Sequence Analysis : An Introduction

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Experiments

- ChIP-seq
- Differential expression
- RNA-seq (alternate splicing)
- Metagenomic
- ...

Technology

Platforms

- Illumina / Genome Analyzer
- Roche / 454
- Applied Biosystems / SOLiD
- Complete Genomics

Pre-processing

Vendor and third-party

- Image processing, base calling
- Machine quality assessment
- Alignment

Bioconductor

- Quality assessment and representation: *ShortRead*, *GenomicRanges*
- Read remediation, trimming, primer removal, specialized manipulation: *IRanges*, *ShortRead*, *Biostrings*
- Specialized alignment tasks: *Biostrings*, *BSgenome*

Bioconductor Sequence Analysis Packages

ChIP-seq

- *BayesPeak*, *chipseq*, *ChIPseqR*, *ChIPsim*, *PICS*

RNA-seq and snRNA discovery

- *Genominator*, *rnaSeqMap*, *segmentSeq*

Metagenomics

- *OTUbase*

Methyl-seq

- *MEDIPS*, *methVisual*

Related: genotyping

- *GGtools*, *VanillaICE* (variant calls from SNP arrays)

Annotation and Integration

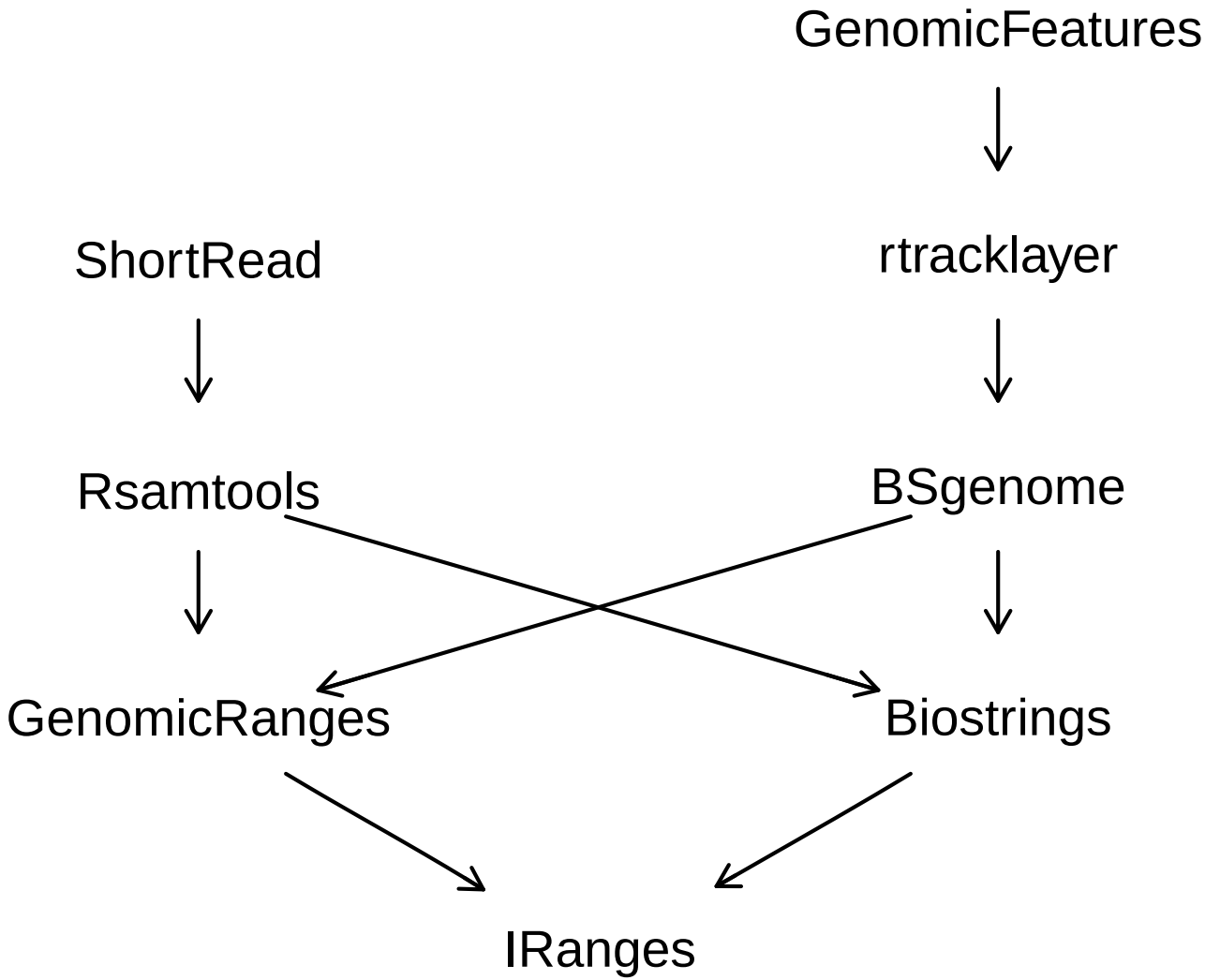
Annotation

- Genome coordinate / gene (and other) relationships, *GenomicFeatures*, *ChIPpeakAnno*
- Resources originally developed for microarray analysis – *AnnotationDbi*, *org.*.db*, *KEGG.db*, *GO.db*, *Category*, *GOstats*

Integration

- Digital and microarray differential expression
- RNAseq and gene ontology / pathway, *goseq*
- HapMap, 1000 genomes, UCSC, Sequence Read Archive, GEO, ArrayExpress, *rtracklayer*, *biomaRt*, *Rsamtools*, *GEOquery*, *SRAdb*

Bioconductor Sequence Packages



ShortRead data input

```
> library(SeattleIntro2010)
> library(ShortRead)
> fl <- system.file("extdata", "SRR002051.chrI-V.bam",
+   package="SeattleIntro2010")
> aln <- readAligned(fl, type = "BAM")
```

The *AlignedRead* class

```
> aln

class: AlignedRead
length: 446075 reads; width: 33 cycles
chromosome: chrI chrI ... chrV chrV
position: 11 1062 ... 576545 576836
strand: - + ... - -
alignQuality: NumericQuality
alignData varLabels: flag

> table(strand(aln), useNA="always")

     +      -      *   <NA>
215256 230819      0      0
```

Accessing reads, base quality, and other data

```
> head(sread(aln), 3)

  A DNAStringSet instance of length 3
    width seq
[1]    33 TGTGGTGTGTGGTG...GTGGGTGTGTGGG
[2]    33 TGCATCTTTAATCT...TTACACTACTCAT
[3]    33 TTAAATAACGTACC...AGTATCGTCTTGA
```

Alphabet by cycle

Expectation: nucleotide use independent of cycle

```
> alnp <- aln[strand(aln) == "+"]
> abc <- alphabetByCycle(sread(alnp))
> class(abc)
```

```
[1] "matrix"
```

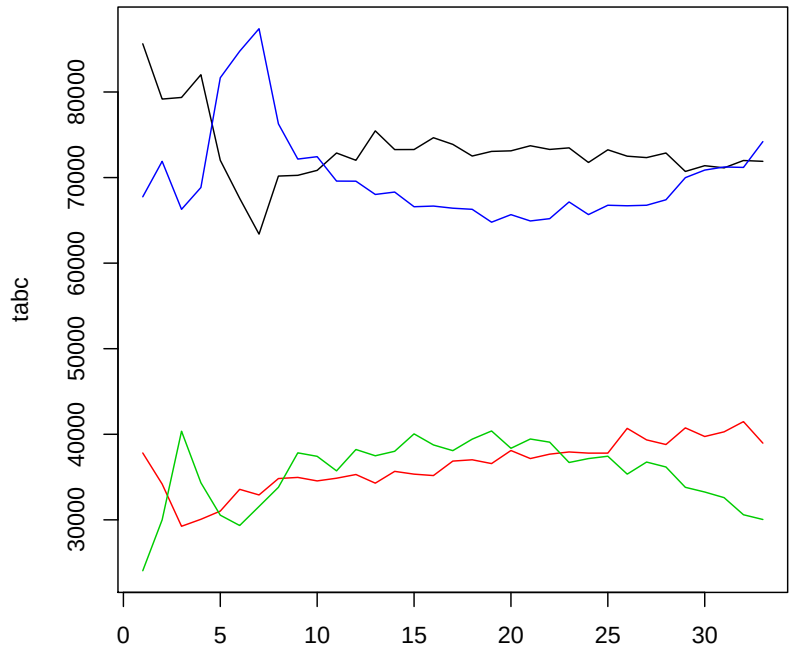
```
> abc[1:6,1:4]
```

```
         cycle
alphabet  [,1]  [,2]  [,3]  [,4]
       A 85632 79174 79363 82020
       C 37815 34182 29250 30064
       G 24054 30002 40356 34334
       T 67755 71896 66287 68838
       M     0     0     0     0
       R     0     0     0     0
```

Alphabet by cycle

`matplot` takes a matrix and plots each column as a set of points

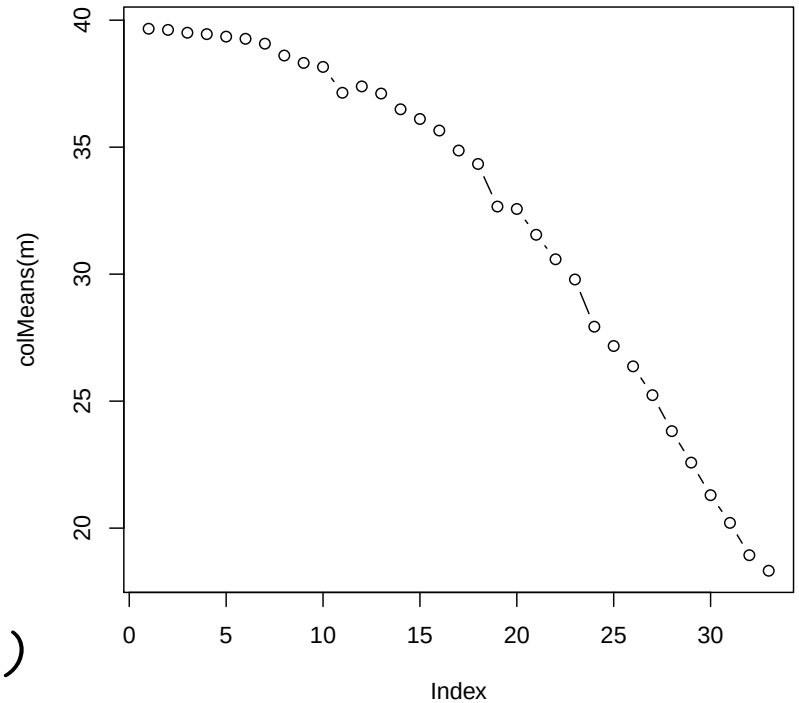
```
> tabc <- t(abc[1:4,])
> matplot(tabc, type="l",
+         lty=rep(1, 4))
```



Quality by cycle

Encoded quality scores can be decoded to their numerical values and represented as a matrix. Calculating the average of the column means creates a vector of average quality scores across cycle.

```
> m <- as(quality(alnp),
+         "matrix")
> plot(colMeans(m), type="b")
```



Recoding and updating

1. Access the chromosome information
2. Extract the chromosome number from the factor level
3. Recode the chromosome number to roman (!), create new levels, and update the chromosome
4. Update the *AlignedRead*

```
> chrom <- chromosome(alnp)
> i <- sub("S288C_([[:digit:]]+)", "\\1", levels(chrom))
> levels(chrom) <- paste("chr", as.roman(i), sep="")
> alnp <- renew(alnp, chromosome=chrom)
```

Rsamtools

Enhancements to *AlignedRead* and *ShortRead*

- Easy to input arbitrary subset of reads
- Not necessary to read sequence, quality, identifier and other information when not necessary
- Reads can be aligned with indels and gaps

samtools and *Rsamtools*

samtools

- Data format – text (SAM) and binary (BAM)
- Tools to manipulate (e.g., merge), analyze (e.g., pileup) and view
- For developers – bindings to other languages, e.g., Picard

Rsamtools

- Input and represent BAM files.
- High-level: `readAligned(..., type="BAM")`; `readGappedAlignments`; `readPileup`
- Flexible: `scanBam`

Input

ScanBamParam

- which: *GRanges* selecting reference, genome coordinates, strand.
- flag: select paired / mapped / mate mapped reads
- what: fields to retrieve, e.g., query name, reference name, strand, position, width, cigar

GenomicRanges : Gapped alignments

The *GappedAlignments* class in *GenomicRanges*

- `readGappedAlignments` uses `scanBam`
- Genomic coordinates, 'cigar', covered intervals
- Cigar: run length encoding; M (match), I, D (insertion, deletion), N (skipped), S, H (soft, hard clip), P (padding). E.g., `35M`, `18M2I15M`
- Accessors, subsetting, narrowing, `pintersect`, `coverage`, ...

Example

```
> ## reads on chr III overlapping 100000-110000
> which <- GRange("chrIII", IRanges(100000, 110000))
> param <- ScanBamParam(which=which)
> bf <- scanBam("path/to/bamfile", param=param)
```

`scanBam` returns a nested list

- One element for each row of `GRanges`
- Nested elements correspond to `what`

454 Microbiome Pre-Processing

```
> library(ShortRead)
> dir <- "/not/public"
> bar <- read454(dir)                       # Input
> code <- narrow(sread(bar), 1, 8) # Extract bar code
> aBar <- bar[code == "AAGCGCTT"]  # Subset one bar code
> noBar <-                                  # Remove bar code
+     narrow(aBar, 11, width(aBar))
> pcrPrimer <- "GGACTACCVGGGTATCTAAT"
> trimmed <-                                # Remove primer
+     trimLRPatterns(pcrPrimer, noBar, Lfixed=FALSE)
> writeFastq(trimmed,                       # Output
+            file.path(dir, "trimmed.fastq"))
```

Digital Gene Expression

```
> library(GenomicFeatures)
> bamFile <- "/path/to/file.bam"
> aligns <- readGappedAlignments(bamFile)
> ## ... txdb: transcripts from UCSC 'knownGenes'
> exonRanges <- exonsBy(txdb, "tx")
> ## ... housekeeping
> counts <- countOverlaps(exonRanges, aligns)
> ## ... normalization --> 'highScores' variable
> txs <- transcripts(txdb,
+             vals=list(tx_id=names(highScores)),
+             columns=c("tx_id","gene_id"))
> systematicNames <- elementMetadata(txs)[["gene_id"]]
```

Resources

Bioconductor Web site

- `http://bioconductor.org`
- 'Installation', 'Software', and 'Mailing lists' links.

Help in *R*

- `help.start()` to view a help browser.
- help(package = "Biostrings")
- `?readAligned`
- `browseVignettes("GenomicRanges")`