

# Annotation and High Throughput Sequencing

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# Annotation Resources – Genes and Genomes

## *AnnotationDbi*

- ▶ Chip, 'org', GO, KEGG, homology
- ▶ Curated from NCBI, GO, other sources for each *Bioconductor* release.
- ▶ SQL 'under the hood'

## *biomaRt*

- ▶ Large online annotation collection
- ▶ Curated by OICR / EMBL-EBI

## *BSgenome*

- ▶ Genome sequences – try `available.genomes`

# Demo

*AnnotationDbi, biomaRt*

# Work Flow: Sequence Analysis

## Prior to analysis

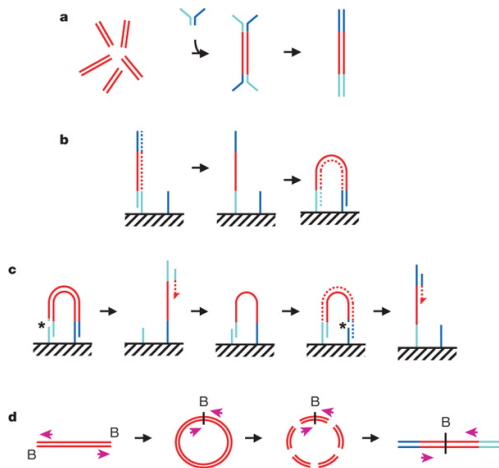
- ▶ Biological experimental design – treatments, replication, etc.
- ▶ Sequencing preparation – library preparation, manufacturer protocol, etc.

## Analysis

1. Pre-processing (sequencing, alignment, quality assessment)
2. Count, e.g., reads per transcript – ChIP-seq; RNA-seq; novel transcript identification; microbiome; ...
3. Differential representation / ChIP-seq / SNP / ...
4. Annotation
5. ...

<http://bioconductor.org/workflows> for common analyses.

# Bridge PCR



Bentley et al., 2008, Nature 456: 53-9

## Bioconductor entry points

- ▶ Quality assessment.
- ▶ Preliminary read processing, e.g., demultiplexing, remediation
- ▶ Specialized alignment, e.g., `matchPDict` in *Biostrings*.
- ▶ 'Upstream' domain-specific work flows, e.g., ChIP-seq peak calling (*chipseq*), RNA-seq reads per transcript (*GenomicRanges* / *IRanges* / ...)
- ▶ Statistical analysis of designed experiments, e.g., *edgeR*, *DESeq*
- ▶ Specialized analysis, e.g., microbiome sequence processing and ecological analysis (*vegan*, *ape*, ...)

# Sequence I/O

## Packages

*Biostrings* DNA sequence, pattern matching

*Rsamtools* BAM manipulation

*ShortRead* 'traditional' aligned reads; quality assessment

*rtracklayer* GFF and other formats; browser interaction

*GenomicRanges* Regions of interest / aligned reads as collections of ranges on genomes

## Functions

- ▶ `readFasta`, `readFastq`, `writeFasta`, `writeFastq`
- ▶ `scanBam` (also `sort`, `index`, `filter` BAM files; `BCF`, `indexed fasta`)
- ▶ `import` / `export` (for GFF & friends)
- ▶ `readAligned`, `readGappedAlignments`

# Representing Sequence Information

## *DNAStringSet*

- ▶ Collections of DNA sequences, e.g., microarray probes, Illumina reads
- ▶ Quality scores

## *GRanges*

- ▶ Genome coordinates – reference sequence name, start and end coordinates, strand; e.g., aligned reads
- ▶ *GRangesList* – hierarchical structure, e.g., exons within transcripts

Additional classes: *AlignedRead*, *GappedAlignment*, ...



# Sequence Annotations

- ▶ Existing infrastructure for gene-level annotation

## *GenomicFeatures*

- ▶ Idea: retrieve annotations from common sources, e.g., UCSC genome browser 'known genes' track; save as a local data base.
- ▶ Query for regions of interest, e.g., exons per transcript

# Demo

*DNAStringSet, GRanges, AlignedRead and GappedAlignment,  
GenomicFeatures*

# Lab activity

Goal: Explore sequences and their annotation

1. Data input and exploration
2. Gapped alignments
3. Transcript annotations
4. Counting reads aligned to regions
5. (Differential representation)
6. Annotation to biological function

## Example Data

Nagalakshmi et al., 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing, *Science* 320: 1344–1349 [?].

- ▶ Original ‘RNA-seq’ experiment
- ▶ Two different primers to generate DNA from poly(A) RNA:
  - RH Random hexamer
  - dT oligo(dT)
- ▶ Biological and technical replicates
- ▶ Illumina GAI – relatively small number (<5 million / lane) of short (33bp) reads; poor trailing base quality.