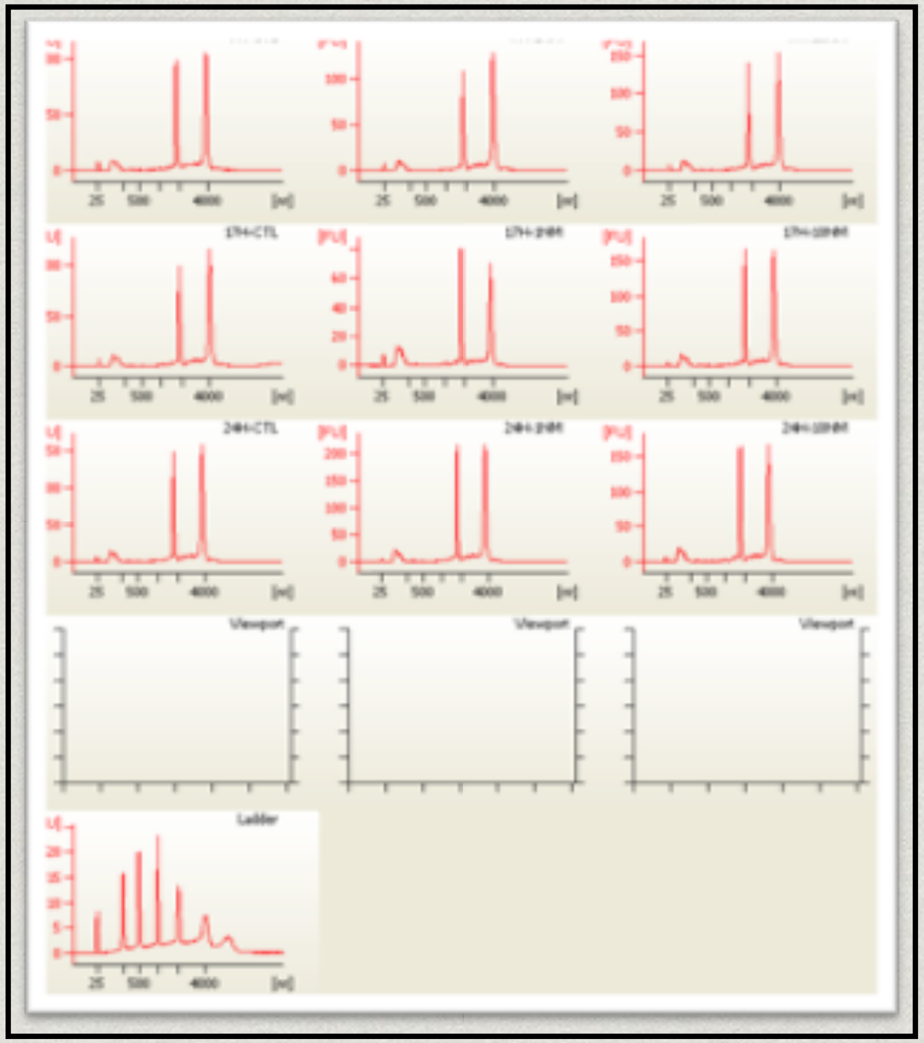
Microbiome 16S	Small Genomes
Targeted Panels	Amplicons

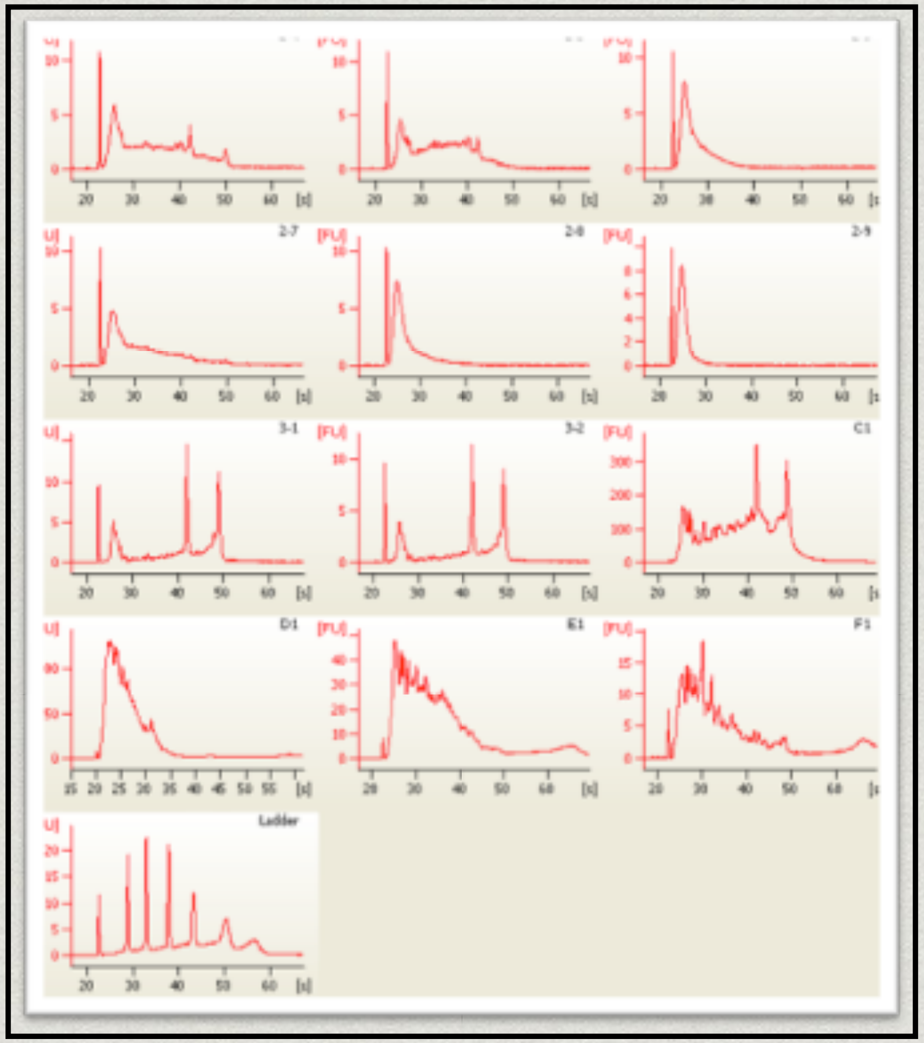
RNA-Sequencing

Advantages of RNA-Seq

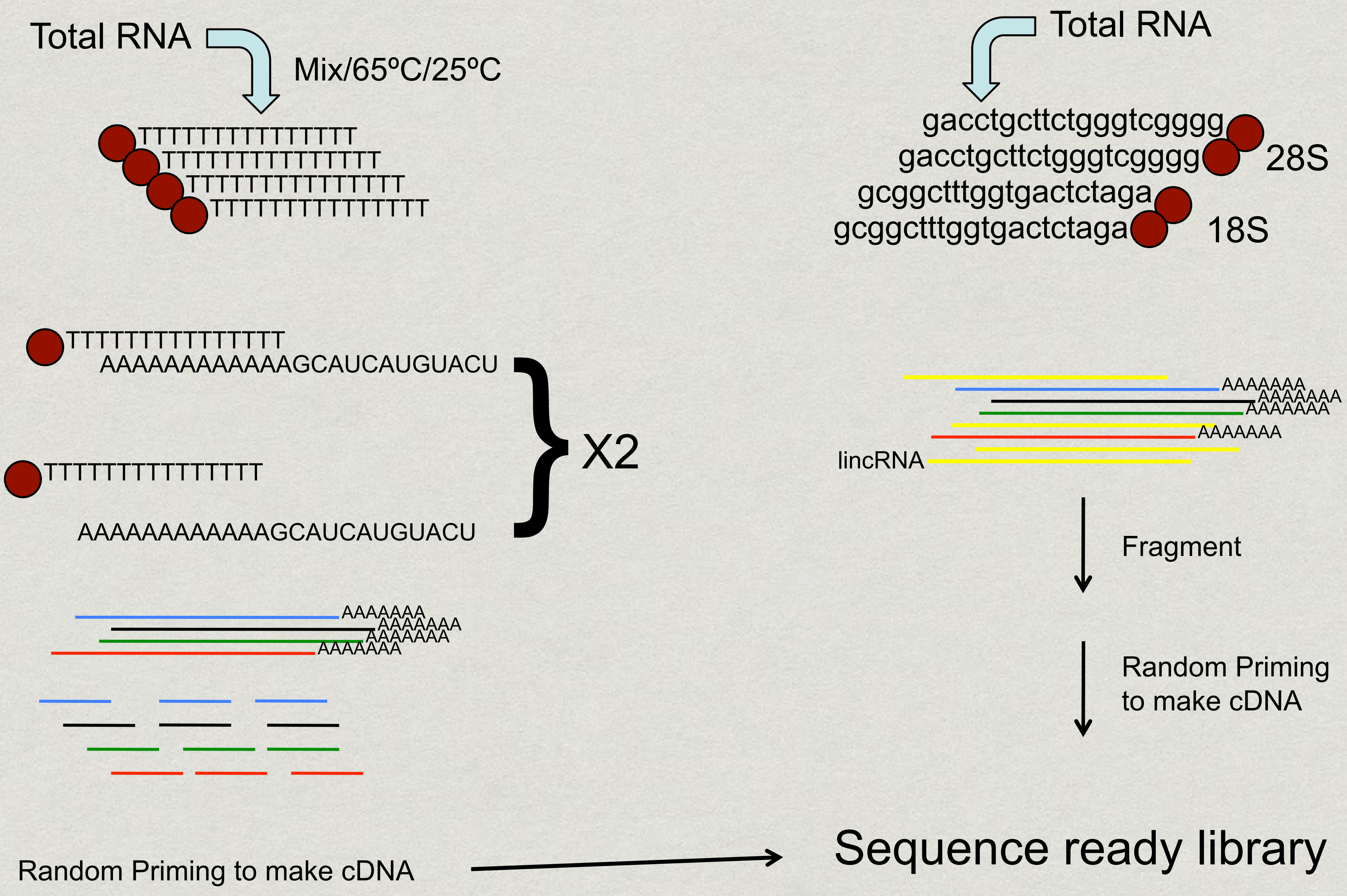
- Digital gene expression
  - simply count the number of reads
- Greater dynamic range
- No hybridization bias
- Not dependent on known content
- Generate Alternative splice/exon usage maps
- Identify variants
- Identify RNA editing

RNA Quality is Very Important





RNA Library Preps



Total RNA

Mix/65ºC/25ºC

Random Priming to make cDNA

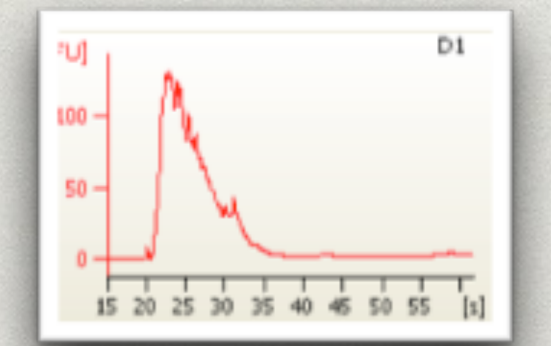
Total RNA

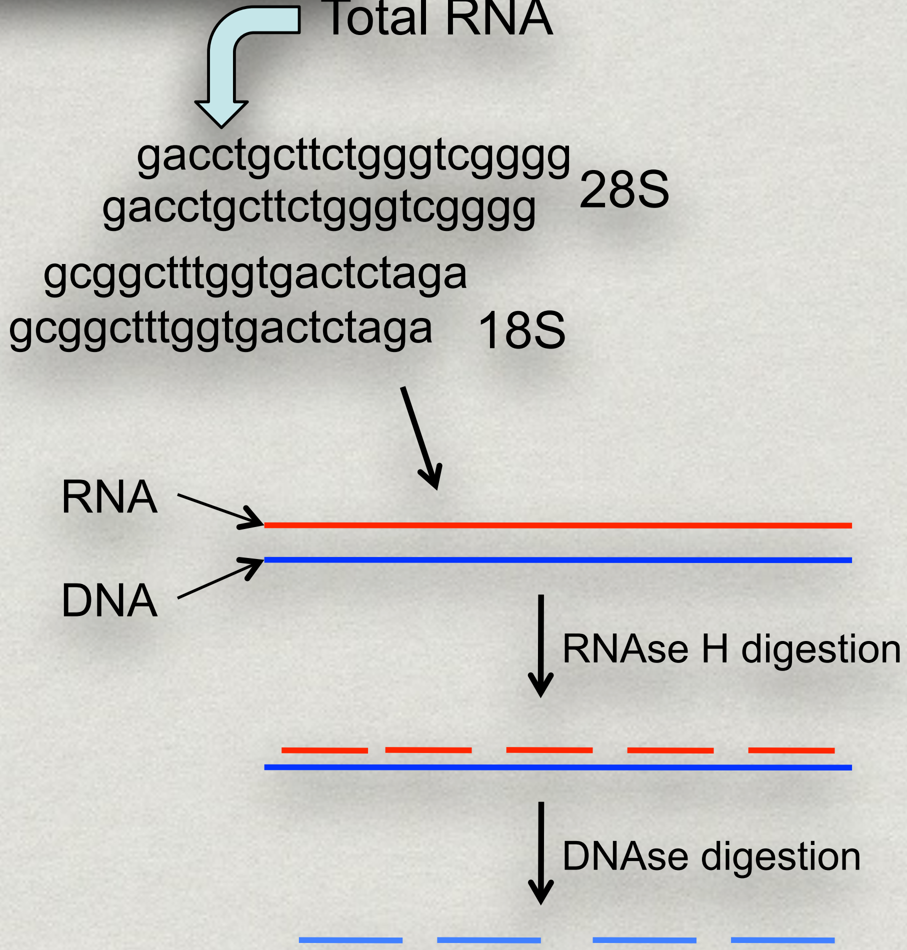
Fragment

Random Priming
to make cDNA

Sequence ready library

Degraded RNA or FFPE





Total RNA

gacctgcttctgggtcgggg
gacctgcttctgggtcgggg 28S

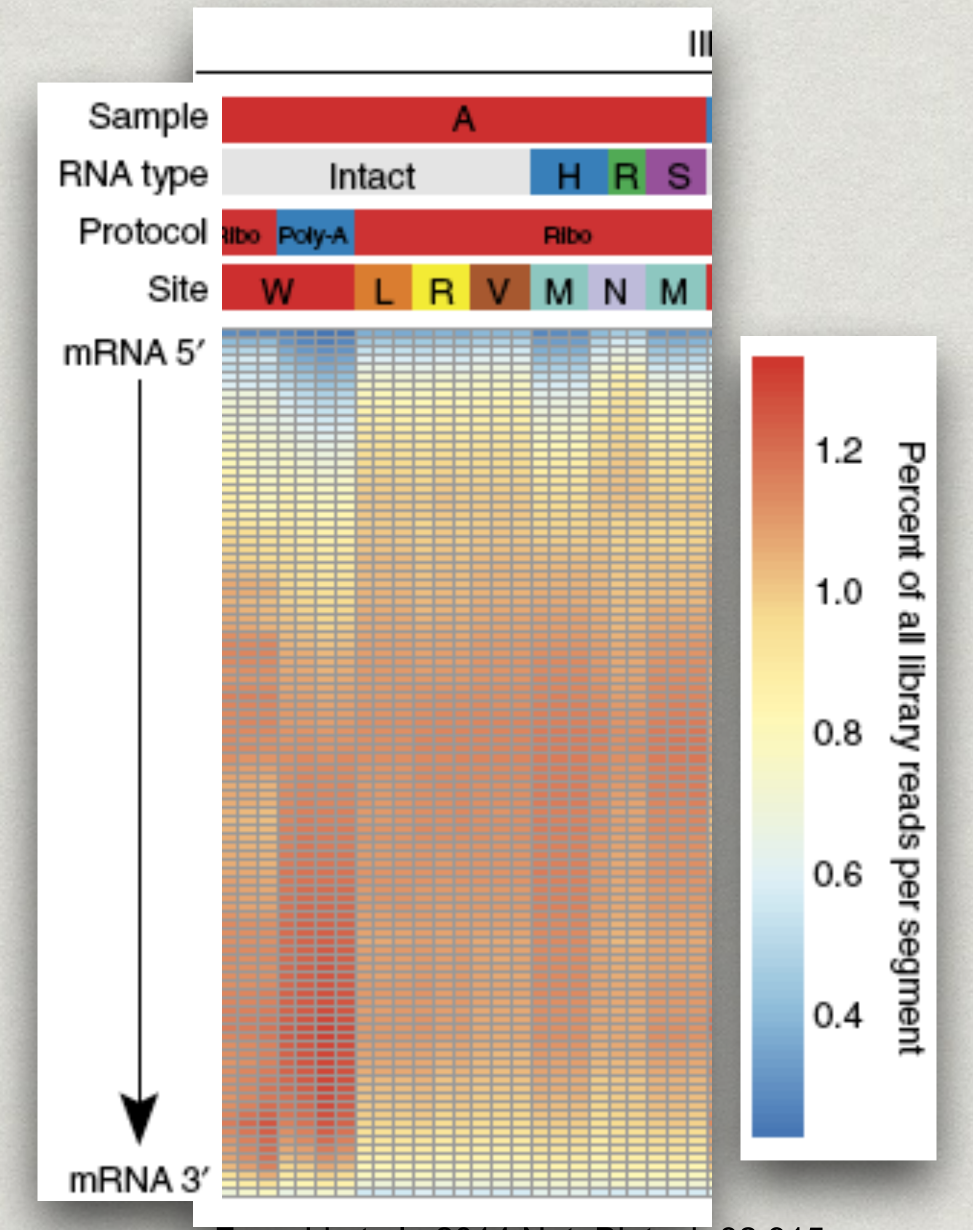
gcggctttggtgactctaga
gcggctttggtgactctaga 18S

RNA

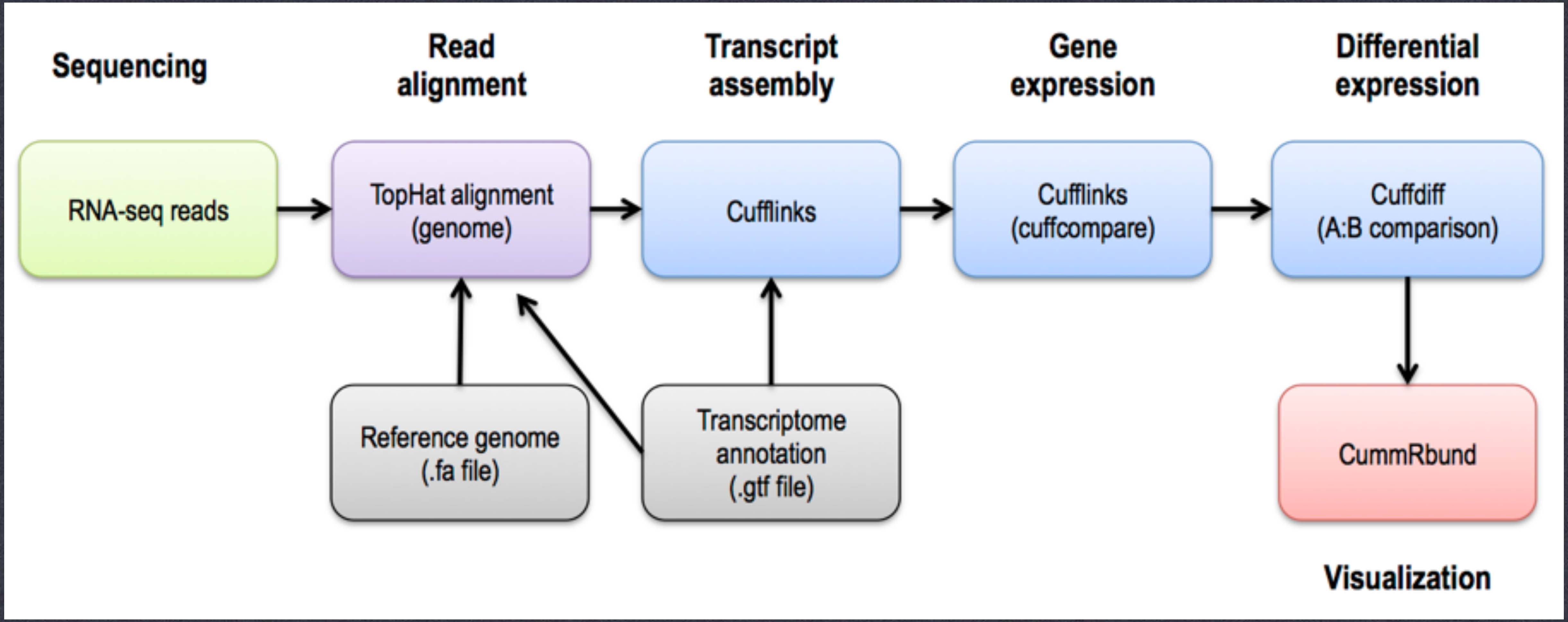
DNA

RNAse H digestion

DNAse digestion



From Li et al., 2014 Nat. Biotech.32:915



Sequencing

RNA-seq reads

Read alignment

TopHat alignment (genome)

Reference genome (.fa file)

Transcript assembly

Cufflinks

Transcriptome annotation (.gtf file)

Gene expression

Cufflinks (cuffcompare)

Differential expression

Cuffdiff (A:B comparison)

CummRbund

Visualization

PROJECT

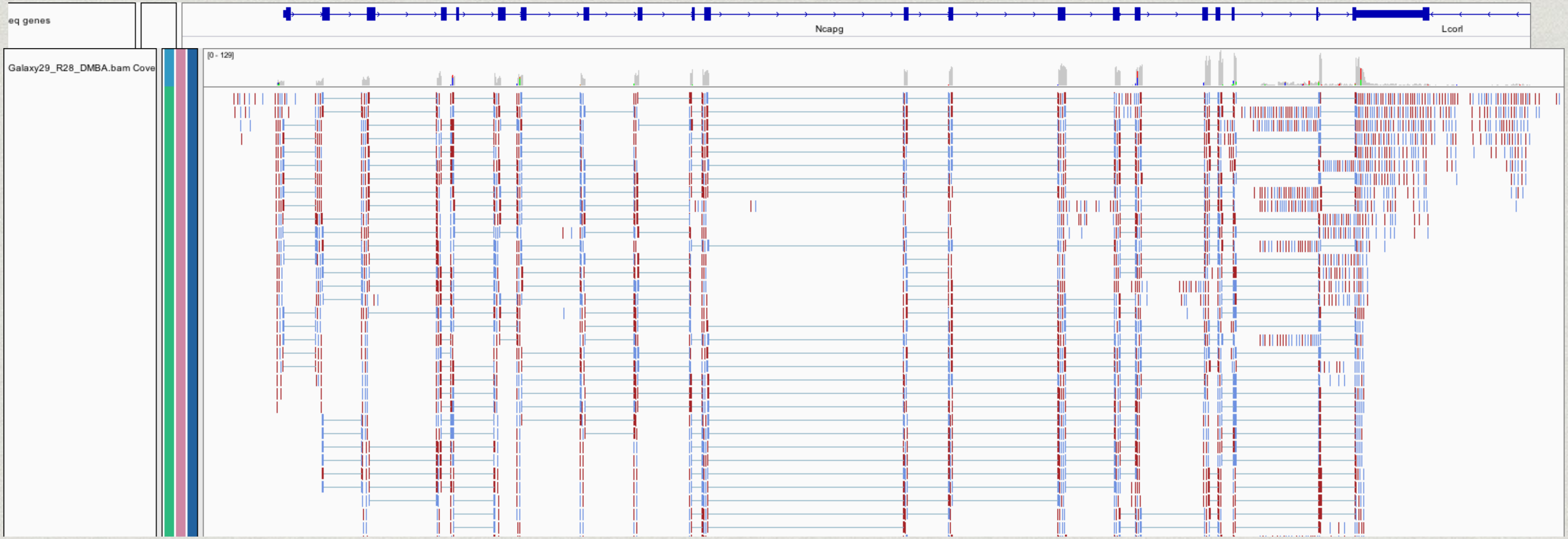
ANALYSIS PIPELINE

RNA-SEQ

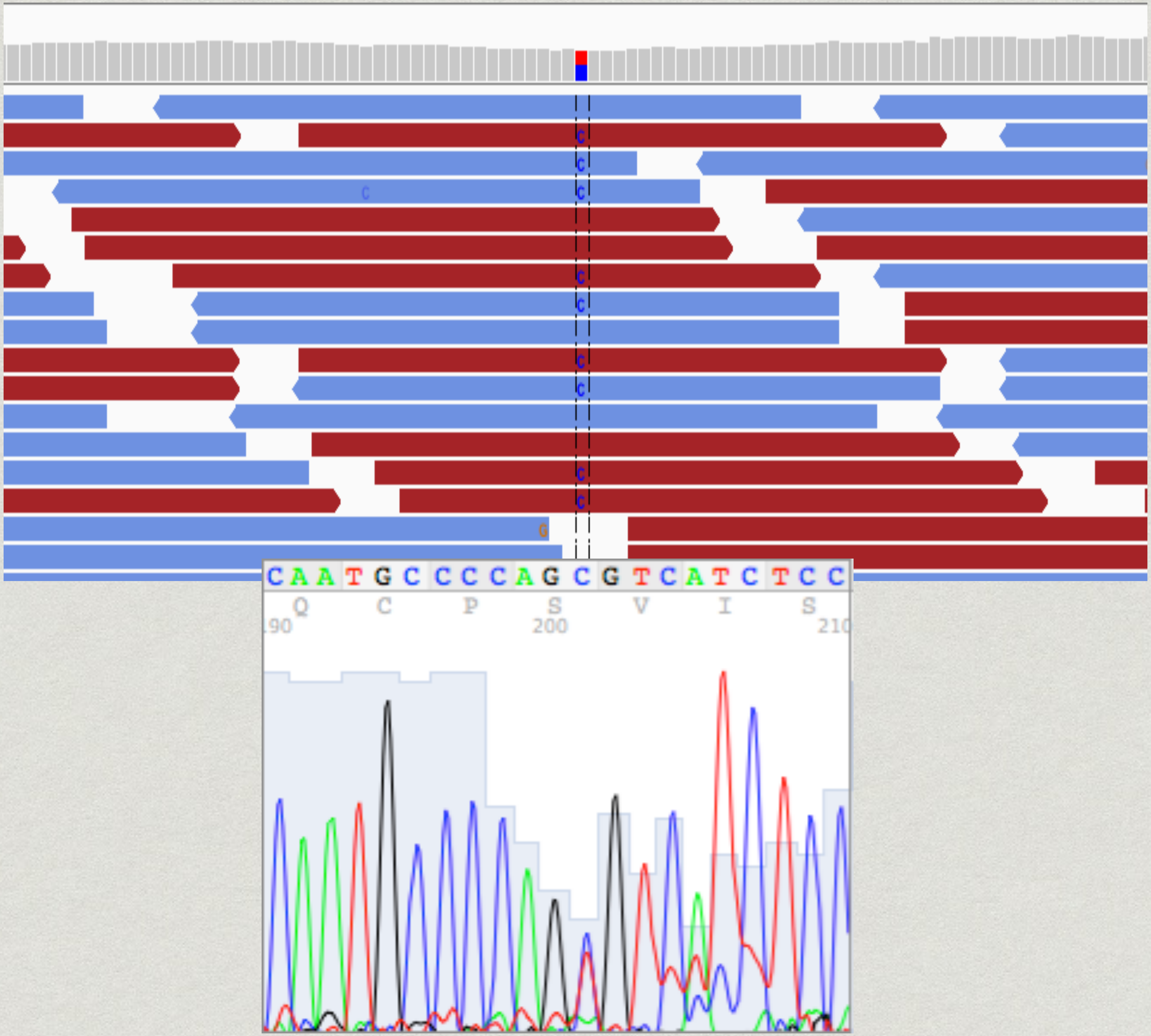
DATE **TODAY**

CLIENT **CROWLEY**

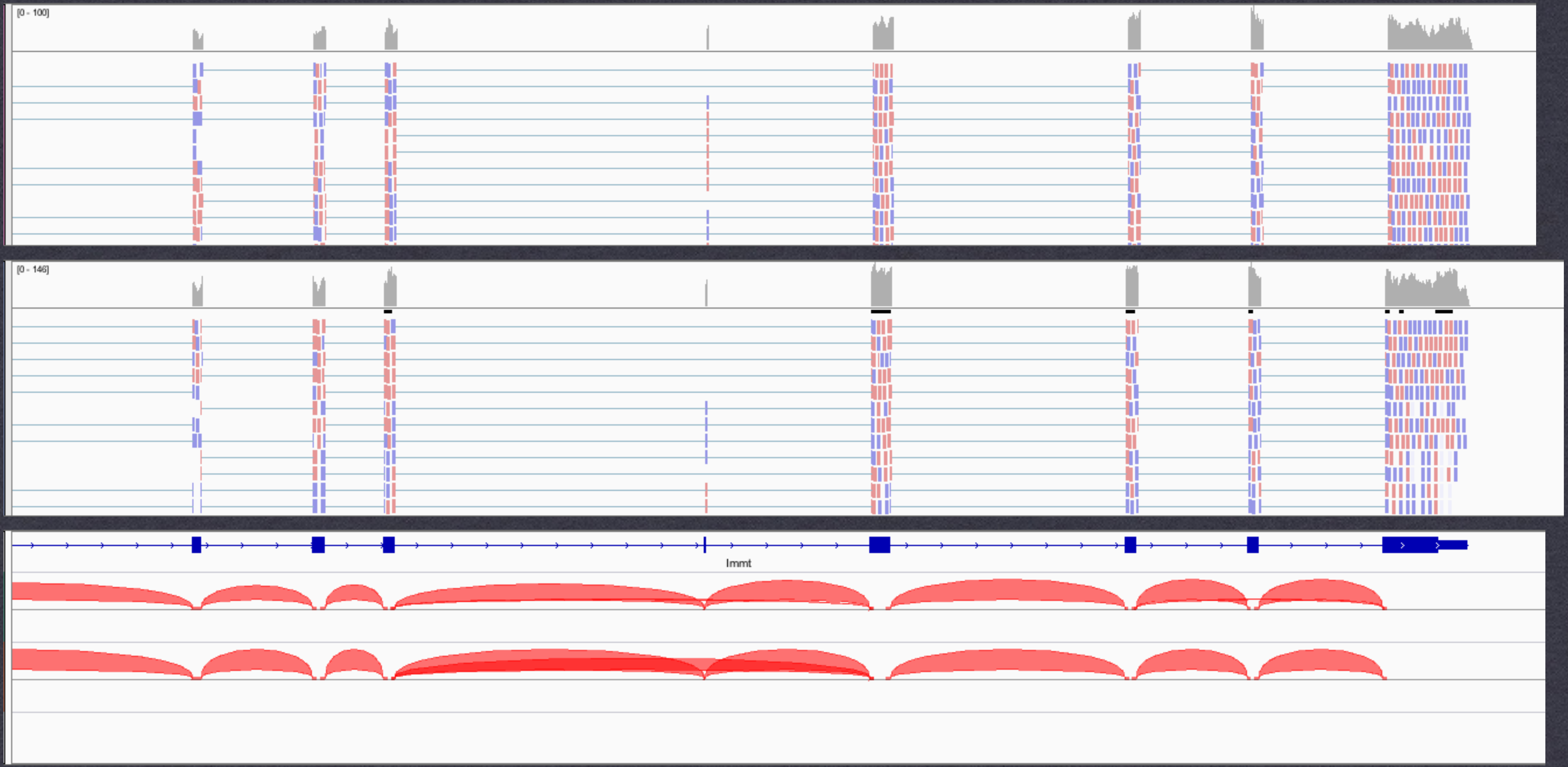
Visualization with IGV



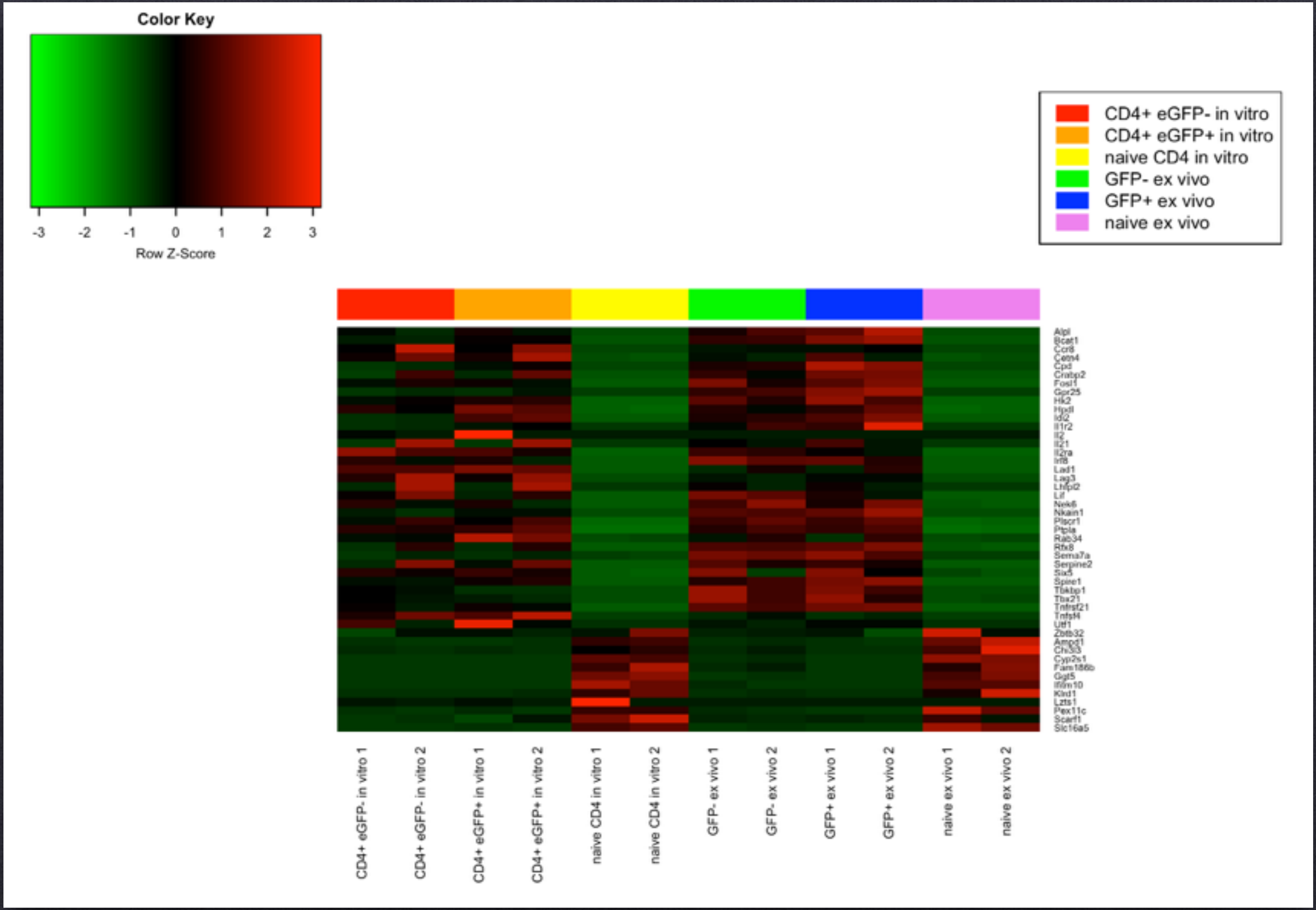
Variant Detection in RNA Seq Data



ALTERNATIVE SPLICING

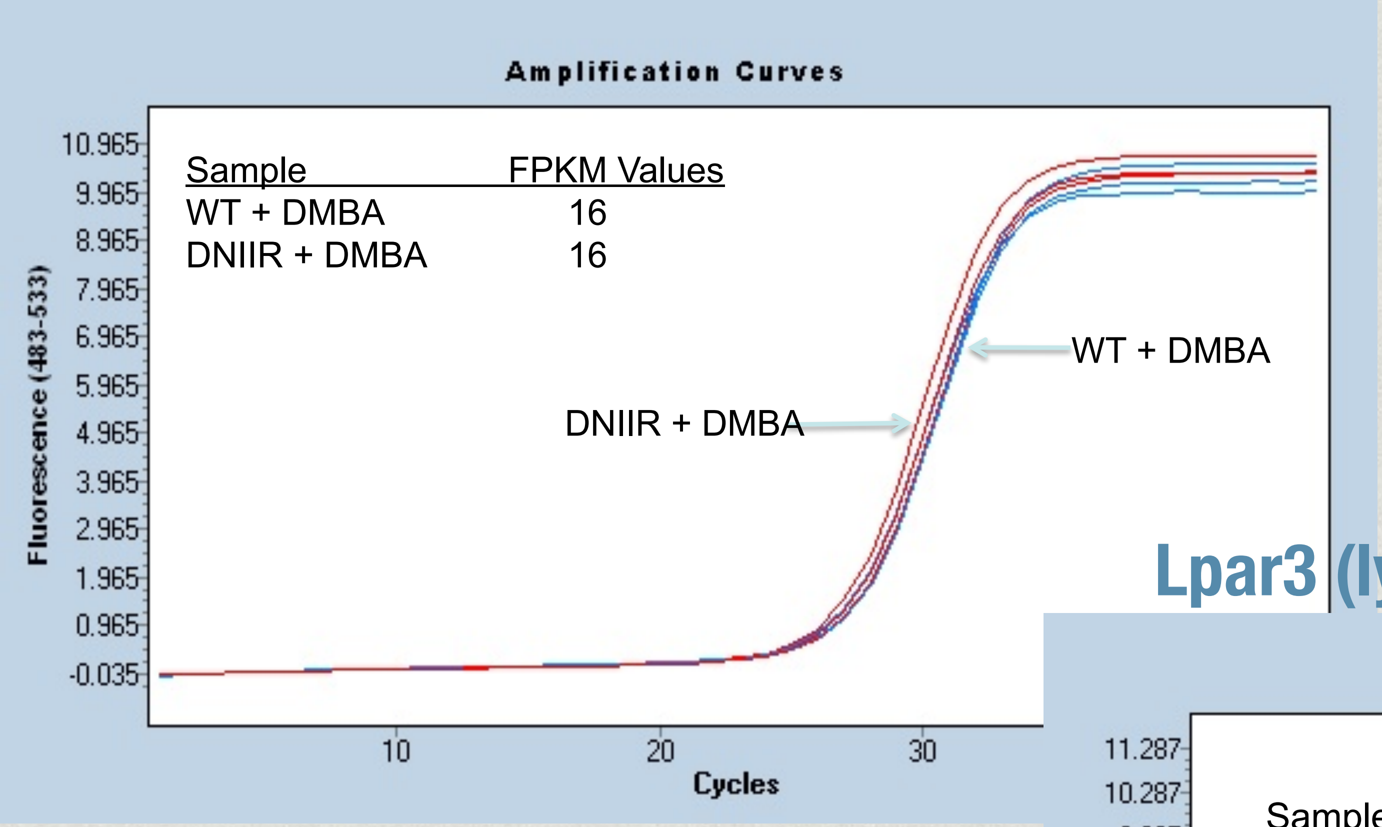


EXPRESSION


Color Key
-3
-2
-1
0
1
2
3
Row Z-Score
CD4+ eGFP- in vitro
CD4+ eGFP+ in vitro
naive CD4 in vitro
GFP- ex vivo
GFP+ ex vivo
naive ex vivo
Alpl
Bcat1
Ccr8
Cetn4
Cpd
Crabp2
Fosl1
Gpr25
Hk2
Hpdl
Idi2
Il1r2
Il2
Il21
Il2ra
Irf8
Lad1
Lag3
Lhfpl2
Lif
Nek6
Nkain1
Plscr1
Ptpla
Rab34
Rfx8
Sema7a
Serpine2
Six5
Spire1
Tbkbp1
Tbx21
Tnfrsf21
Tnfsf4
Utf1
Zbtb32
Amotl1
Chi3l3
Cyp2s1
Fam188b
Ggt5
Ifitm10
Klrd1
Lzts1
Pex11c
Scarf1
Slc16a5
CD4+ eGFP- in vitro 1
CD4+ eGFP- in vitro 2
CD4+ eGFP+ in vitro 1
CD4+ eGFP+ in vitro 2
naive CD4 in vitro 1
naive CD4 in vitro 2
GFP- ex vivo 1
GFP- ex vivo 2
GFP+ ex vivo 1
GFP+ ex vivo 2
naive ex vivo 1
naive ex vivo 2

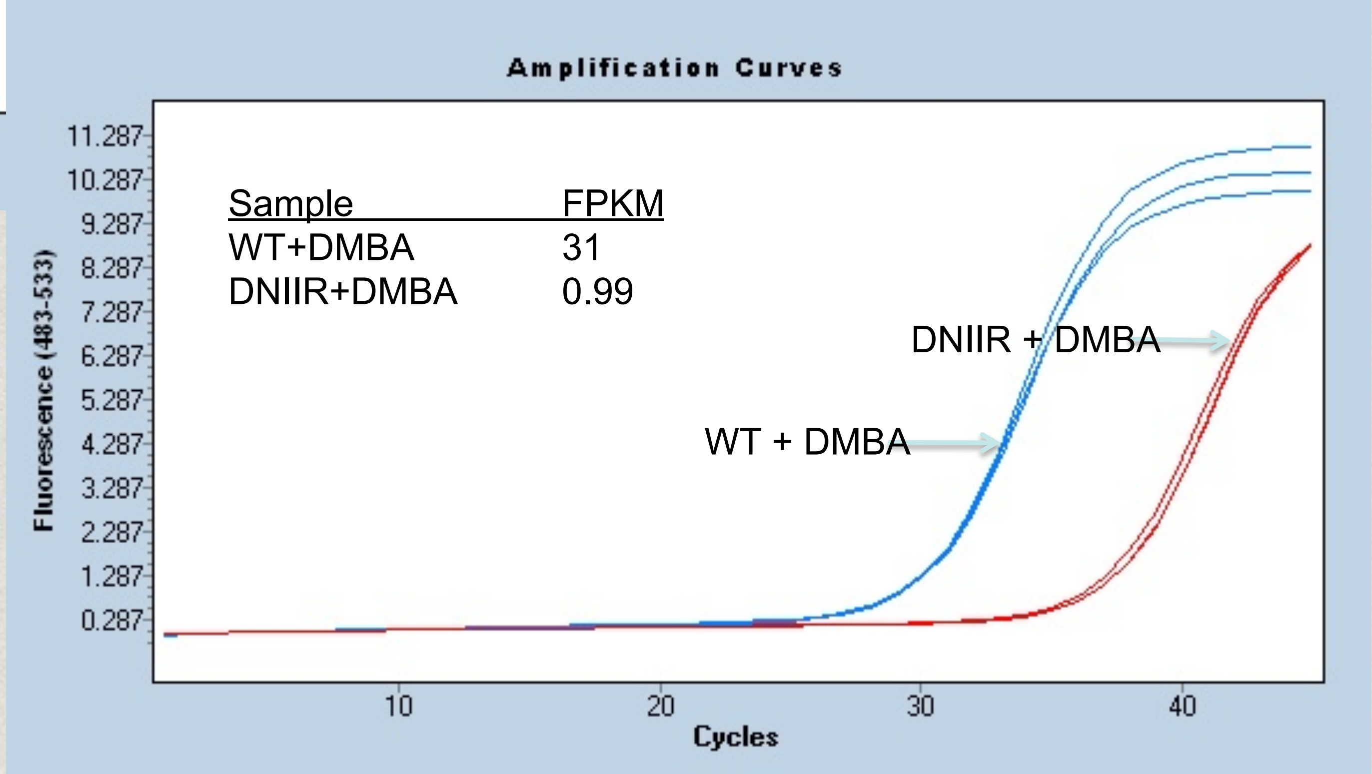
Validation

Lmf1 (lipase maturation factor 1)



Sample	FPKM Values
WT + DMBA	16
DNIIR + DMBA	16

Lpar3 (lysophosphotitic acid receptor 3)



Sample	FPKM
WT+DMBA	31
DNIIR+DMBA	0.99

A WORD ABOUT MICRORNA STUDIES

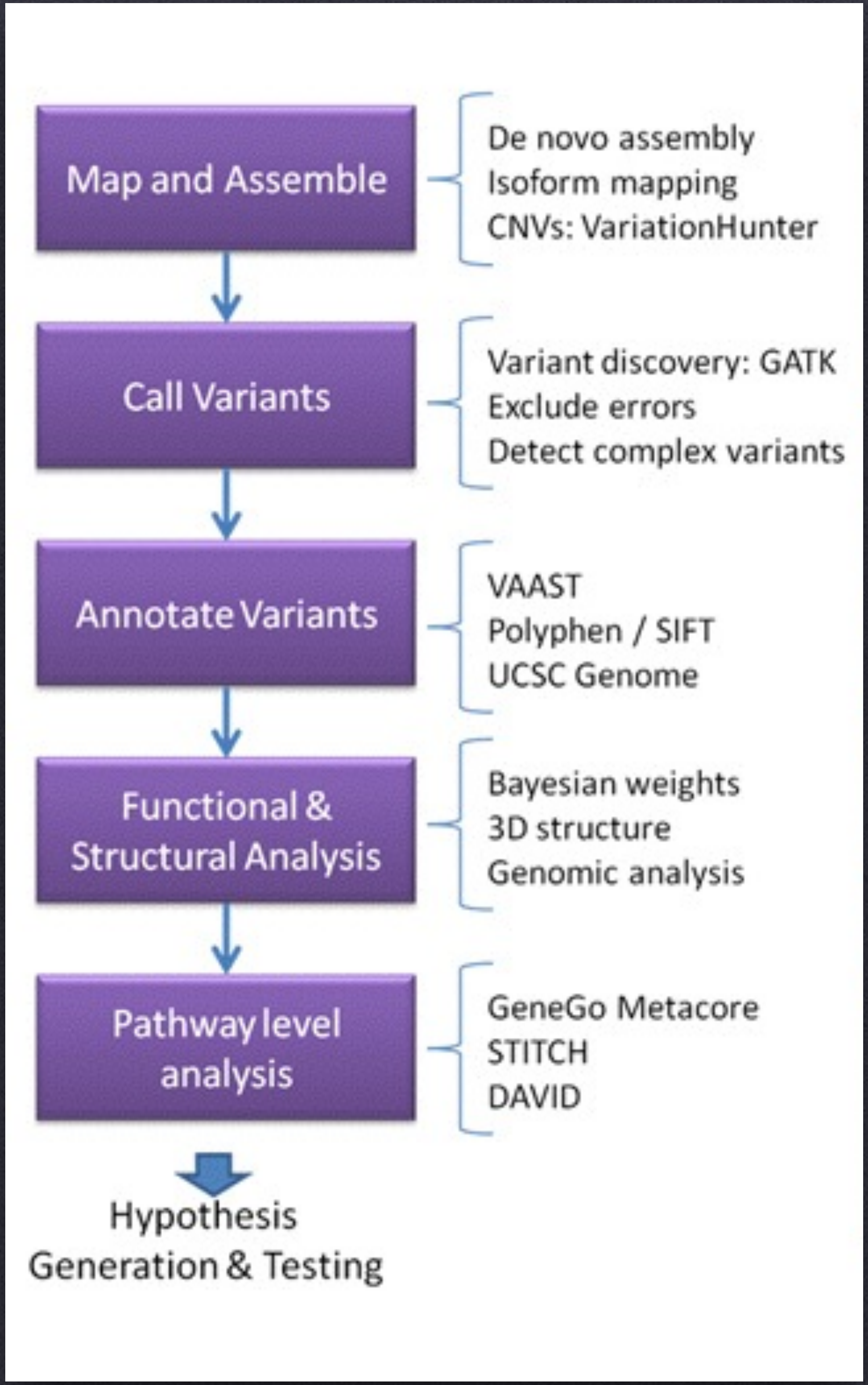
DNA SEQUENCING

APPLICATIONS

- Whole Genome Sequencing
- Whole Exome Sequencing
- Targeted Genomic Sequencing
- Mitochondrial DNA Sequencing
- Chromatin-IP Sequencing
- Methyl Sequencing
- Microbiome 16S Sequencing
- Metagenomics Sequencing

WHOLE GENOME OR EXOME ANALYSIS PIPELINE

DAVID CROSSMAN, PH.D.
BIOINFORMATICS



Map and Assemble
- De novo assembly
- Isoform mapping
- CNVs: VariationHunter

Call Variants
- Variant discovery: GATK
- Exclude errors
- Detect complex variants

Annotate Variants
- VAAST
- Polyphen / SIFT
- UCSC Genome

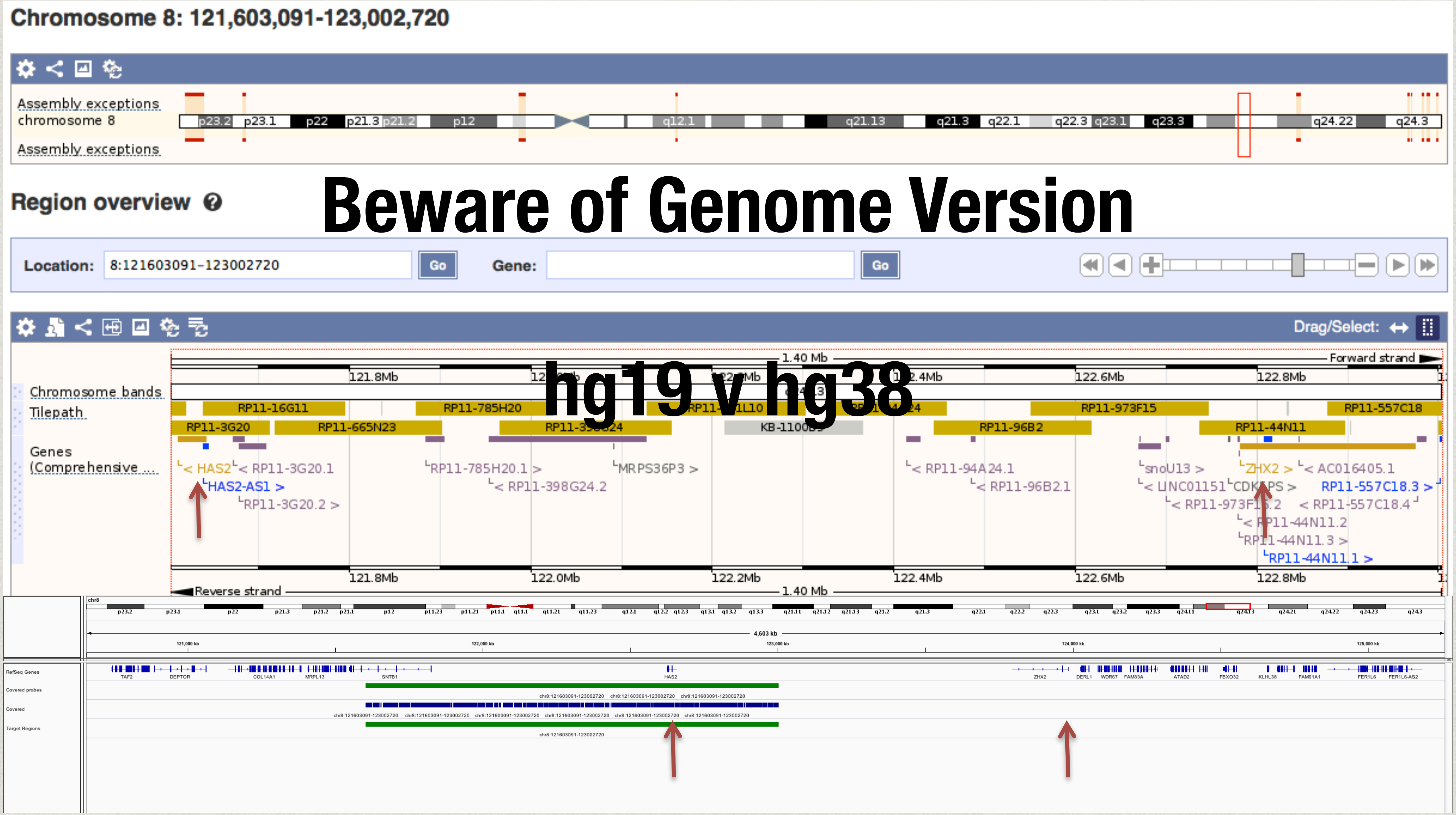
Functional & Structural Analysis
- Bayesian weights
- 3D structure
- Genomic analysis

Pathway level analysis
- GeneGo Metacore
- STITCH
- DAVID

Hypothesis Generation & Testing

www. smd.qmul.ac.uk

Targeted Re-Sequencing



Chromosome 8: 121,603,091-123,002,720

Region overview

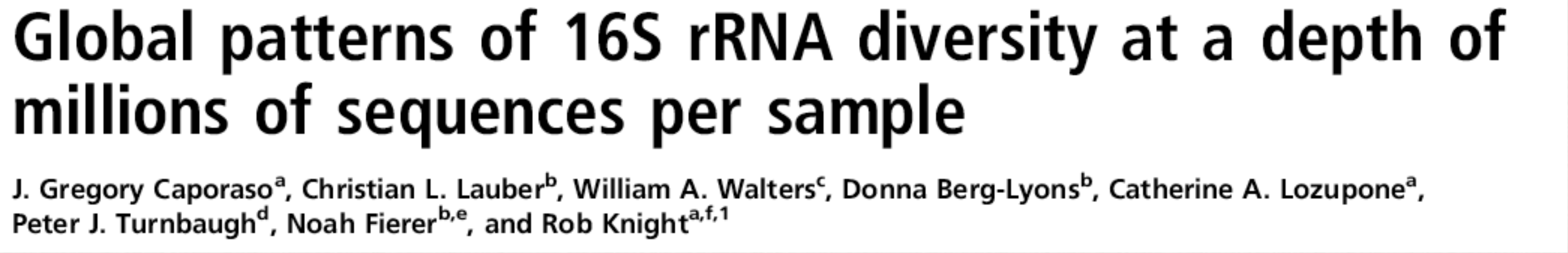
Location: 8:121603091-123002720

Gene:

Beware of Genome Version

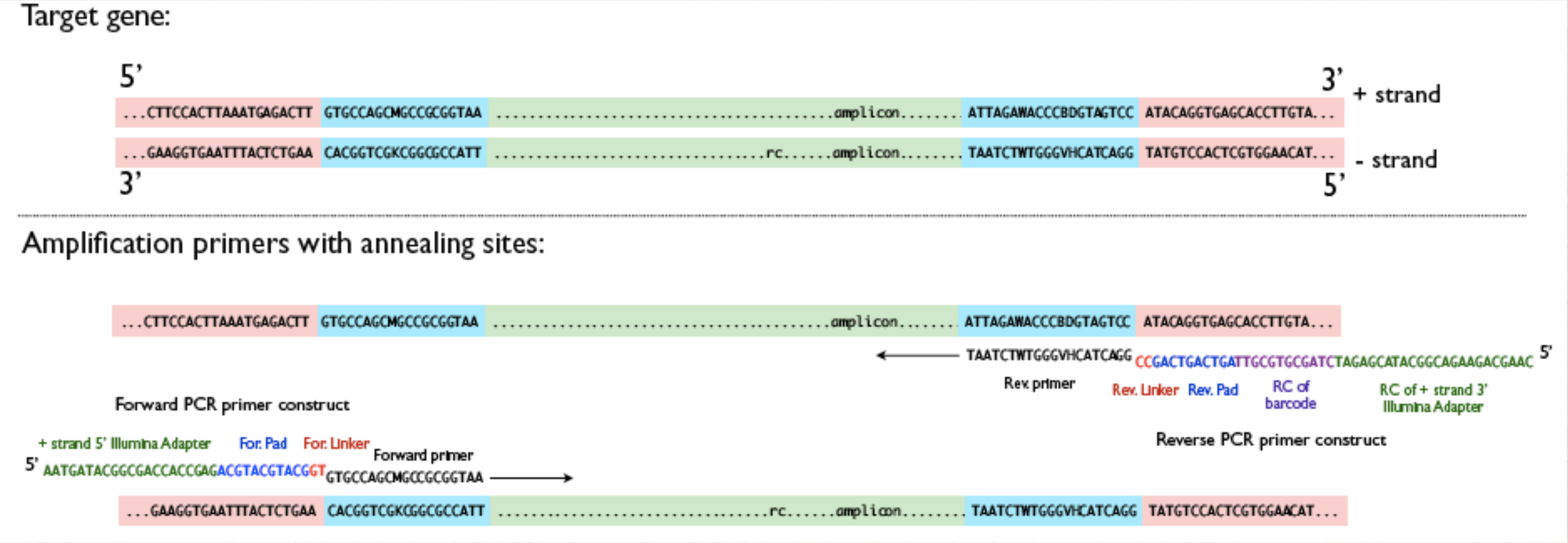
hg19 v hg38

Microbiome

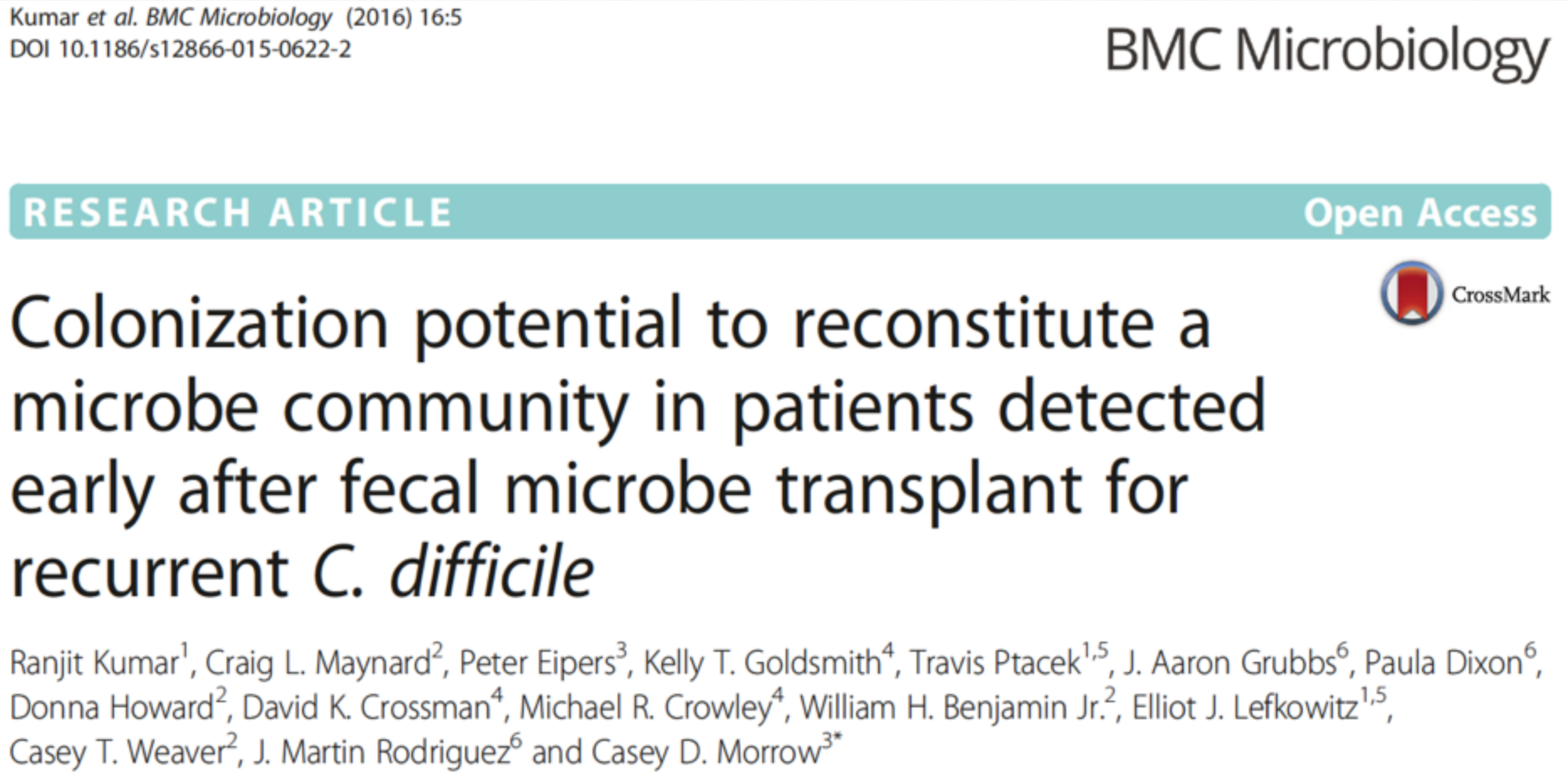


Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample

J. Gregory Caporaso[a], Christian L. Lauber[b], William A. Walters[c], Donna Berg-Lyons[b], Catherine A. Lozupone[a], Peter J. Turnbaugh[d], Noah Fierer[b,e], and Rob Knight[a,f,1]



Microbiome Shared Facility directed by Casey Morrow, Ph.D.



Kumar *et al. BMC Microbiology* (2016) 16:5
DOI 10.1186/s12866-015-0622-2

BMC Microbiology

RESEARCH ARTICLE **Open Access**

Colonization potential to reconstitute a microbe community in patients detected early after fecal microbe transplant for recurrent *C. difficile*

Ranjit Kumar[1], Craig L. Maynard[2], Peter Eipers[3], Kelly T. Goldsmith[4], Travis Ptacek[1,5], J. Aaron Grubbs[6], Paula Dixon[6], Donna Howard[2], David K. Crossman[4], Michael R. Crowley[4], William H. Benjamin Jr.[2], Elliot J. Lefkowitz[1,5], Casey T. Weaver[2], J. Martin Rodriguez[6] and Casey D. Morrow[3*]

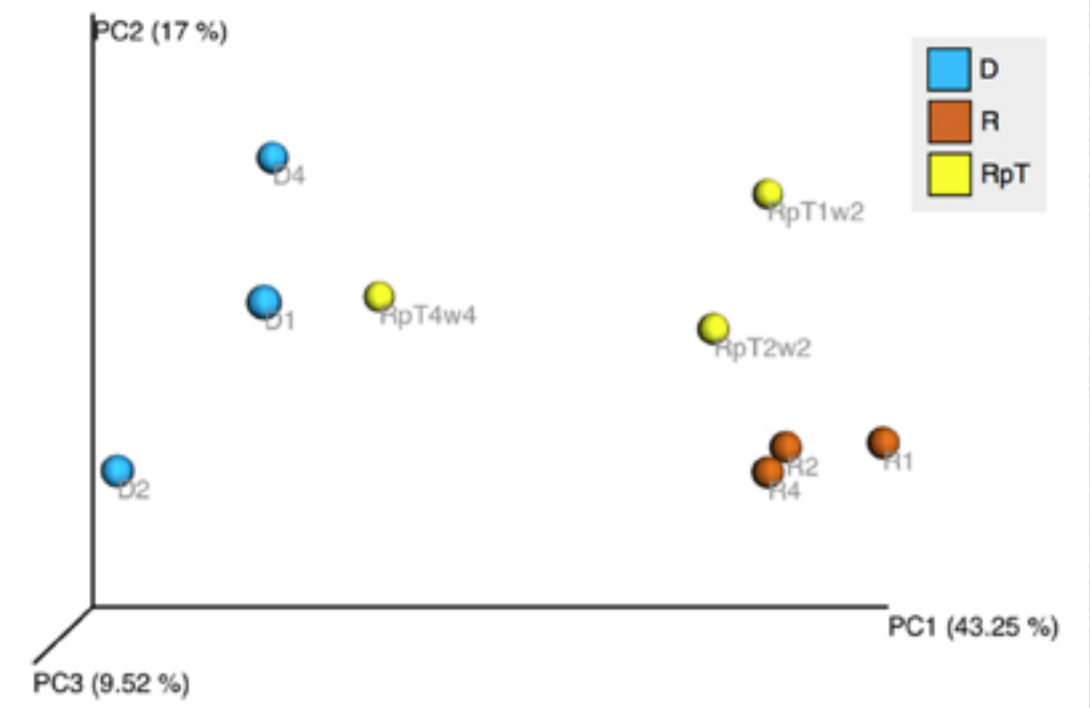


Fig. 1 Comparison of the microbial diversity of human donors and recipients pre and post FMT. Principal Coordinate analysis is used to generate 3D PCoA plot (using weighted UniFrac distance metrics) for the fecal samples from the donor (D) and recipient (R) and post FMT (RpT)

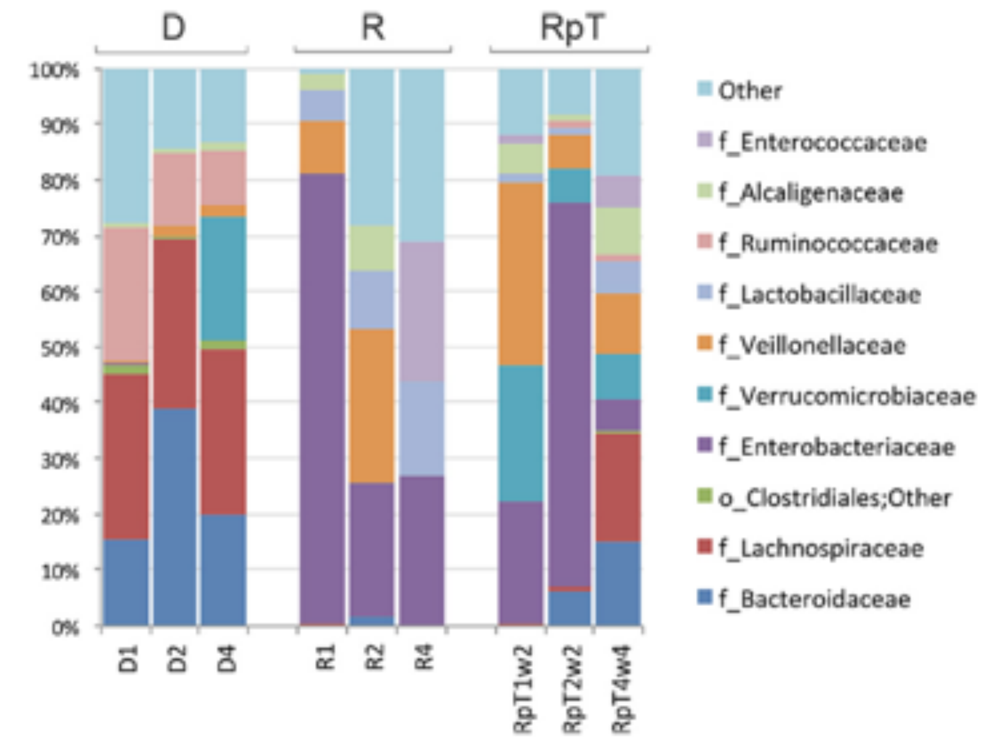
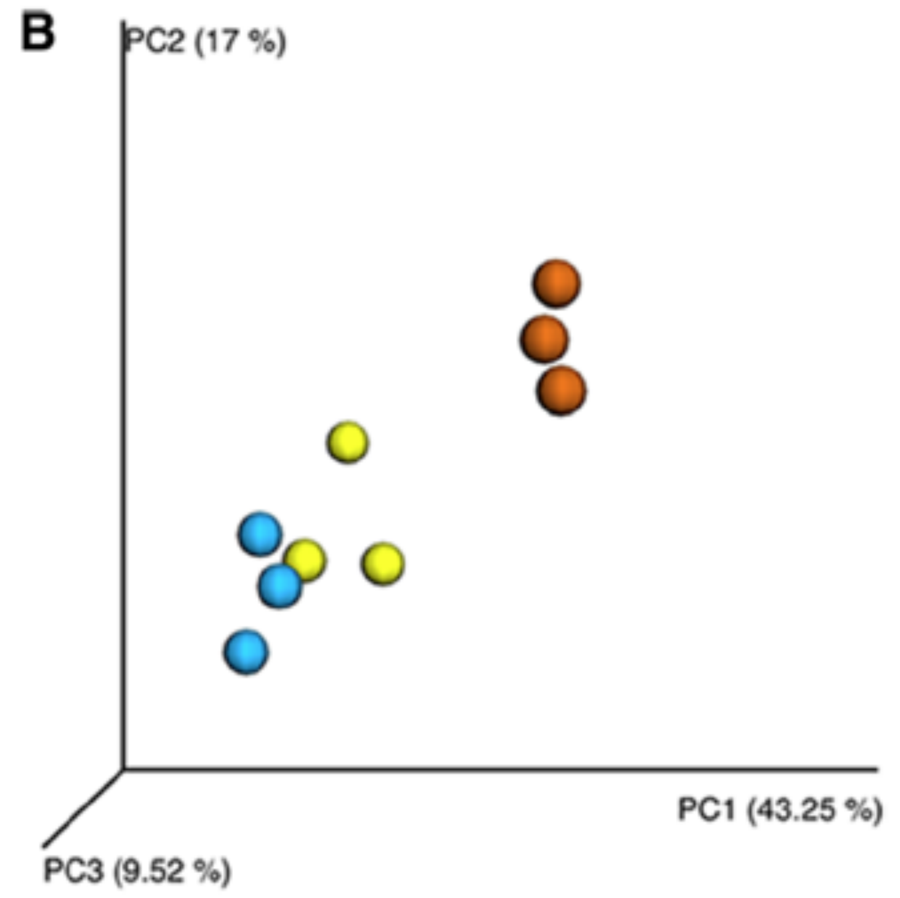
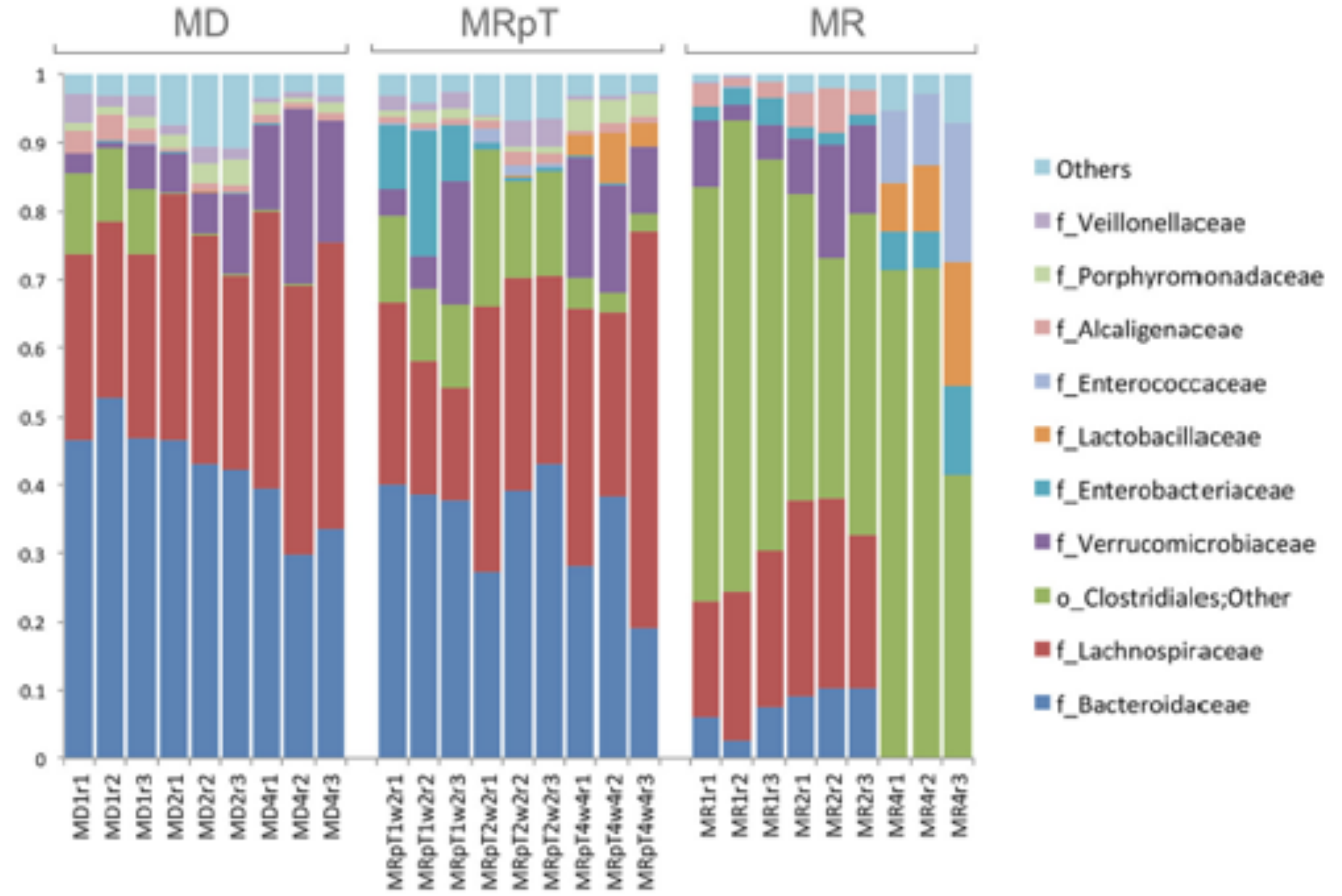


Fig. 2 Taxa distribution of human donors and recipients pre and post transplant. A stacked bar plot depicting the taxa distribution at the *family* level of the fecal samples of individual samples from the donors and recipient pre and post FMT





PRICING

A lot goes into calculating the price of an assay including:

- price of the reagents
- shipping of the reagents
- consumables for the prep (tubes, tips, Q.C., library quant, etc.)
- labor
- maintenance agreement costs ($86,000/year on HiSeq2500 alone)

RNA-Seq library prep:	$350/sample (standard PolyA selection)
ribosome reduction:	$100/sample
DNA Library prep:	$300/sample
Library Quantitation:	$25/library

Sequencing Costs:

Single End 50bp	$9.75/million reads
Paired End 50bp	$13/million reads
Paired End 100bp	$15.75/million reads

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Caitlin Cox

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