

Bioconductor packages for short read analyses

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Foreword

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General
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Objectives

- The “core” packages for integrating NGS data analysis represents a massive structure.
- It is under very active development and often different ways exist to achieve one goal.
 - *e.g RangedData vs. GRanges*
- The trunk of this core starts to reach maturity and redundant branches might be pruned.

- Introduce all the necessary packages to perform the QA and the pre-processin of NGS rawdata:
 - **biomRt**
 - **rtracklayer**
 - **Biostrings**
 - **BSgenome**
 - **GenomicFeatures**
 - **GenomicRanges**
 - **IRanges**
 - **Rsamtools**
 - **ShortRead**

Before we “really” start: the Classes in R

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- Two kinds: S3 and S4
 - S3 are old and informal, setting the class attribute is enough to “convert” an object into a class
 - S4 is an attempt at making R more object oriented
 - they have specific definitions
 - they define “fields” called “**slots**”
 - they can inherit and be inherited from
 - they can have prototypes, validators
 - they can be virtual
 - etc.
 - Most of the classes described here are of S4 type, except when backward compatibility with the R core required otherwise
 - More information can be found in the R help page: `?classRepresentation`

Methods to browse S4 classes

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(Load the IRanges library to run the following example)

```
> require(IRanges)
> ?Classes
> ?Methods
> getClass("RleList")
```

Virtual Class "RleList" [package "IRanges"]

Slots:

Name:	elementType	elementMetadata	metadata
Class:	character	DataTableORNULL	list

Extends:

Class "AtomicList", directly
Class "List", by class "AtomicList", distance 2
Class "Vector", by class "AtomicList", distance 3
Class "Annotated", by class "AtomicList", distance 4

Known Subclasses: "RleViews", "CompressedRleList", "SimpleRleList"

Methods to browse S4 classes

```
> names(completeSubclasses(getClass("RleList")))
[1] "RleViews"          "CompressedRleList" "SimpleRleList"
> head(showMethods(classes="RleList",printTo=FALSE))
[1] ""                  "Function \"activeView\": \"
[3] " <not an S4 generic function>" ""
[5] "Function \"activeView<-\": \" " <not an S4 generic function>"
> showMethods("values",includeDefs=TRUE)
Function: values (package IRanges)
x="QASummary"
function (x, ...)
{
  x@values
}

x="RangedData"
function (x, ...)
{
  .local <- function (x)
    x@values
  .local(x, ...)
}

x="SummarizedExperiment"
function (x, ...)
{
  values(rowData(x), ...)
}
```

Packages dependencies

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- Sometimes packages define the same function resulting in one of the function to be inaccessible anymore.
- When this happens, one needs to contact the packages authors for them to find an appropriate solution
- In the meanwhile, the hack described on the next slides might help
- load the GenomicRanges and the genomeIntervals in that order

Packages dependencies

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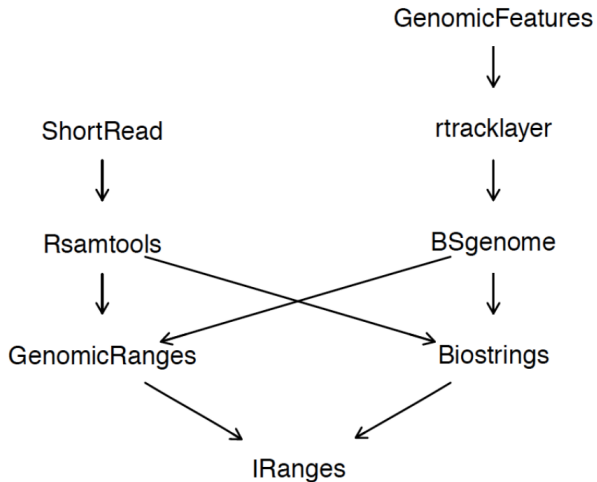
- For the purpose of the example it is not necessary to understand the actual objects that are created. We'll come back to them later.
- Create the necessary object
 - `grngs <- GRanges (seqnames=c("chr1","chr2","chr3"),
ranges=IRanges(start=c(3,4,1),end=c(7,5,3)),
strand=c("+","+","-"), seqleths=c("chr1"=24,"chr2"=18))`

Bottom - up approach

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Infrastructure package

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■ IRanges

- Long sequences, compressed and pointer referenced
- **Views** on long sequences
- Integer overlap functions; e.g. interval overlap
- Used to define genomic intervals (i.e. RangedData)

■ GenomicRanges

Recent

- IRanges extension
- Adds discontinuous genomic interval sets (useful for gapped alignments)

■ genomeIntervals

Not Core

- Very similar to IRanges
- Extremely efficient at interval calculations; e.g. interval overlap

Infrastructure Views

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- Issue
 - DNA sequences can be very large (think of the human genome)
 - Duplicating them in memory is contra-efficient
- Therefore the views!
 - Views is yet another IRanges class
 - a virtual class for storing set of views (pointers) on a single Sequence object
 - available as RleViews, XStringViews, XIntegerViews, XStringSetViews, etc.
 - it stores the sequence using a “pass-by-reference” semantic and associates ranges to select the subsequences

Infrastructure Running Length Encodings (RLEs)

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- Issue
 - Again, memory is the limit. holding a coverage vector at a single bp resolution is inefficient.
- Therefore the concept of RLEs
 - a common compression technique for piecewise constant data
 - 0 0 0 1 1 1 2 2 3 3 3 ... can be compressed in
 - 0(3), 1(3), 2(2), 3(3),...
 - it couples values e.g. 0 with a run length i.e. 3
 - Can be partitioned into RleList, e.g. for storing the coverage of different chromosomes

Infrastructure methods

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- get the methods for the Rle S4 class
 - `f.list <- showMethods(classes="Rle", printTo=FALSE)`
- process the result to extract the function name
 - `sapply(strsplit(f.list[grep("Function",f.list,"")],
function(l)gsub(' ','',l[[2]]))`

```
> f.list<-showMethods(classes="Rle", printTo=FALSE)
> length(sapply(strsplit( f.list[grep("Function",
+   f.list)],' '),function(l){gsub('\ '|:','',l[[2]]}))

[1] 340
```

Infrastructure methods, some examples

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- **Arithmetic** $+$, $-$, $*$, $'$ $\%\%$, $\%/ \%$, $/$
- **Compare** $==$, $>$, $<$, $!=$, $<=$, $>=$
- **Logic** $\&$, $|$
- **Math** abs , sign , sqrt , ceiling , floor , trunc , cummax , cummin , cumprod , cumsum , log , log10 , log2 , log1p , acos , acosh , asin , asinh ,...
- **Math2** round , signif
- **Summmary** max , min , range , prod , sum , any , all

Looks intimidating

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- Still the point is:
 - whenever you think about a functionality, it probably already exists.

Example 1: coverage

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■ Coverage calculation

```
> require("ShortRead")
> fl<-system.file("extdata","GSM424494_wt_G2_orc_chip_rep1_S288C_14.mapview.txt.gz",
+               package="EatonEtAlChIPseq")
> aln<-readAligned(fl,type="MAQMapview")
> cover<-coverage(aln);cover
> cover[["S288C_14"]]
> head(runValue(cover[["S288C_14"]]))
> as.integer(cover[["S288C_14"]])
> smoothCover<-round(runmean(cover,75,endrule="constant"))
> class(smoothCover)
> smoothCover
```

Example 2: slice

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■ Finding wide regions with elevated coverage

```
> islands<-slice(smoothCover,lower=10)
> islandsWithWidePeaks<- islands[vienMaxs(islands)>=20L &width(islands)>=500L]
> islandsWithWidePeaks
```

What comes on top of IRanges

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- We've "covered" **IRanges** and it's low level capabilities.
- Still, High Throughput methods in biology, especially sequencing, are more about sequences than maths.
- Therefore the **Biostrings** package, build on top of IRanges



Biostrings

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- All the classes in that package derives from the XString class

```
> require(Biostrings)
> getClass("XString")
```

```
Virtual Class "XString" [package "Biostrings"]
```

Slots:

Name:	shared	offset	length	elementMetadata
Class:	SharedRaw	integer	integer	DataTableORNULL

Name:	metadata
Class:	list

Extends:

```
Class "XRaw", directly
Class "XVector", by class "XRaw", distance 2
Class "Vector", by class "XRaw", distance 3
Class "Annotated", by class "XRaw", distance 4
```

Known Subclasses: "BString", "DNABString", "RNABString", "AABString"

- There are 4 subclasses:
 - **BString**: store strings without alphabet
 - **DNABString**: store strings with an DNA alphabet
 - **RNABString**: store strings with an RNA alphabet
 - **AABString**: store strings with an Amino Acid alphabet

An DNAString example

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■ The **Biostring** package contains many example datasets

```
> data(package="Biostrings")
> data(yeastSEQCHR1)
> class(yeastSEQCHR1)

[1] "character"

> nchar(yeastSEQCHR1)

[1] 230208

> DNAString(yeastSEQCHR1)

230208-letter "DNAString" instance
seq: CCACACCACACCCACACACCCACACACCACACCACA...GGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGTGGG
```

■ The obtained DNAString is defined by the DNA alphabet

```
> alphabet(DNAString(yeastSEQCHR1))

[1] "A" "C" "G" "T" "M" "R" "W" "S" "Y" "K" "V" "H" "D" "B" "N" "-" "+" "."
```

The alphabets

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■ The **Biostring** package implements the possible alphabets

```
> GENETIC_CODE
TTT TTC TTA TTG TCT TCC TCA TCG TAT TAC TAA TAG TGT TGC TGA TGG CTT CTC CTA CTG
"F" "F" "L" "L" "S" "S" "S" "S" "Y" "Y" "*" "*" "C" "C" "*" "W" "L" "L" "L" "L"
CCT CCC CCA CCG CAT CAC CAA CAG CGT CGC CGA CGG ATT ATC ATA ATG ACT ACC ACA ACG
"P" "P" "P" "P" "H" "H" "Q" "Q" "R" "R" "R" "R" "I" "I" "I" "M" "T" "T" "T" "T"
AAT AAC AAA AAG AGT AGC AGA AGG GTT GTC GTA GTG GCT GCC GCA GCG GAT GAC GAA GAG
"N" "N" "K" "K" "S" "S" "R" "R" "V" "V" "V" "V" "A" "A" "A" "A" "D" "D" "E" "E"
GGT GGC GGA GGG
"G" "G" "G" "G"

> AMINO_ACID_CODE
      A      R      N      D      C      Q      E      G      H      I      L      K      M
"Ala" "Arg" "Asn" "Asp" "Cys" "Gln" "Glu" "Gly" "His" "Ile" "Leu" "Lys" "Met"
      F      P      S      T      W      Y      V      U      O      B      Z      X
"Phe" "Pro" "Ser" "Thr" "Trp" "Tyr" "Val" "Sec" "Pyl" "Asx" "Glx" "Xaa"

> RNA_GENETIC_CODE
UUU UUC UUA UUG UCU UCC UCA UCG UAU UAC UAA UAG UGU UGC UGA UGG CUU CUC CUA CUG
"F" "F" "L" "L" "S" "S" "S" "S" "Y" "Y" "*" "*" "C" "C" "*" "W" "L" "L" "L" "L"
CCU CCC CCA CCG CAU CAC CAA CAG CGU CGC CGA CGG AUU AUC AUA AUG ACU ACC ACA ACG
"P" "P" "P" "P" "H" "H" "Q" "Q" "R" "R" "R" "R" "I" "I" "I" "M" "T" "T" "T" "T"
AAU AAC AAA AAG AGU AGC AGA AGG GUU GUC GUA GUG GCU GCC GCA GCG GAU GAC GAA GAG
"N" "N" "K" "K" "S" "S" "R" "R" "V" "V" "V" "V" "A" "A" "A" "A" "D" "D" "E" "E"
GGU GGC GGA GGG
"G" "G" "G" "G"

> IUPAC_CODE_MAP
      A      C      G      T      M      R      W      S      Y      K      V
"A" "C" "G" "T" "AC" "AG" "AT" "CG" "CT" "GT" "ACG"
      H      D      B      N
"ACT" "AGT" "CGT" "ACGT"
```

Set of Strings

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- XStrings and subclasses instances can all be grouped into Sets

```
> names(completeSubclasses(getClass("XStringSet")))
[1] "BStringSet"           "DNAStringSet"
[3] "RNAStringSet"         "AAStringSet"
[5] "QualityScaledXStringSet" "XStringQuality"
[7] "QualityScaledBStringSet" "QualityScaledDNAStringSet"
[9] "QualityScaledRNAStringSet" "QualityScaledAAStringSet"
[11] "QualityScaledBStringSet" "QualityScaledDNAStringSet"
[13] "QualityScaledRNAStringSet" "QualityScaledAAStringSet"
[15] "PhredQuality"         "SolexaQuality"
[17] "IlluminaQuality"
```

- Again, there are data examples within the **Biostring** package to play with

```
> data(srPhiX174)
> class(srPhiX174)
```

```
[1] "DNAStringSet"
attr(,"package")
[1] "Biostrings"
```

```
> head(srPhiX174)
```

```
A DNAStringSet instance of length 6
width seq
[1] 35 GTTATTATACCGTCAAGGACTGTGTGACTATTGAC
[2] 35 GGTGGTTATTATACCGTCAAGGACTGTGTGACTAT
[3] 35 TACCGTCAAGGACTGTGTGACTATTGACGTCCTTC
[4] 35 GTACGCCGGCAATAATGTTTATGTTGGTTTCATG
[5] 35 GGTTTCATGGTTTGGTCTAACTTTACCGCTACTAA
[6] 35 GGGCAATAATGTTTATGTTGGTTTCATGGTTTGGT
```

XString Methods

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- Basic utilities
 - subsequence selection
 - subseq, Views, narrow (XStringSet, IRanges package)
 - letter frequencies
 - alphabetFrequency, dinucleotideFrequency (tri..., oligo...), uniqueLetters
 - letter consensus
 - consensusMatrix, consensusString
 - letter transformation
 - reverse, complement, reverseComplement, translate, chartr
 - Input/Output
 - read.DNAStringSet (...B...,...RNA...,...AA..)
 - write.XStringSet, save.XStringSet

Xstrings Methods (c'ed)

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■ Advanced

- **alignment utilities**: pairwiseAlignment, stringDist

- **string matching**

- (v)matchPDict (on a reference or a reference set (v))
- (v)matchPDict, (v)countPDict,(v)whichPDict
- matchPattern
- (v)matchPattern,(v)countPattern, neditStartingAt, neditEndingAt, (which.) isMatchingStartingAt, (which.)isMatchingEndingAt
- matchPWM(Position Weight Matrix, e.g. for transcription factor binding sites)
- matchPWM,countPWM

- **Others**

- matchLRPatterns, trimLRPatterns,matchProbePair, findPalindromes, findComplementedPalindromes

Example 1: Letter/ alphabet frequencies

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■ Single-letter frequencies

```
> alphabetFrequency(DNAString(yeastSEQCHR1))
```

	A	C	G	T	M	R	W	S	Y	K	V	H	D
69830	44643	45765	69970	0	0	0	0	0	0	0	0	0	0
	B	N	-	+	.								
	0	0	0	0	0								

```
> alphabetFrequency(DNAString(yeastSEQCHR1),baseOnly=TRUE)
```

	A	C	G	T	other
69830	44643	45765	69970	0	

■ Multi-letter frequencies

```
> dinucleotideFrequency(DNAString(yeastSEQCHR1))
```

	AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA
23947	12493	13621	19769	15224	9218	7089	13112	14478	8910	9438	12938	16181	
	TC	TG	TT										
14021	15617	24151											

```
> head(trinucleotideFrequency(DNAString(yeastSEQCHR1)),20)
```

	AAA	AAC	AAG	AAT	ACA	ACC	ACG	ACT	AGA	AGC	AGG	AGT	ATA	ATC	ATG	ATT
8576	4105	4960	6306	3924	2849	2186	3534	4537	2680	2707	3697	5242	3849	4294	6384	

Example 2: String manipulation

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■ Standard transformations

```
> head(narrow(srPhiX174,1,9))
```

A DNAStringSet instance of length 6
width seq

```
[1] 9 GTTATTATA
[2] 9 GGTGGTTAT
[3] 9 TACCGTCAA
[4] 9 GTACGCCGG
[5] 9 GGTTTCATG
[6] 9 GGGCAATAA
```

```
> head(reverse(narrow(srPhiX174,1,9)))
```

A DNAStringSet instance of length 6
width seq

```
[1] 9 ATATTATTG
[2] 9 TATTGGTGG
[3] 9 AACTGCCAT
[4] 9 GGCCGCATG
[5] 9 GTACTTTGG
[6] 9 AATAACGGG
```

```
> head(reverseComplement(narrow(srPhiX174,1,9)))
```

A DNAStringSet instance of length 6
width seq

```
[1] 9 TATAATAAC
[2] 9 ATAACCACC
[3] 9 TTGACGGTA
[4] 9 CCGGCGTAC
[5] 9 CATGAAACC
[6] 9 TTATTGCCC
```

```
> head(translate(narrow(srPhiX174,1,9)))
```

A AAStringSet instance of length 6
width seq

```
[1] 3 VII
[2] 3 GGY
[3] 3 YRQ
[4] 3 VRR
[5] 3 GFM
[6] 3 GQ*
```

Example 2: String manipulation

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■ Bisulffite transformation

```
> alphabetFrequency(chartr("C","T", DNASTring(yeastSEQCHR1)),baseOnly=TRUE)
```

A	C	G	T	other
69830	0	45765	114613	0

```
> alphabetFrequency(DNASTring(yeastSEQCHR1),baseOnly=TRUE)
```

A	C	G	T	other
69830	44643	45765	69970	0

Example 3: Consensus

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■ Consensus matrix

```
> snippet<-subseq(head(sort(srPhiX174),5),1,10);snippet
```

```
A DNAStringSet instance of length 5  
width seq
```

```
[1] 10 AAATAATGTT  
[2] 10 AACGTTATAT  
[3] 10 AAGGAATGTG  
[4] 10 AAGGACTGTG  
[5] 10 AAGGACTGTG
```

```
> consensusMatrix(snippet,baseOnly=TRUE)
```

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]
A	5	5	1	0	4	2	1	0	1	0
C	0	0	1	0	0	2	0	0	0	0
G	0	0	3	4	0	0	0	4	0	3
T	0	0	0	1	1	1	4	1	4	2
other	0	0	0	0	0	0	0	0	0	0

■ Consensus string

```
> consensusString(snippet)
```

```
[1] "AAGGAMTGTK"
```

Example 4: String Matching

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■ Match counting

```
> data(phiX174Phage)
> phiX174Phage
```

```
A DNASTringSet instance of length 6
      width seq                      names
[1]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA Genbank
[2]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA RF70s
[3]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA SS78
[4]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA Bull
[5]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA G97
[6]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA NEB03
```

```
> genome<- phiX174Phage[["NEB03"]]
> negPhiX174<- reverseComplement(srPhiX174)
> posCounts<- countPDict(PDict(srPhiX174),genome)
> negCounts<- countPDict(PDict(negPhiX174),genome)
> table(posCounts,negCounts)
```

```
      negCounts
posCounts    0
0    1030
1     83
```

Example 4: String Matching

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- So we have 1030 reads that do not align either way to the genome and only 83 aligning.
- The match locations can be found using:

```
[[2]] IRanges of length 1 start end width [1] 2746 2780 35  
[[3]] IRanges of length 1 start end width [1] 2757 2791 35  
  
... <80 more elements>
```

Example 5: Pairwise alignment

■ alignment scores

```
> posScore <- pairwiseAlignment(srPhiX174, genome, type="global-local", scoreOnly=TRUE)
> negScore <- pairwiseAlignment(negPhiX174, genome, type="global-local", scoreOnly=TRUE)
> which(pmin(posScore)<pmin(negScore))
```

```
[1] 932
```

■ alignment

```
> pairwiseAlignment(srPhiX174[932],genome,type="global-local")
```

```
Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
pattern: [1] GCAATAACCTTGCGAGTCATTCTTTGATTGGTC
subject: [2804] GCAATAATGTTTATGTTGGTTTCATGG-TTTGGTC
score: -33.31176
```

```
> pairwiseAlignment(negPhiX174[932],genome,type="global-local")
```

```
Global-Local PairwiseAlignmentsSingleSubject (1 of 1)
pattern: [1] GACCAAATCAAAGAAATGACTCGCAAGGTTATTGC
subject: [3666] GACCAAATCAAAGAAATGACTCGCAAGGTTAGTGC
score: 61.4804
```


What next?

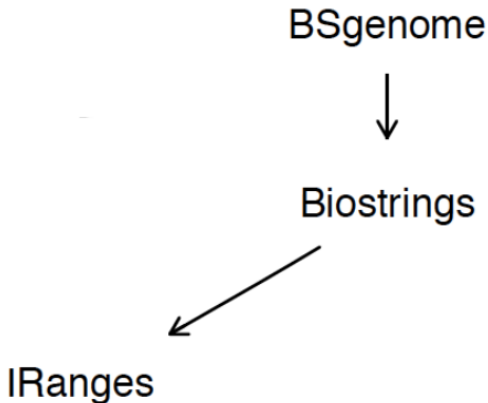
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- We now have seen how to deal with biologically meaningful intervals and objects.
- Many organism have been sequenced and their genome is know.
- An interface in R to easily acces and manipulate such information would be very useful; this is the **BSgenome** package.

- It is not just a data package; it leverages the functionalities introduced in **Biostrings**



Available genomes

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■ Easy to find out

```
> require(BSgenome)
> head(available.genomes())
```

```
[1] "BSgenome.Alyrata.JGI.v1"
[2] "BSgenome.Amelliifera.BeeBase.assembly4"
[3] "BSgenome.Amelliifera.UCSC.apiMel2"
[4] "BSgenome.Amelliifera.UCSC.apiMel2.masked"
[5] "BSgenome.Athaliana.TAIR.04232008"
[6] "BSgenome.Athaliana.TAIR.TAIR9"
```

- However, large genomes(i.e. human, mouse, ...) packages might take log to transfer.

BSgenome Class overview (c'ed)

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- Important:
 - proper S4 class usage ban accessing a slot through the “@” accessor, except within a package scope.
 - Hence, it is nowhere to be seen on the present slide

```
> library(BSgenome.Dmelanogaster.UCSC.dm3)
> # Dmelanogaster@seqs_dir
> #Dmelanogaster@mask_dir      ERROR
> #dir(Dmelanogaster@mask_dir)
```

BSgenome methods

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- **Sequence selection:** `[[, $`
- **Subsequence selection:** `getSeq`
- **Accessors:** `length`, `names/seqnames`, `mseqnames`, `seqlengths`, `masknames`, `sourceUrl`
- **Matching:** all Biostings methods
- **SNPs:** `injectSNPs`, `SNPlocspkgname`, `SNPcount`, `SNPlocs`

Sequence information

Bioconductor
packages for
short read
analyses

Alex Sánchez

General
Information
Objectives

■ operation that do not load sequences

```
> require(BSgenome.Dmelanogaster.UCSC.dm3)
> head(seqnames(Dmelanogaster))
```

```
[1] "chr2L" "chr2R" "chr3L" "chr3R" "chr4"  "chrX"
```

```
> head(seqlengths(Dmelanogaster))
```

chr2L	chr2R	chr3L	chr3R	chr4	chrX
23011544	21146708	24543557	27905053	1351857	22422827

■ operation that do

```
> alphabetFrequency(Dmelanogaster[["chr4"]],baseOnly=TRUE)
```

A	C	G	T	other
430227	238155	242039	441336	100

Extending Biostrings. Example 1

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packages for
short read
analyses

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General
Information
Objectives

■ Applying the Biostrings matching functions:

```
> exclude<-setdiff(seqnames(Hsapiens),c("chr1","chr2"))  
> vcountPattern("ACYTANCAGT", Hsapiens, fixed=c(pattern=FALSE, subject=TRUE), exclude=exclude)
```

	seqname	strand	count
1	chr1	+	1546
2	chr1	-	1545
3	chr2	+	1722
4	chr2	-	1684

```
> #vmatchPattern("ACYTANCAGT",Hsapiens, fixed=c(pattern=FALSE, subject=TRUE), exclude=exclude)  
> #asRangedData=FALSE)
```


Example 2

■ Using a Pattern Dictionary, e.g. a library of microarray probes

```
> library(hgu95av2probe)
> probes<-DNASTringSet(hgu95av2probe$sequence[1:100])
> probes[1:10]
```

```
A DNASTringSet instance of length 10
width seq
[1] 25 TGGCTCCTGCTGAGGTCCCCTTTCC
[2] 25 GGCTGTGAATTCCTGTACATATTC
[3] 25 GCTTCAATTCATTATGTTTAAATG
[4] 25 GCCGTTTGACAGAGCATGCTCTGCG
[5] 25 TGACAGAGCATGCTCTGCGTTGTTG
[6] 25 CTCTGCGTTGTTGGTTTCACCAGCT
[7] 25 GGTTTCACCAGCTTCTGCCCTCACA
[8] 25 TTCTGCCCTCACATGCACAGGGATT
[9] 25 CCTCACATGCACAGGGATTTAACAA
[10] 25 TCCTTGGTACTCTGCCCTCCTGTCA
```

Example 2

Bioconductor
packages for
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General
Information
Objectives

```
> counts<-vcountPDict(probes,Hsapiens,exclude=exclude);counts
```

```
DataFrame with 400 rows and 4 columns
```

	seqname	strand	index	count
	<Rle>	<Rle>	<integer>	<Rle>
1	chr1	+	1	0
2	chr1	+	2	0
3	chr1	+	3	0
4	chr1	+	4	0
5	chr1	+	5	0
...
396	chr2	-	96	0
397	chr2	-	97	0
398	chr2	-	98	0
399	chr2	-	99	0
400	chr2	-	100	0

```
> #whichMatch<-seqselect(counts$index,counts$count>0);whichMatch No existeix seqselect!!  
> #matchedProbes<- probes[WhichMatch];matchedProbes  
> #matchLocs<-matchPDict(PDict(matchedProbes),Hsapiens$chr2);matchLocs  
> #extractAllMatches(Hsapiens$chr2,matchLocs)
```

Example 5

Bioconductor
packages for
short read
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General
Information
Objectives

- A new interesting feature is the possibility to inject SNPs!

```
> cat(available.SNPs(),sep="\n")

SNPlocs.Hsapiens.dbSNP.20090506
SNPlocs.Hsapiens.dbSNP.20100427
SNPlocs.Hsapiens.dbSNP.20101109
SNPlocs.Hsapiens.dbSNP.20110815
SNPlocs.Hsapiens.dbSNP.20111119
SNPlocs.Hsapiens.dbSNP.20120608

> library("SNPlocs.Hsapiens.dbSNP.20090506")
> HsWithSNPs<-injectSNPs(Hsapiens,
+   "SNPlocs.Hsapiens.dbSNP.20090506")
>
>
```

Example 5

Bioconductor
packages for
short read
analyses

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General
Information

Objectives

```
> HsWithSNPs
```

```
Human genome
```

```
|  
| organism: Homo sapiens (Human)  
| provider: UCSC  
| provider version: hg19  
| release date: Feb. 2009  
| release name: Genome Reference Consortium GRCh37  
| with SNPs injected from package: SNPlocs.Hsapiens.dbSNP.20090506
```

```
| single sequences (see '?seqnames'):
```

```
| chr1          chr2          chr3  
| chr4          chr5          chr6  
| chr7          chr8          chr9  
| chr10         chr11         chr12  
| chr13         chr14         chr15  
| chr16         chr17         chr18  
| chr19         chr20         chr21  
| chr22         chrX          chrY  
| chrM          chr1_gl000191_random chr1_gl000192_random  
| chr4_ctg9_hap1 chr4_gl000193_random chr4_gl000194_random  
| chr6_apd_hap1  chr6_cox_hap2        chr6_dbb_hap3  
| chr6_mann_hap4 chr6_mcf_hap5        chr6_qb1_hap6  
| chr6_ssto_hap7 chr7_gl000195_random chr8_gl000196_random  
| chr8_gl000197_random chr9_gl000198_random chr9_gl000199_random  
| chr9_gl000200_random chr9_gl000201_random chr11_gl000202_random  
| chr17_ctg5_hap1 chr17_gl000203_random chr17_gl000204_random  
| chr17_gl000205_random chr17_gl000206_random chr18_gl000207_random  
| chr19_gl000208_random chr19_gl000209_random chr21_gl000210_random
```

What next?

Bioconductor
packages for
short read
analyses

Alex Sánchez

General
Information
Objectives

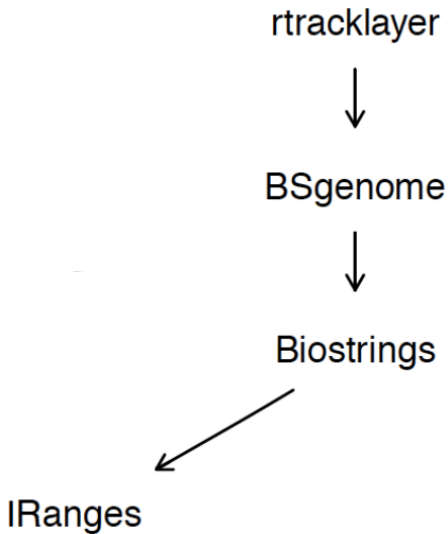
- Now that we can access genomic information, it would be useful to import the related annotation. That's (one of) the purpose of the following packages:
 - **rtracklayer**
 - **GenomicFeatures**
 - **biomaRt**
 - **genomeIntervals**
- **rtracklayer** offers export function too and as already presented, **genomeIntervals** offers interval utilities similar to **IRanges**

rtracklayer

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analyses

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General
Information
Objectives



Methods

Bioconductor
packages for
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analyses

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General
Information
Objectives

- There are two high level methods
 - import
 - export
- Both accept the following formats:
 - BED: bed, bedGraph, bed15
 - GFF: gff1, 2 and 3
 - WIG
- export works with *RangedData* objects
- import returns a *RangedData* object or *GRanges* object, depending on the (asRangedData) boolean argument.

Methods (c'ed)

Bioconductor
packages for
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analyses

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General
Information
Objectives

- When exporting
 - The naming convention of the *RangedData* column names is crucial.
 - The following column names
 - **names**: for exporting the feature names
 - **scores**: for exporting the feature scores
 - **strand**: for exporting the feature strands
 - **see** ?export.bed for the complete details

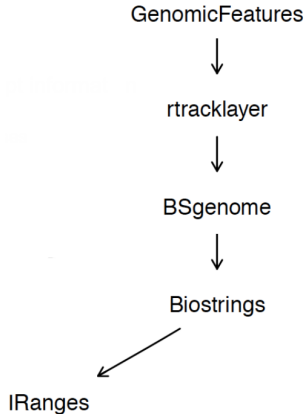
GenomicFeatures

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packages for
short read
analyses

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General
Information
Objectives

- management of transcript information
 - using **GenomicRanges**
 - stored into SQLite databases



Constructors and Class

Bioconductor
packages for
short read
analyses

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General
Information
Objectives

- makeTranscriptDbFromBiomart
- makeTrascriptDbFromUCSC

```
> library(GenomicFeatures)
> head(supportedUCSCTables())
```

	track	subtrack
knownGene	UCSC Genes	<NA>
knownGeneOld3	Old UCSC Genes	<NA>
ccdsGene	CCDS	<NA>
refGene	RefSeq Genes	<NA>
xenoRefGene	Other RefSeq	<NA>
vegaGene	Vega Genes	Vega Protein Genes

```
> mm9KG<-makeTranscriptDbFromUCSC(genome="mm9",tablename="knownGene")
> saveFeatures(mm9KG,file="mm9KG.sqlite")
```

Constructors and Class

Bioconductor
packages for
short read
analyses

Alex Sánchez

General
Information
Objectives

```
> mm9KG<-loadFeatures("mm9KG.sqlite")  
> mm9KG
```

```
TranscriptDb object:  
| Db type: TranscriptDb  
| Supporting package: GenomicFeatures  
| Data source: UCSC  
| Genome: mm9  
| Organism: Mus musculus  
| UCSC Table: knownGene  
| Resource URL: http://genome.ucsc.edu/  
| Type of Gene ID: Entrez Gene ID  
| Full dataset: yes  
| miRBase build ID: NA  
| transcript_nrow: 55419  
| exon_nrow: 246570  
| cds_nrow: 213117  
| Db created by: GenomicFeatures package from Bioconductor  
| Creation time: 2014-06-07 22:03:43 +0200 (Sat, 07 Jun 2014)  
| GenomicFeatures version at creation time: 1.16.2  
| RSQLite version at creation time: 0.11.4  
| DBSCHEMAVERSION: 1.0
```

Extractors

Bioconductor
packages for
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analyses

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General
Information
Objectives

- ungrouped
 - transcriptBy
 - exonsBy
 - intronsByTranscript
 - fiveUTRsByTranscript
 - threeUTRsByTranscript

Extractors

Bioconductor
packages for
short read
analyses

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General
Information
Objectives

```
> library(GenomicFeatures)
> txExons<-exonsBy(mm9KKG)
> head(txExons)
```

GRangesList of length 6:

\$1

GRanges with 8 ranges and 3 metadata columns:

	seqnames	ranges	strand	exon_id	exon_name	exon_rank
	<Rle>	<IRanges>	<Rle>	<integer>	<character>	<integer>
[1]	chr1	[4797974, 4798063]	+	1	<NA>	1
[2]	chr1	[4798536, 4798567]	+	2	<NA>	2
[3]	chr1	[4818665, 4818730]	+	3	<NA>	3
[4]	chr1	[4820349, 4820396]	+	4	<NA>	4
[5]	chr1	[4822392, 4822462]	+	5	<NA>	5
[6]	chr1	[4827082, 4827155]	+	6	<NA>	6
[7]	chr1	[4829468, 4829569]	+	7	<NA>	7
[8]	chr1	[4831037, 4832908]	+	9	<NA>	8

\$2

GRanges with 9 ranges and 3 metadata columns:

	seqnames	ranges	strand	exon_id	exon_name	exon_rank
[1]	chr1	[4797974, 4798063]	+	1	<NA>	1
[2]	chr1	[4798536, 4798567]	+	2	<NA>	2
[3]	chr1	[4818665, 4818730]	+	3	<NA>	3
[4]	chr1	[4820349, 4820396]	+	4	<NA>	4
[5]	chr1	[4822392, 4822462]	+	5	<NA>	5
[6]	chr1	[4827082, 4827155]	+	6	<NA>	6
[7]	chr1	[4829468, 4829569]	+	7	<NA>	7
[8]	chr1	[4831037, 4831213]	+	8	<NA>	8
[9]	chr1	[4835044, 4836816]	+	10	<NA>	9

Usage

Bioconductor
packages for
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analyses

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General
Information
Objectives

- Overlapping with transcripts
 - findOverlaps
 - countOverlaps
 - match
 - %in%
 - subsetByOverlaps
- More about these in the following part about **GenomicRanges**

- Side note to get help from within R:
 - `vignette("biomaRt",package="biomaRt")`
- biomaRt is an interface to the collection of databases that implements the bioMart software suite:
 - `http://biomart.org`
 - allow retrieval of huge datasets from different sources through a common interface
 - examples are: Ensembl, HapMap, Uniprot, ...

biomaRt, an example

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General
Information
Objectives

■ Connect the mart database

```
> require(biomaRt)
> ensembl<- useMart("ensembl")
> head(listDatasets(ensembl))
```

	dataset	description
1	oanatinus_gene_ensembl	Ornithorhynchus anatinus genes (OANA5)
2	cporcellus_gene_ensembl	Cavia porcellus genes (cavPor3)
3	gaculeatus_gene_ensembl	Gasterosteus aculeatus genes (BROADS1)
4	lafricana_gene_ensembl	Loxodonta africana genes (loxAfr3)
5	itridecemlineatus_gene_ensembl	Ictidomys tridecemlineatus genes (spetri2)
6	choffmanni_gene_ensembl	Choloepus hoffmanni genes (choHof1)

```
version
1 OANA5
2 cavPor3
3 BROADS1
4 loxAfr3
5 spetri2
6 choHof1
```

```
> ensembl<- useMart("ensembl",dataset="dmelanogaster_gene_ensembl")
> head(listAttributes(ensembl))
```

	name	description
1	ensembl_gene_id	Ensembl Gene ID
2	ensembl_transcript_id	Ensembl Transcript ID
3	ensembl_peptide_id	Ensembl Protein ID
4	ensembl_exon_id	Ensembl Exon ID
5	description	Description
6	chromosome_name	Chromosome Name

biomaRt, an example (c'ed)

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General
Information
Objectives

■ query the database

```
> exon.annotation<-getBM(c("ensembl_gene_id","strand",  
+                           "chromosome_name","ensembl_exon_id",  
+                           "exon_chrom_start","exon_chrom_end"),  
+                           mart=ensembl,filters="chromosome_name",  
+                           values="4")
```

■ convert into a RangedData / Granges

genomeIntervals

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General
Information
Objectives

- Similar interval implementation to IRanges
 - (+) overall faster, gff function more robust to 'incorrect' format
 - (-) less integrated in R
- Two classes:
 - Genome_intervals
 - Genome_intervals_stranded
- Methods
 - input
 - **readGff3**, **getGffAttributes**, **parseGffAttributes**
 - intervals utilities
 - **interval_overlap**, **interval_complement**, **interval_union**, **interval_intersection**

What next?

Bioconductor
packages for
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analyses

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General
Information
Objectives

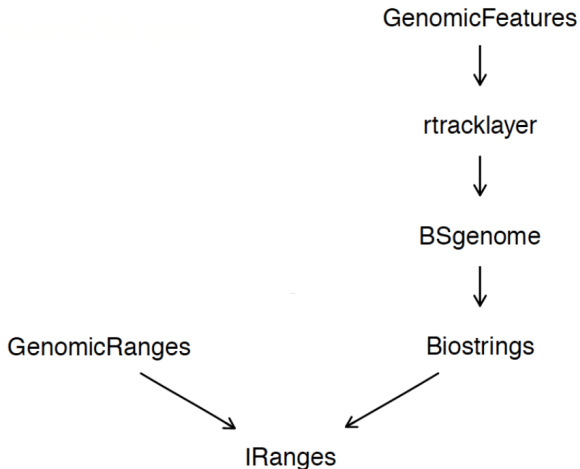
- We have seen how to get genomic sequences and their annotation
- For processing NGS data, we are now missing the other half of the workflow: loading and manipulating the actual data. For this, three packages are available.
 - **GenomicRanges**
 - **Rsamtools**
 - **ShortRead**

GenomicRanges

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General
Information
Objectives



Naive approach

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General
Information
Objectives

- Genomic coordinates consist of
 - chromosome
 - position
 - strand
 - additional information
 - GC content
 - etc.
- This can be represented by a data.frame
 - fine for organism information (~ 100k exons, 20k genes)
 - not for million of reads

BIOC representation for intervals with data

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General
Information
Objectives

- *RangedData*
 - used by **rtracklayer**
 - interval grouped by chromosome/contig
 - strand unaware
- *GRanges*
 - used by **GenomicFeatures**
 - intervals not required to be grouped by chromosome/contig
 - strand aware
 - *GRangesList* can hold exons with spliced transcripts

GRanges constructor and slots

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General
Information
Objectives

- starts and ends defined in an *IRanges* object
- strand, seqnames (chromosome) and seqlenghts (chromosome size) to be provided

```
> grngs<-GRanges(seqnames=c("chr1","chr2","chr1"),  
+               ranges=IRanges(start=c(3,4,1),end=c(7,5,3)),  
+               strand=c("+","+","-"),seqlengths = c("chr1"=24,"chr2"=18))  
> grngs
```

GRanges with 3 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[3, 7]	+
[2]	chr2	[4, 5]	+
[3]	chr1	[1, 3]	-

```
---  
seqlengths:  
chr1 chr2  
24   18
```

- additional slots can contain mtadata information

```
> getSlots("GRanges")
```

seqnames	ranges	strand	elementMetadata	seqinfo
"Rle"	"IRanges"	"Rle"	"DataFrame"	"Seqinfo"
metadata				
"list"				

Interval operations

Bioconductor
packages for
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analyses

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General
Information
Objectives

- Intra-interval
 - flank,resize,shift
- Inter-interval
 - disjoint, gaps, reduce, range
 - coverage
- Between intervals sets
 - union, intersect, setdiff
 - punion,pintersectm psetdiff
 - findOverlaps, countOverlaps, %in%, match
- Low Level
 - start,end,width

Other functions

■ Selecting

- seqselect, [
■ head, tail, window
■ subset, subsetByOverlaps

```
> grngs[strand(grngs)=="-"]
```

```
GRanges with 1 range and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[1, 3]	-

```
---
```

```
seqlengths:
```

chr1	chr2
24	18

```
> # seqselect(grngs,strand(grngs)=="-") NO EXISTEIX seqselect
```

Example 1: Intra-interval

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analyses

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General
Information
Objectives

■ shift

```
> grngs
```

```
GRanges with 3 ranges and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[3, 7]	+
[2]	chr2	[4, 5]	+
[3]	chr1	[1, 3]	-

```
---
seqlengths:
chr1 chr2
24 18
```

```
> shift(grngs,1)
```

```
GRanges with 3 ranges and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[4, 8]	+
[2]	chr2	[5, 6]	+
[3]	chr1	[2, 4]	-

```
---
seqlengths:
chr1 chr2
24 18
```

■ resize

```
> resize(grngs,10)
```

```
GRanges with 3 ranges and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[3, 12]	+
[2]	chr2	[4, 13]	+
[3]	chr1	[1, 3]	-

```
---
seqlengths:
chr1 chr2
24 18
```

■ flank

```
> flank(grngs,2)
```

```
GRanges with 3 ranges and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	[1, 2]	+
[2]	chr2	[2, 3]	+
[3]	chr1	[4, 5]	-

```
---
seqlengths:
chr1 chr2
24 18
```

Overlap detection

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packages for
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analyses

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General
Information
Objectives

- *findOverlap* and *countOverlaps* produce a mapping and a tabulation of interval overlaps, respectively

```
> ol<-findOverlaps(grngs,reduce(grngs))  
> ol
```

```
Hits of length 3  
queryLength: 3  
subjectLength: 3  
  queryHits subjectHits  
    <integer>    <integer>  
1           1           1  
2           2           3  
3           3           2
```

Rsamtools

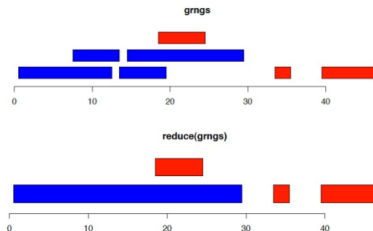
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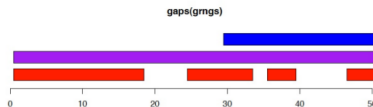
General
Information
Objectives

Blue represents the "+" strand, red the "-" strand

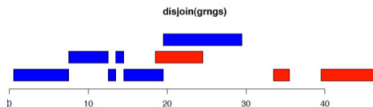
Reduce



Gaps



Disjoin



samtools and Rsamtools

Bioconductor
packages for
short read
analyses

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General
Information
Objectives

- samtools
 - Data Format: SAM(text) and BAM (binary)
 - Tools: merge, sort, pileup, view, etc.
- Rsamtools
 - Reads and represents BAMfiles
 - high level: readAligned (type=BAM), readPileup
 - lower level: scanBam, scanBamParam, ScanBamWhat
 - utilities: countBam, sortBam, indexBam, filterBam, scanBamHeader
 - views: BamViews

Input

Bioconductor
packages for
short read
analyses

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General
Information
Objectives

- `readAligned` returns an *alignedRead* class
 - described in the following section on **ShortRead**
- `scanBam` returns a list of list i.e.. one list per column in the SAM file.
 - `qname`: a *BStringSet* containing the read id
 - `seq`: a *DNASTringSet* containing the read sequence
 - etc.
 - The possible fields can be found with `scanBamWhat()`

```
> require(Rsamtools)
> scanBamWhat()

[1] "qname"      "flag"      "rname"      "strand"     "pos"
[6] "qwidth"    "mapq"      "cigar"      "mrnm"       "mpos"
[11] "isize"     "seq"       "qual"       "groupid"    "mate_status"
```
- `scanBam` is the function called by the **GenomicRanges** `readGappedAlignments` method

Input (c'ed)

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General
Information
Objectives

- The input can be controlled using ScanBamParam
 - it has three fields
 - **which**: *GRanges* selecting references, genomic loci, strand, ...
 - **flag**: use the SAM flag to selected paired, mapped, etc. reads.

```
> names(formals(scanBamFlag))  
  
[1] "isPaired"                "isProperPair"  
[3] "isUnmappedQuery"         "hasUnmappedMate"  
[5] "isMinusStrand"           "isMateMinusStrand"  
[7] "isFirstMateRead"         "isSecondMateRead"  
[9] "isNotPrimaryRead"        "isNotPassingQualityControls"  
[11] "isDuplicate"             "isValidVendorRead"
```

- **what**: fields to retrieve (cf. scanBamWhat)

GappedAlignments vs AlignedRead

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General
Information
Objectives

■ *AlignedRead*

- reads complete files
- include sequence, quality, identifier, etc.
- reads are assumed to be ungapped

■ *GappedAlignments*

- use scanBam
- genomic coordinates, 'cigar', covered intervals
- Cigar: an RLE; M(match), I (insertion), D (deletion), N (skipped), P (padding), S/H (soft/hard clip)
- direct IRanges accessors (sub-setting, narrowing, coverage)

BamViews

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General
Information
Objectives

- Access a set of experiments stored in BAM files
 - for example to query a specific loci
- Check the vignette ("leeViews")
- Still very unstable!

BamViews

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General
Information
Objectives

```
> library(leeBamViews)
> bpaths=dir(system.file("bam",package="leeBamViews"),full=TRUE, patt="bam$")
> gt<- do.call(rbind, strsplit(basename(bpaths),"_"))[,1]
> geno<-substr(gt,1,nchar(gt)-1)
> lane<- substr(gt,nchar(gt),nchar(gt))
> pd=DataFrame(geno=geno, lane=lane, row.names=paste(geno,lane,sep="."))
> bs1=BamViews(bamPaths=bpaths, bamSamples=pd, bamExperiment=list(annotation="org.Sc.sgd.db")
> bamPaths(bs1)
```

```
isowt.5
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/isowt5_13e.bam"
isowt.6
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/isowt6_13e.bam"
rlp.5
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/rlp5_13e.bam"
rlp.6
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/rlp6_13e.bam"
ssr.1
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/ssr1_13e.bam"
ssr.2
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/ssr2_13e.bam"
xrn.1
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/xrn1_13e.bam"
xrn.2
"/home/alex/R/x86_64-pc-linux-gnu-library/3.1/leeBamViews/bam/xrn2_13e.bam"
```

BamViews

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```
> bamSamples(bs1)
```

```
DataFrame with 8 rows and 2 columns
```

	geno	lane
	<character>	<character>
isowt.5	isowt	5
isowt.6	isowt	6
rlp.5	rlp	5
rlp.6	rlp	6
ssr.1	ssr	1
ssr.2	ssr	2
xrn.1	xrn	1
xrn.2	xrn	2

```
> sel<-GRanges(seqnames="Scchr13",IRanges(start=861250,end=863000),strand="+")
```

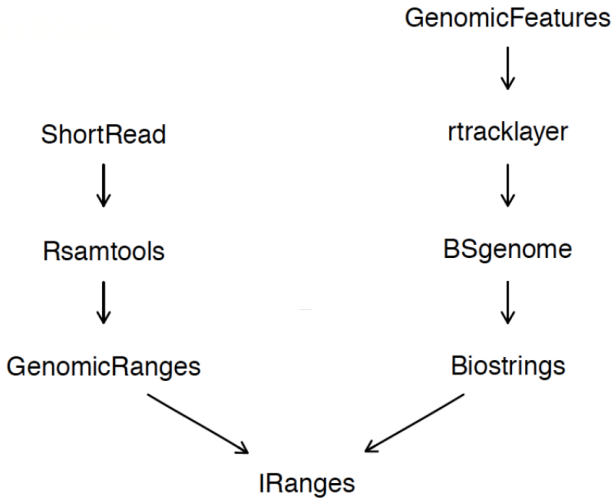
```
> # covex=RleList(lapply(bamPaths(bs1),function(x) coverage(readGappedAlignments(x))[[1]]))
```

ShortRead

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ShortRead

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General
Information
Objectives

- Input
 - read most sequence proprietary formats
 - read fastq
 - read BAM
- Exploration
 - contains sequence, quality, id, etc. information
- Manipulation
 - allow the manipulation of the fields with a limited memory impact
- Quality assessment
 - offers quality assessment functionalities

AlignedReadClass

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Objectives

■ The main class to store the read information

```
> require(ShortRead)
> showClass("AlignedRead")
```

```
Class "AlignedRead" [package "ShortRead"]
```

```
Slots:
```

Name:	chromosome	position	strand	alignQuality
Class:	factor	integer	factor	QualityScore

Name:	alignData	quality	sread	id
Class:	AlignedDataFrame	QualityScore	DNASTringSet	BStringSet

```
Extends:
```

```
Class "ShortReadQ", directly
```

```
Class "ShortRead", by class "ShortReadQ", distance 2
```

```
Class ".ShortReadBase", by class "ShortReadQ", distance 3
```

■ All slots can be accessed through accordingly named accessors

SRFilterclass

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■ Useful tools to filter the reads during or after the import

```
> showClass("SRFilter")
```

```
Class "SRFilter" [package "ShortRead"]
```

```
Slots:
```

```
Name:          .Data          name
```

```
Class:         function ScalarCharacter
```

```
Extends:
```

```
Class "function", from data part
```

```
Class ".SRUtil", directly
```

```
Class "OptionalFunction", by class "function", distance 2
```

```
Class "PossibleMethod", by class "function", distance 2
```

```
Class "expressionORfunction", by class "function", distance 2
```

```
Class "functionORNULL", by class "function", distance 2
```

■ many already implemented

- idFilter
- chromosomeFilter
- positionFilter
- strandFilter
- etc.

■ They can be combined using compose

- `compose(idFilter,chromosomeFilter)`

Other classes

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Objectives

- The package implements many classes to hold the different kind of data

```
> getClasses(where="package:ShortRead")

[1] "AlignedDataFrame"      "AlignedRead"          "ArrayIntensity"
[4] "BAMQA"                  "BowtieQA"             "ExperimentPath"
[7] "FastqFile"             "FastqFileList"        "FastqFileReader"
[10] "FastqQA"               "FastqQuality"         "FastqSampler"
[13] "FastqSamplerList"      "FastqStreamer"        "FastqStreamerList"
[16] "IntegerQuality"        "Intensity"            "IntensityInfo"
[19] "IntensityMeasure"      "MAQMapQA"            "MatrixQuality"
[22] "NumericQuality"        "QA"                   ".QA"
[25] ".QA2"                  "QAAdapterContamination" "QACollate"
[28] "QAData"                "QAFastqSource"        "QAFiltered"
[31] "QAFlagged"             "QAFrequentSequence"   "QANucleotideByCycle"
[34] "QANucleotideUse"       "QAQualityByCycle"     "QAQualityUse"
[37] "QAReadQuality"        "QASequenceUse"        "QASource"
[40] "QASummary"            "QualityScore"         ".Roche"
[43] "RochePath"            "RocheSet"             "RtaIntensity"
[46] "SFastqQuality"        "ShortRead"            ".ShortReadBase"
[49] "ShortReadFile"        "ShortReadQ"           "ShortReadQQA"
[52] "Snapshot"             "SnapshotFunction"     "SnapshotFunctionList"
[55] ".Solexa"              "SolexaExportQA"       "SolexaIntensity"
[58] "SolexaIntensityInfo"  "SolexaPath"           "SolexaRealignQA"
[61] "SolexaSet"            "SpTrellis"           "SRError"
[64] "SRFilter"             "SRFilterResult"       "SRLList"
[67] "SRSet"                ".SRUtil"              "SRVector"
[70] "SRWarn"              "trellis"
```


Input and accessor examples

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■ Simple walk through

```
> require("EatonEtAlChIPseq")
> fl<-system.file("extdata","GSM424494_wt_G2_orc_chip_rep1_S288C_14.mapview.txt.gz",p
> aln<-readAligned(fl,type="MAQMapview");aln

class: AlignedRead
length: 478774 reads; width: 39 cycles
chromosome: S288C_14 S288C_14 ... S288C_14 S288C_14
position: 2 4 ... 784295 784295
strand: + - ... + +
alignQuality: IntegerQuality
alignData varLabels: nMismatchBestHit mismatchQuality nExactMatch24 nOneMismatch24

> head(sread(aln))

A DNAStringSet instance of length 6
width seq
[1] 39 CGGCTTTTCTGACCGAAATTAAAAAAAAAAAAATGAAAATG
[2] 39 GATTATGAAAGAAATTAAAAAAAAAAAAATGAAAATGAA
[3] 39 CTTTCTGACCGAAATTAAAAAAAAAAAAATGAAAATGAAA
[4] 39 TTTCTGACCGAAATTAAAAAAAAAAAAATGAAATTGAAAC
[5] 39 TTTATGAAAGAAATAAAAAAAAAAAAATGAAAATGAAAC
[6] 39 TTTCTGAAAGAAATAAAAAAAAAAAAATGAAAATGAAAC
```

Input and accessor examples

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■ with filters

```
> filter<- compose(chromosomeFilter("S288C_14"),positionFilter(min=1,max=1000))
> alnF<-readAligned(fl,type="MAQMapView",filter=filter);alnF

class: AlignedRead
length: 715 reads; width: 39 cycles
chromosome: S288C_14 S288C_14 ... S288C_14 S288C_14
position: 2 4 ... 997 999
strand: + - ... - -
alignQuality: IntegerQuality
alignData varLabels: nMismatchBestHit mismatchQuality nExactMatch24 nOneMismatch24
```

Input and accessor examples

```
> head(quality(aln))
```

```
class: FastqQuality
```

quality:

A BStringSet instance of length 6

width seq

```
[1] 39 >>>>>>>><>><<<>5><><<<<<44444///.
```

[2] 39 .%/4&/14&&:<<<.(>>>>>>>>>>>>>>>>>>>

[3] 39 >>>>>>>>>>>>>>><><><>><<<<444444////

[4] 39 <>>>>:<<><::>><>:><<<><:::;<44%-4//\$/

[5] 39 .,/ ,1+41+4<<<4<>>>>>>>>>>>>>>>>>>

[illegible]

```
> head(id(aln))
```

A BStringSet instance of length 6

width seq

[1] 23 X8193_200:5:175:690:668

[2] 22 X8193_200:5:62:612:145

[3] 23 X8193_200:5:206:446:786

[4] 22 X8193_200:5:12:950:859

[5] 23 X8193_200:5:230:400:822

[6] 23 X8193_200:5:258:160:889

Manipulation example

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■ For example to rename chromosome

```
> chrom<-chromosome(alnF)
> i<-sub("S288C_[[:digit:]]+)", "\\1", levels(chrom));i

[1] "14"

> levels(chrom)

[1] "S288C_14"

> levels(chrom)<-paste("chr",as.roman(i),sep="")
> levels(chrom)

[1] "chrXIV"

> alnF<-renew(alnF,chromosome=chrom);alnF

class: AlignedRead
length: 715 reads; width: 39 cycles
chromosome: chrXIV chrXIV ... chrXIV chrXIV
position: 2 4 ... 997 999
strand: + - ... - -
alignQuality: IntegerQuality
alignData varLabels: nMismatchBestHit mismatchQuality nExactMatch24 nOneMismatch24

>
```

Quality assessment

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- Many functions are available in **ShortRead** that can be used for performing QA

```
> f.list<-showMethods(  
+ where="package:ShortRead"  
+ printTo=FALSE)
```

```
> sapply(strsplit(f.list[grepl("Function",f.list)],' '),  
+ function(x)x[2])
```

```
[1] "alphabet"  
[3] "alphabetFrequency"  
[5] "annTrack"  
[7] "!"  
[9] "[["  
[11] "$"  
[13] "chromosome"  
[15] "coerce"  
[17] "to=\"OptionalFunction\""  
[19] "to=\"OptionalFunction\""  
[21] "countLines"  
[23] "dim"  
[25] "encoding"  
[27] "fac"  
[29] "FastqQuality"  
[31] "FastqStreamerList"  
[33] "files"  
[35] "functions"  
[37] "id"  
[39] "%in%"  
[41] "lapply"  
[43] "names<-"  
[45] "name"  
[47] "pan"  
[49] "phenoData"  
[51] "qa2"  
[53] "qa"  
[55] "read454"  
[57] "readBaseQuality"  
"alphabetByCycle"  
"alphabetScore"  
"append"  
"["  
"$<-"  
"c"  
"clean"  
"to=\"classGeneratorFun"  
"to=\"genericFunction\""  
"to=\"genericFunction\""  
"coverage"  
"dustyScore"  
"experimentPath"  
"FastqFileList"  
"FastqSamplerList"  
"FastqStreamer"  
"flag"  
"getTrellis"  
"ignore.strand"  
"laneNames"  
"length"  
"names"  
"narrow"  
"pData"  
"position"  
"QACollate"  
"rbind"  
"readAligned"  
"readFastqQual"
```

QA example (yet another one...)

■ Using independent functions

```
> abc<- alphabetByCycle(sread(alnF))  
> abc[1:4,1:12]
```

	cycle											
alphabet	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]	[,11]	[,12]
A	239	246	251	236	244	244	223	207	184	212	217	230
C	197	172	180	178	169	194	192	194	202	212	185	182
G	103	88	87	105	89	93	108	101	114	90	103	83
T	176	209	197	196	213	184	192	213	215	201	210	220

```
> abc<-abc[1:4, ]  
> par(mfrow=c(1,2))  
> matplot(t(abc),type="l",lty=rep(1,4))  
> m<-as (quality(alnF),"matrix")  
> plot(colMeans(m),type="b")
```

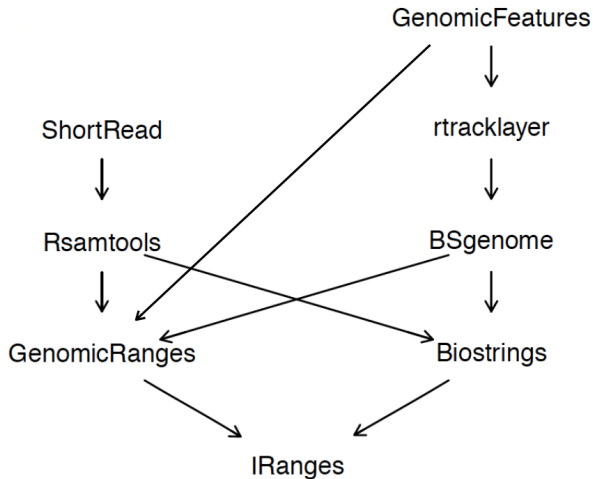
- All these and more are combined into the function: `qa()`
- These can then be reported using the `report()` function

Conclusion

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General
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Objectives



Conclusion

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Objectives

- We have seen the two “branches” of the core packages:
 - the one used to get genomic sequence and annotation
 - the one used to load and manipulate NGS data
- Actually, the cit is not so clear ad the packages of these two branches are interacting at different levels.
- They provide numerous functionalities and are getting into a “production” (stable development) state.
- Higher level packaages are being developed to wrap these functionalities into more user friendly packages.

Conclusion

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Objectives

- If you would start today using these packages:
 - go for the BAM format
 - go for GRanges objects
 - Be on the lookout, especially for the *SummarizedExperiment* class in the **GenomicRanges** package.
 - It is a concept similar to the *ExpressionSet* class developed for microarray and aims at normalizing the output of NGS experiments within R/Bioconductor

If we were fast..

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- Another couple of package to mention
 - Rsubread (only on linux)
 - easyRNASeq (self-promotion)

Rsubread

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General
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Objectives

- a package to align short read in R!
- If you have a session on vuori you can try that code slightly modified in the R file to use only chromosome 1

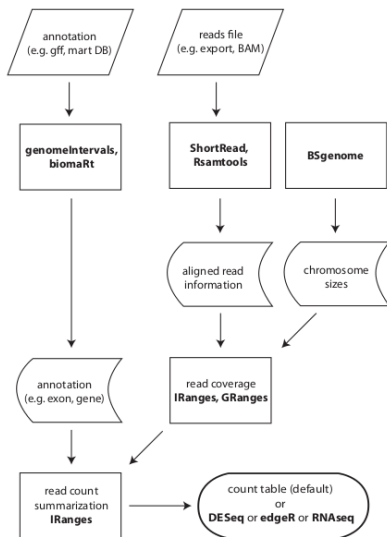
```
> ## write the human genome sequences
> writeXStringSet(Reduce(append,
+ lapply(seqnames(Hsapiens),
+ function(nam)
+ {dss<-DNASTringSet(unmasked(Hsapiens[[nam]]))
+ names(dss)<-nam
+ dss})),file="hg19.fa")
> ##create the indexes
> require(Rsubread)
> dir.create("indexes")
> buildindex(basename=file.path("indexes","hg19"),
+           reference="hg19.fa")
> ## align the reads
> sapply(dir(pattern="*\\.gz$"),function(fil){
+   ## decompress the files
+   gunzip(fil)
+   ##align
+   align(index=file.path("indexes","hg19"),
+         readfile1=sub("\\.gz$", "",fil),
+         nsubreads=2, TH1=1,
+         output_file=sub("\\.fastq\\.gz$", "\\,sam",fil))
+   ## create bam files
+   asBAM(file=sib("\\.fastq\\.gz$", "\\,sam",fil),
+         destination=sub("\\.fastq\\.gz$", "",fil),
+         indexDestination=TRUE)
+ })
```

easyRNASeq package

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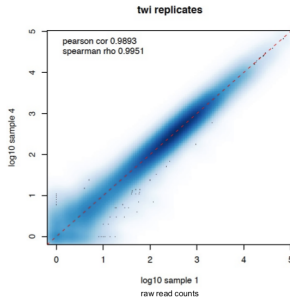
Replicate comparison

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Objectives

- The simplest output is a matrix
 - Comparing replicates is therefore easy
 - Can be done automatically if the user provides the sample information
 - GRAFIC



Normalization

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Objectives

- Three types can be applied
 - **R**eads **P**er feature **K**b per **M**ilion reads in the library
- DESeq
 - based on Negative Binomial
 - fit a model to correct for the library sizes
- edgeR
 - based on Negative Binomial
 - use a trimmed mean of M-values to correct for the library sizes

