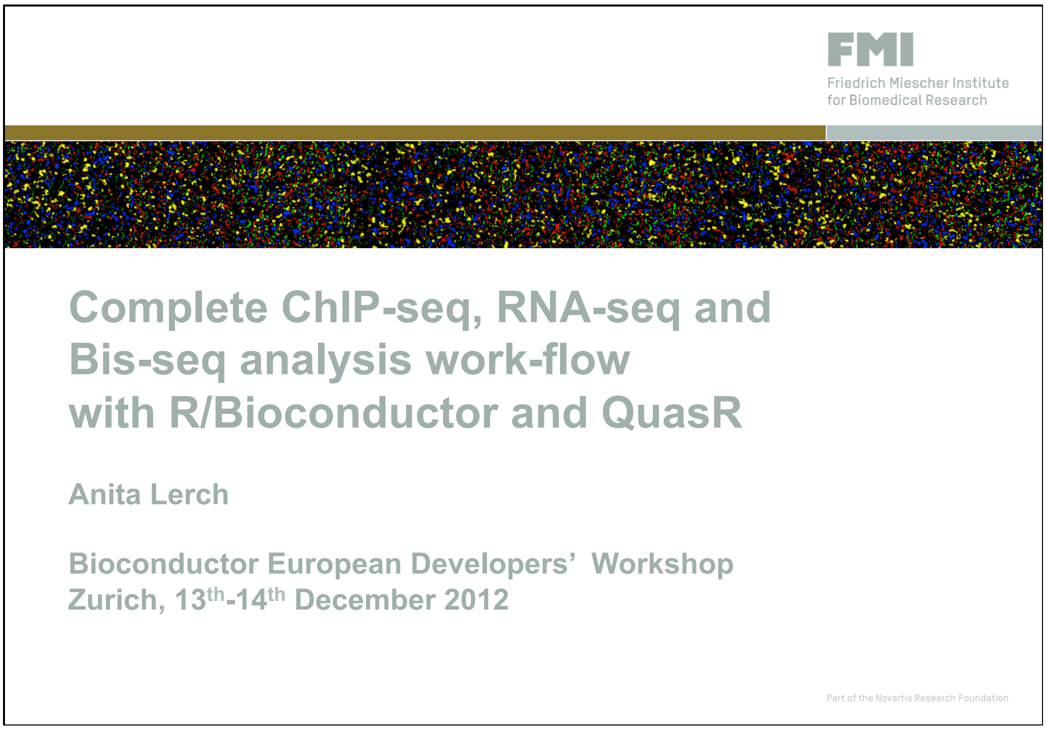

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Complete ChIP-seq, RNA-seq and Bis-seq analysis work-flow with R/Bioconductor and QuasR

Anita Lerch

Bioconductor European Developers' Workshop
Zurich, 13th-14th December 2012

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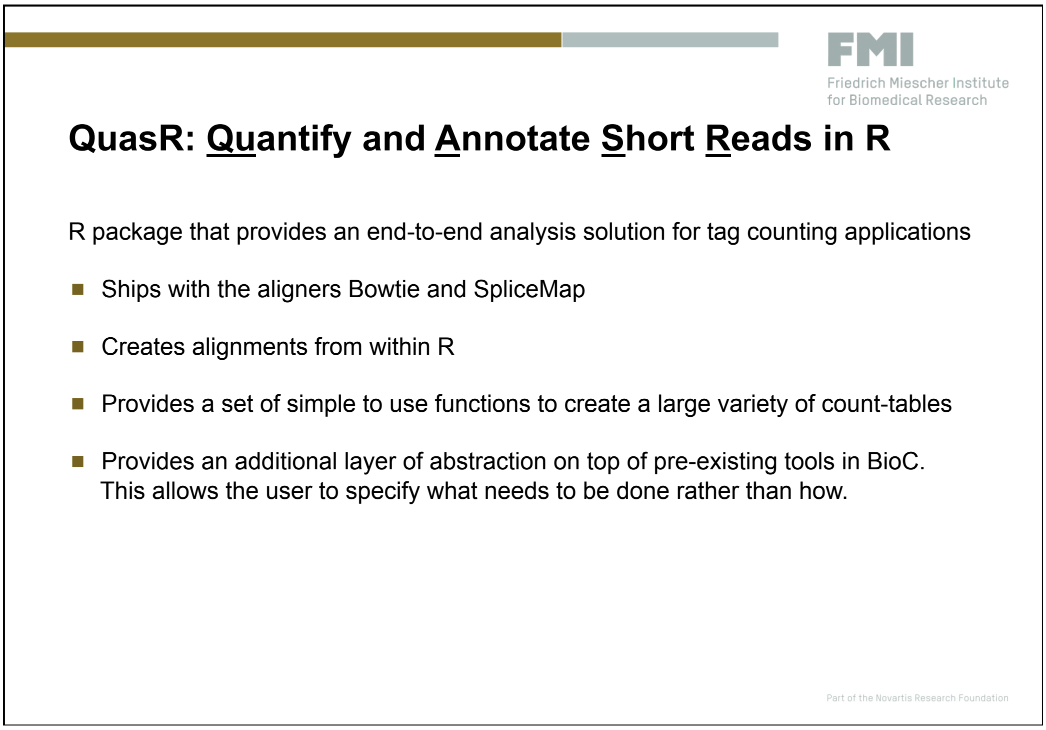

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QuasR: Quantify and Annotate Short Reads in R

R package that provides an end-to-end analysis solution for tag counting applications

- Ships with the aligners Bowtie and SpliceMap
- Creates alignments from within R
- Provides a set of simple to use functions to create a large variety of count-tables
- Provides an additional layer of abstraction on top of pre-existing tools in BioC. This allows the user to specify what needs to be done rather than how.

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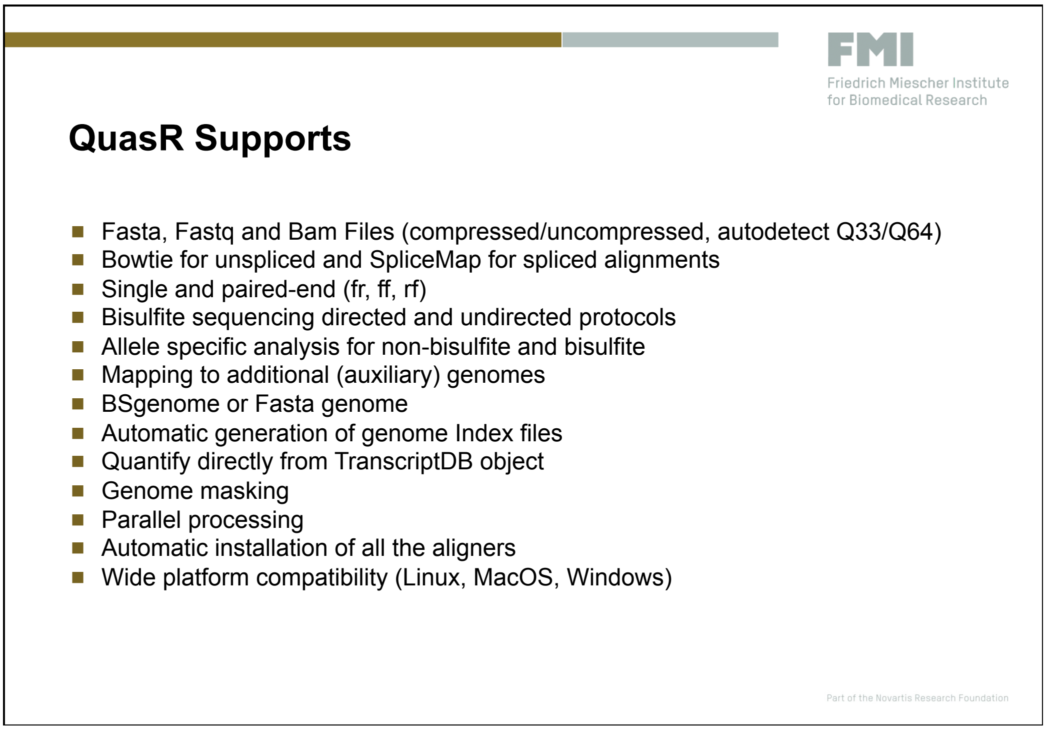

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QuasR Supports

- Fasta, Fastq and Bam Files (compressed/uncompressed, autodetect Q33/Q64)
- Bowtie for unspliced and SpliceMap for spliced alignments
- Single and paired-end (fr, ff, rf)
- Bisulfite sequencing directed and undirected protocols
- Allele specific analysis for non-bisulfite and bisulfite
- Mapping to additional (auxiliary) genomes
- BSgenome or Fasta genome
- Automatic generation of genome Index files
- Quantify directly from TranscriptDB object
- Genome masking
- Parallel processing
- Automatic installation of all the aligners
- Wide platform compatibility (Linux, MacOS, Windows)

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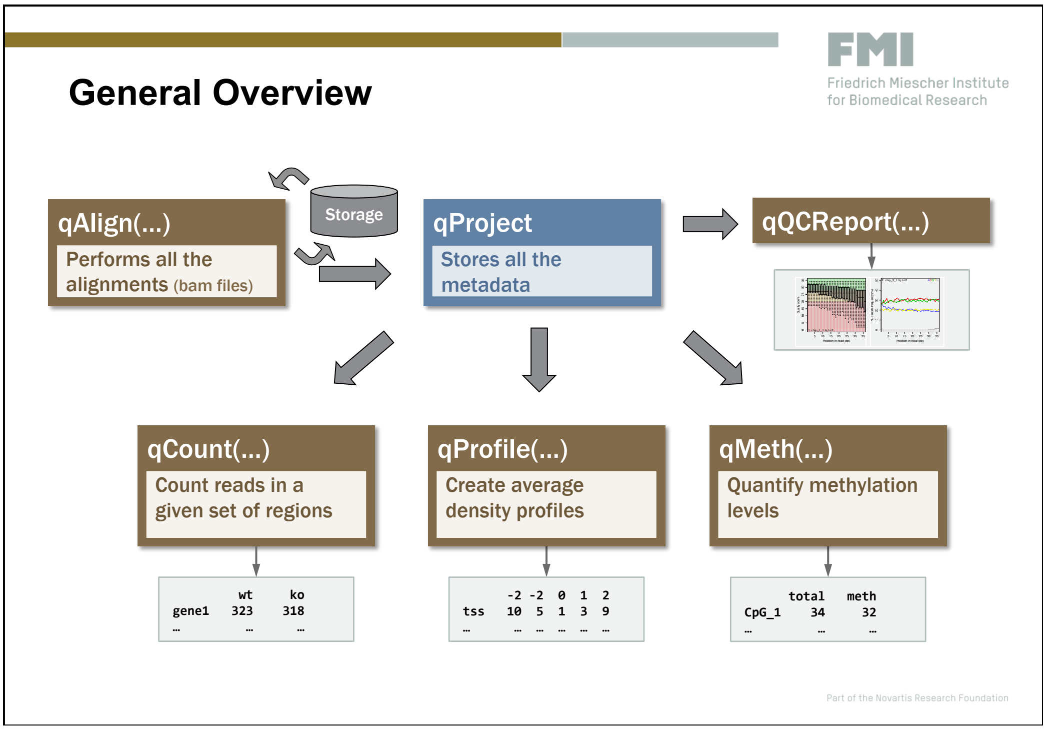

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General Overview

qAlign(...)
Performs all the alignments (bam files)
Storage
qProject
Stores all the metadata
qQCReport(...)
qCount(...)
Count reads in a given set of regions
qProfile(...)
Create average density profiles
qMeth(...)
Quantify methylation levels

```
        wt    ko
gene1  323   318
…        …     …
```

```
     -2 -2  0  1  2
tss  10  5  1  3  9
…     …  …  …  …  …
```

```
       total  meth
CpG_1    34    32
…         …     …
```

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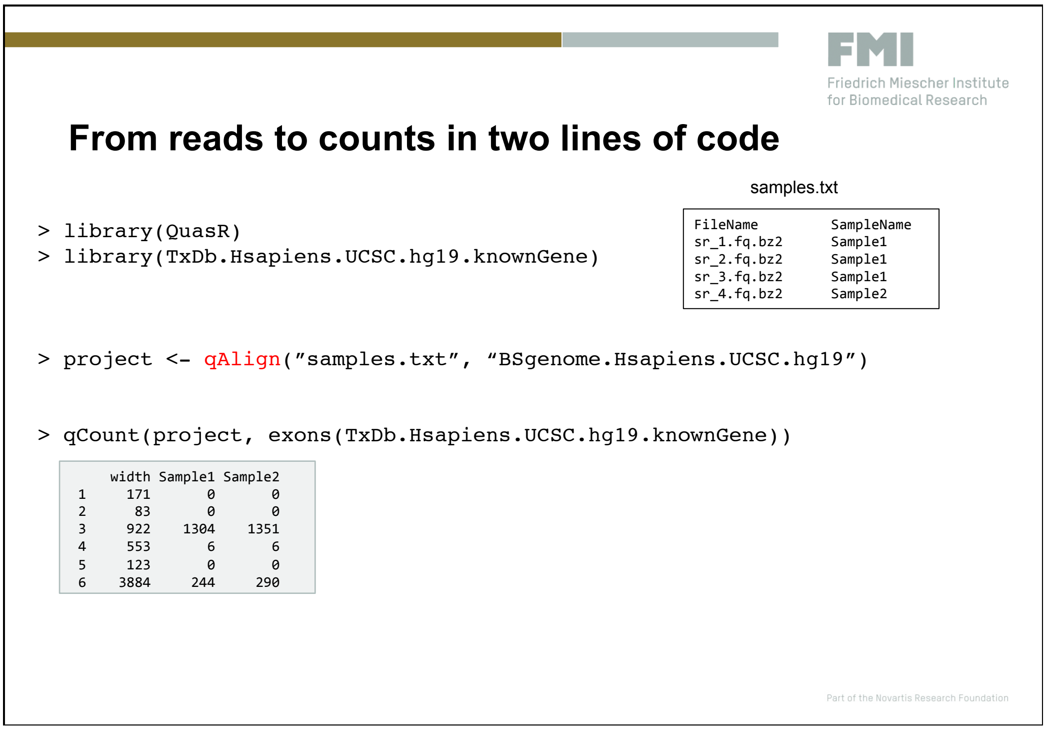

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From reads to counts in two lines of code

samples.txt

```
FileName        SampleName
sr_1.fq.bz2     Sample1
sr_2.fq.bz2     Sample1
sr_3.fq.bz2     Sample1
sr_4.fq.bz2     Sample2
```

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)

> qCount(project, exons(TxDb.Hsapiens.UCSC.hg19.knownGene))
```

```
   width Sample1 Sample2
1    171       0       0
2     83       0       0
3    922    1304    1351
4    553       6       6
5    123       0       0
6   3884     244     290
```

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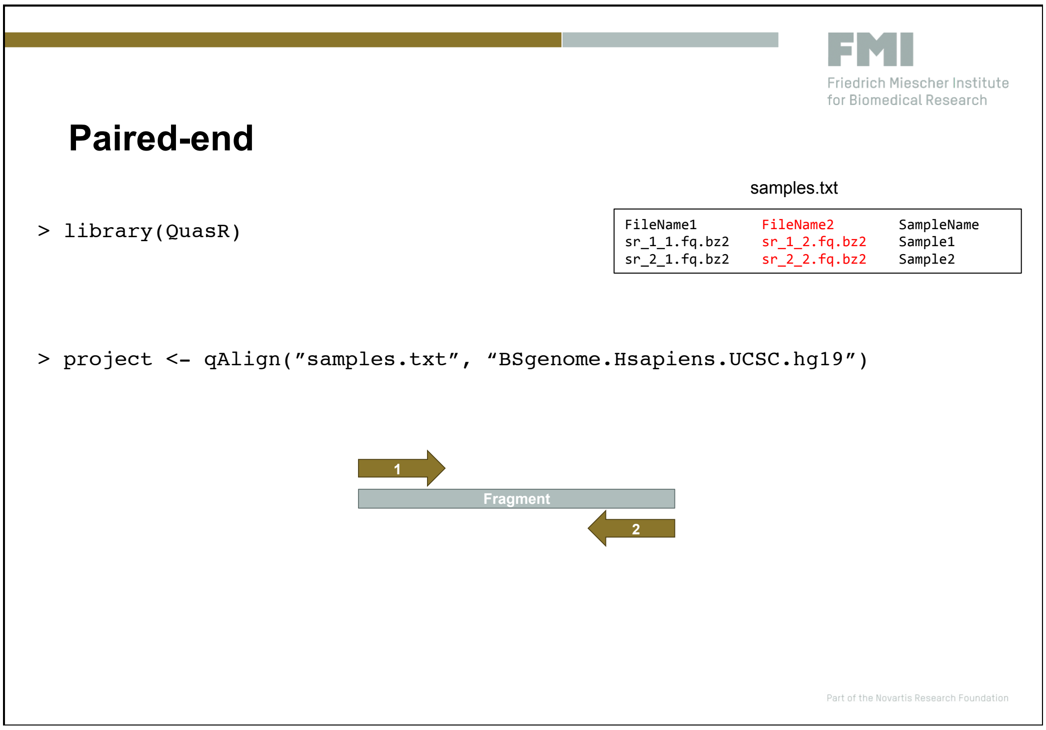

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Paired-end

samples.txt

```
FileName1       FileName2       SampleName
sr_1_1.fq.bz2   sr_1_2.fq.bz2   Sample1
sr_2_1.fq.bz2   sr_2_2.fq.bz2   Sample2
```

```
> library(QuasR)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)
```

1
Fragment
2

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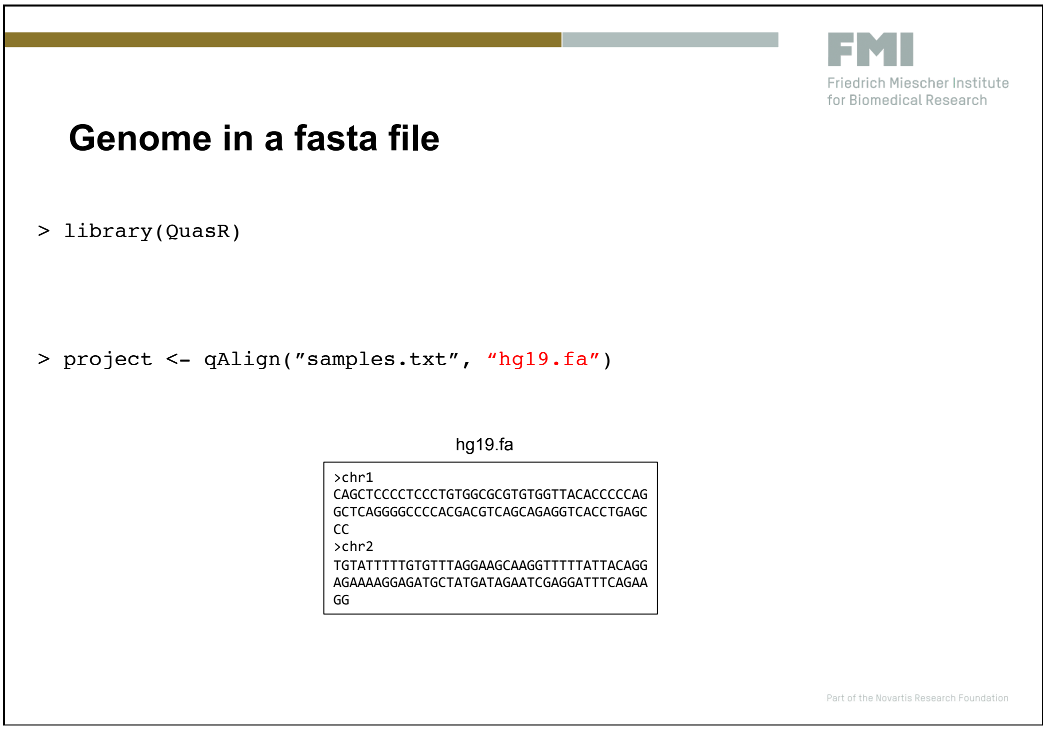

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Genome in a fasta file

```
> library(QuasR)

> project <- qAlign(”samples.txt”, “hg19.fa”)
```

hg19.fa

```
>chr1
CAGCTCCCCTCCCTGTGGCGCGTGTGGTTACACCCCCAG
GCTCAGGGGCCCCACGACGTCAGCAGAGGTCACCTGAGC
CC
>chr2
TGTATTTTTGTGTTTAGGAAGCAAGGTTTTTATTACAGG
AGAAAAGGAGATGCTATGATAGAATCGAGGATTTCAGAA
GG
```

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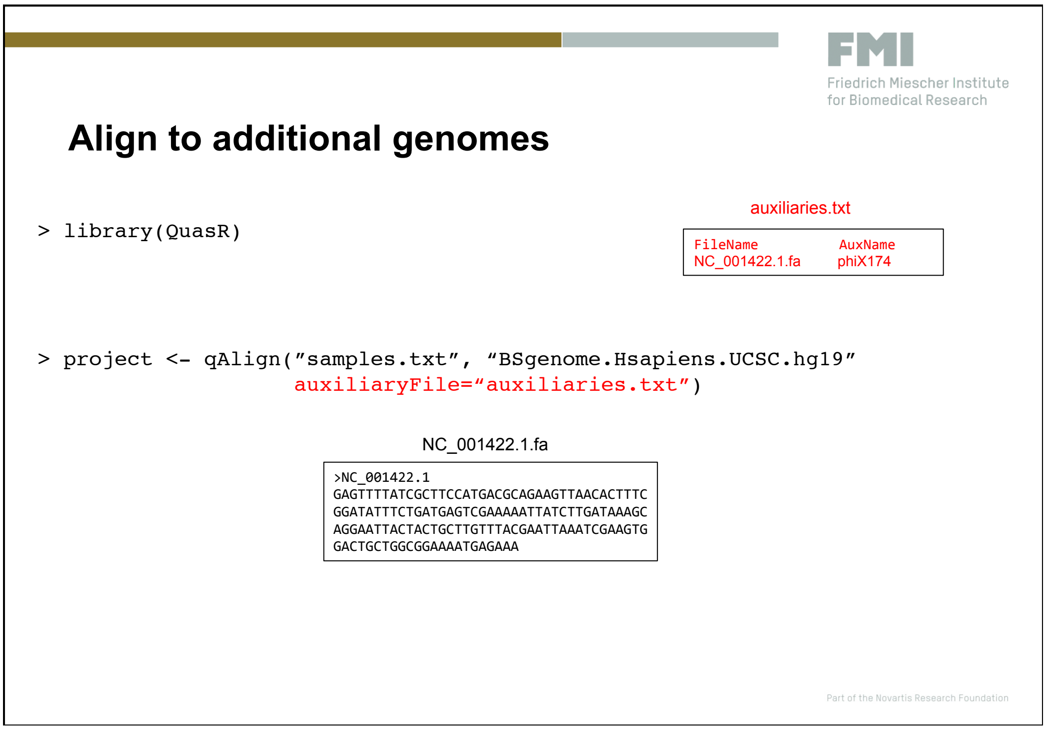

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Align to additional genomes

```
> library(QuasR)
```

auxiliaries.txt

```
FileName          AuxName
NC_001422.1.fa    phiX174
```

```
> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”
                    auxiliaryFile=“auxiliaries.txt”)
```

NC_001422.1.fa

```
>NC_001422.1
GAGTTTTATCGCTTCCATGACGCAGAAGTTAACACTTTC
GGATATTTCTGATGAGTCGAAAAATTATCTTGATAAAGC
AGGAATTACTACTGCTTGTTTACGAATTAAATCGAAGTG
GACTGCTGGCGGAAAATGAGAAA
```

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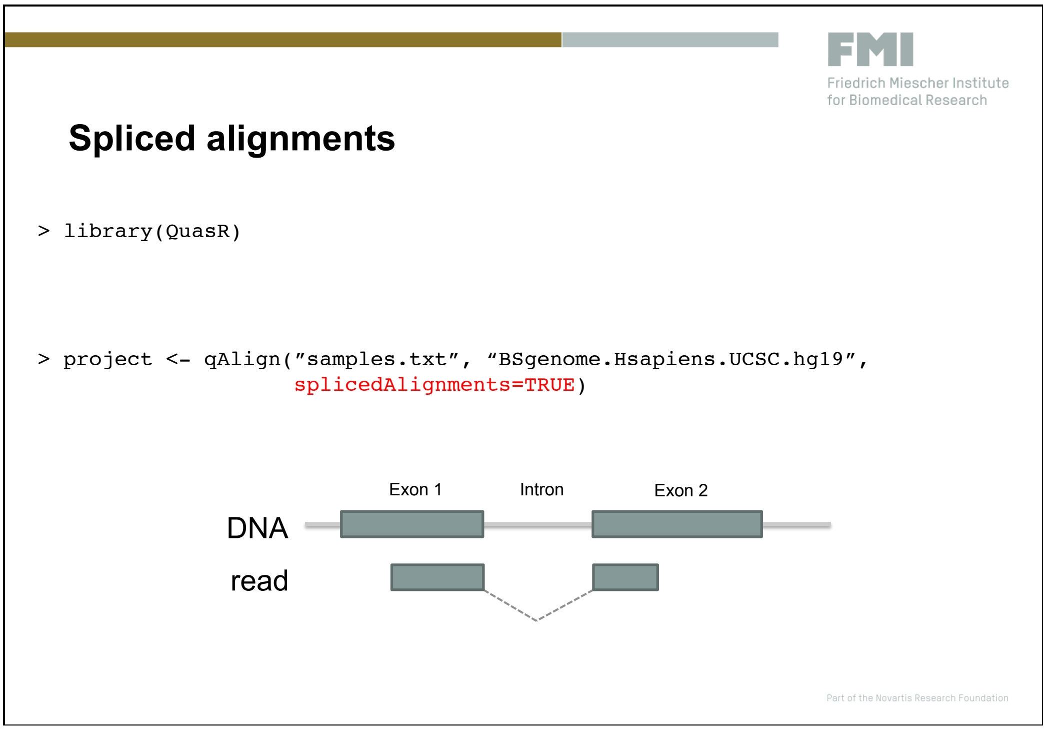

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Spliced alignments

```
> library(QuasR)
```

```
> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”,
                    splicedAlignments=TRUE)
```

Exon 1
Intron
Exon 2
DNA
read

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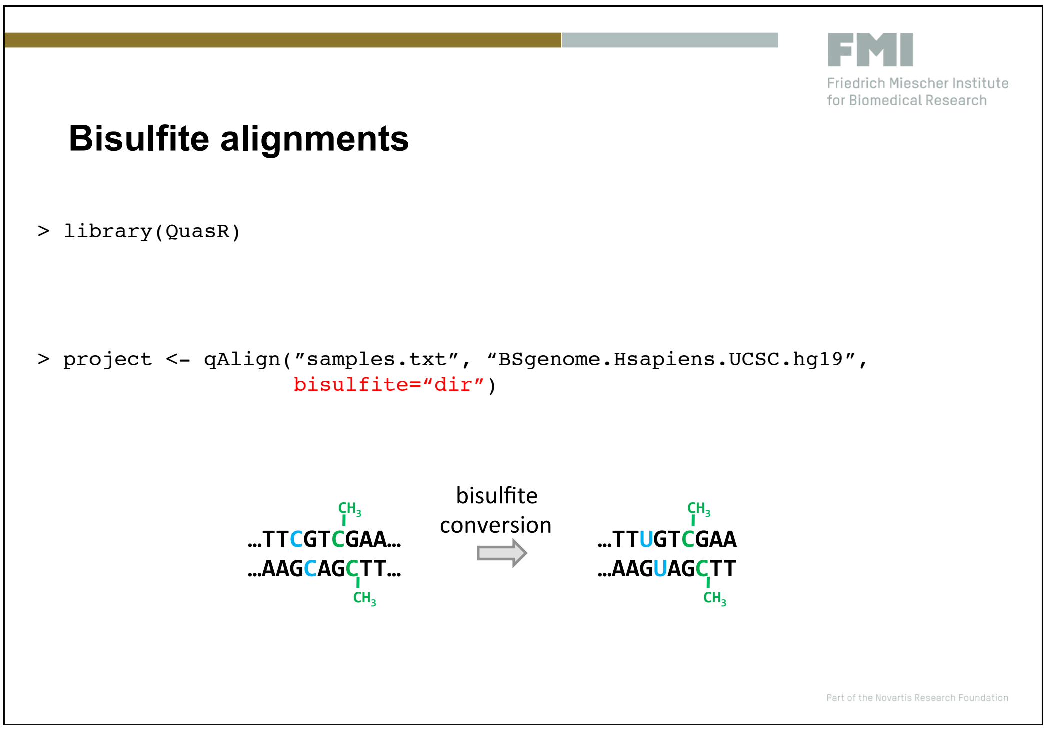

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Bisulfite alignments

```
> library(QuasR)
```

```
> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”,
                    bisulfite=“dir”)
```

CH_3
...TTCGTCGAA...
...AAGCAGCTT...
CH_3
bisulfite
conversion
CH_3
...TTUGTCGAA
...AAGUAGCTT
CH_3

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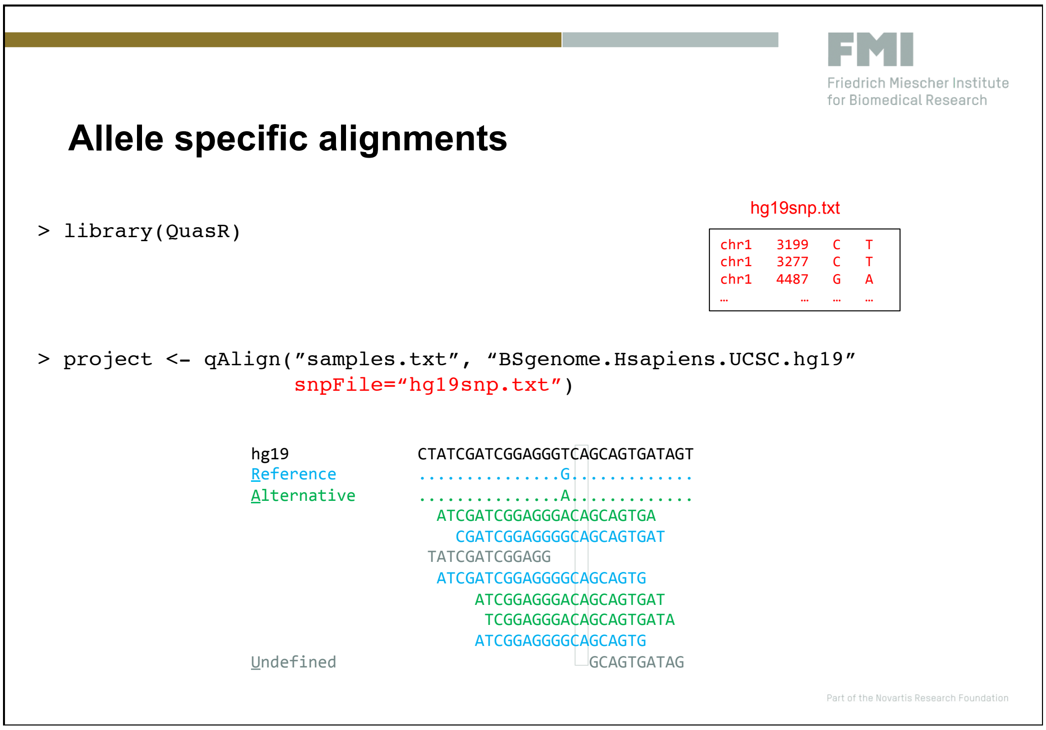

Allele specific alignments

```
> library(QuasR)
```

hg19snp.txt

```
chr1   3199   C   T
chr1   3277   C   T
chr1   4487   G   A
…         …   …   …
```

```
> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”
                   snpFile=“hg19snp.txt”)
```

```
hg19              CTATCGATCGGAGGGTCAGCAGTGATAGT
Reference         ................G.............
Alternative       ................A.............
                    ATCGATCGGAGGGACAGCAGTGA
                      CGATCGGAGGGGCAGCAGTGAT
                   TATCGATCGGAGG
                    ATCGATCGGAGGGGCAGCAGTG
                        ATCGGAGGGACAGCAGTGAT
                         TCGGAGGGACAGCAGTGATA
                        ATCGGAGGGGCAGCAGTG
Undefined                           GCAGTGATAG
```

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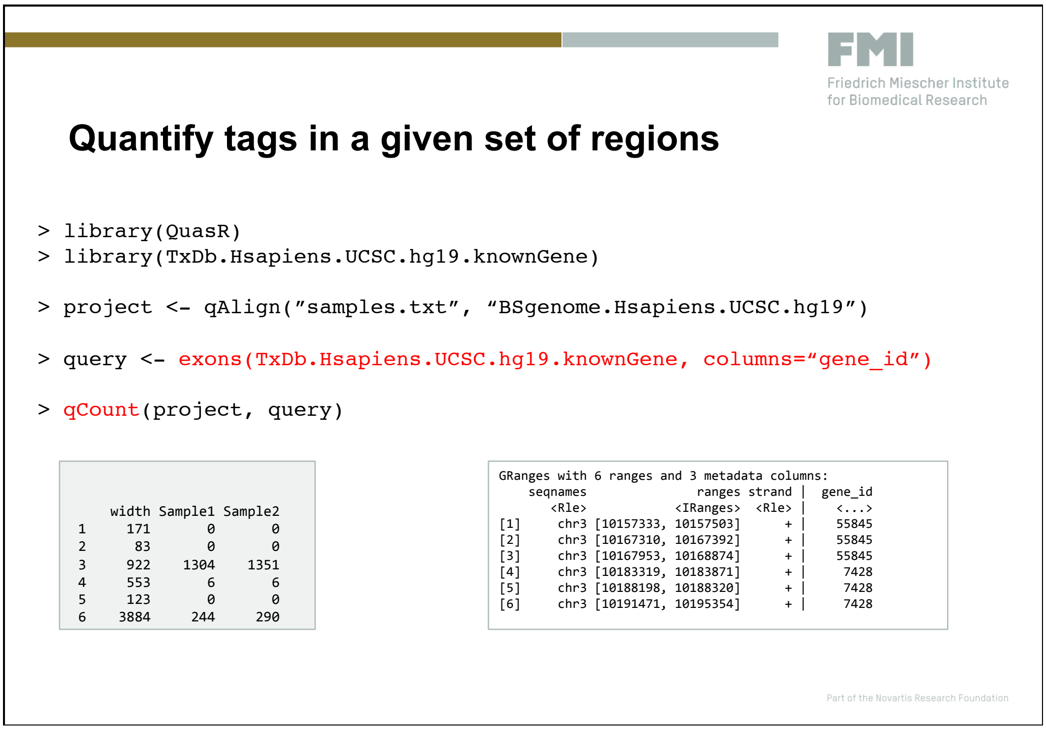

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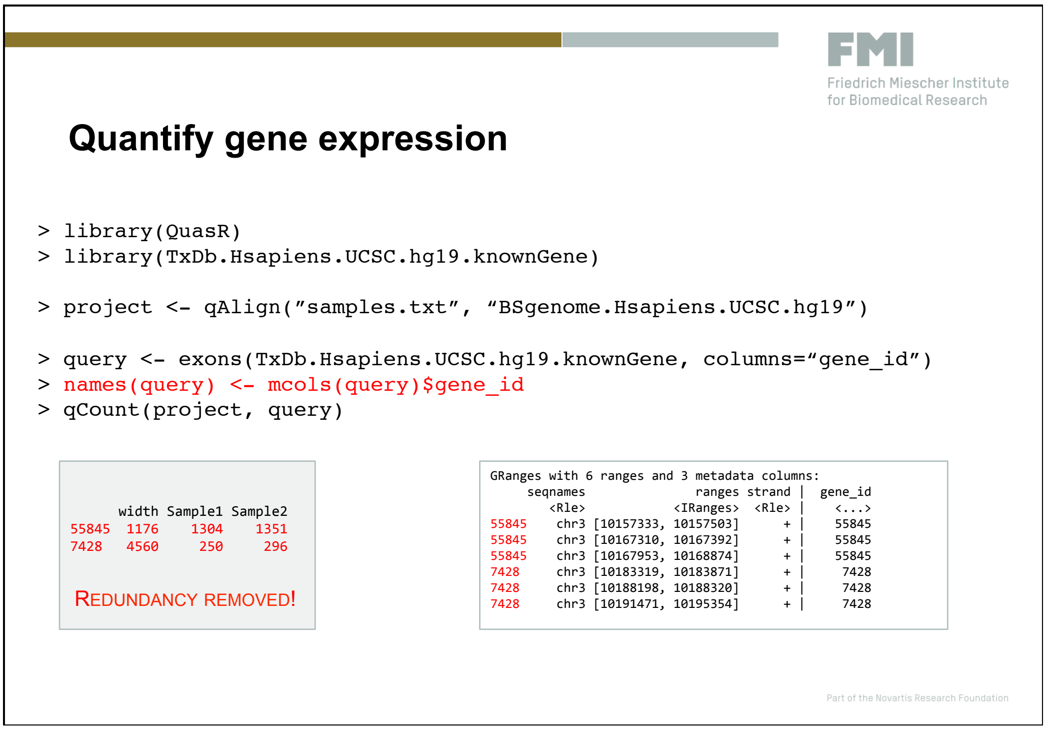
Quantify tags in a given set of regions

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)

> query <- exons(TxDb.Hsapiens.UCSC.hg19.knownGene, columns=“gene_id”)

> qCount(project, query)
```

```
   width Sample1 Sample2
1    171       0       0
2     83       0       0
3    922    1304    1351
4    553       6       6
5    123       0       0
6   3884     244     290
```

```
GRanges with 6 ranges and 3 metadata columns:
    seqnames                 ranges strand |   gene_id
       <Rle>              <IRanges>  <Rle> |     <...>
[1]     chr3 [10157333, 10157503]      + |     55845
[2]     chr3 [10167310, 10167392]      + |     55845
[3]     chr3 [10167953, 10168874]      + |     55845
[4]     chr3 [10183319, 10183871]      + |      7428
[5]     chr3 [10188198, 10188320]      + |      7428
[6]     chr3 [10191471, 10195354]      + |      7428
```



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Quantify gene expression

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)

> query <- exons(TxDb.Hsapiens.UCSC.hg19.knownGene, columns=“gene_id”)
> names(query) <- mcols(query)$gene_id
> qCount(project, query)
```

```
      width Sample1 Sample2
55845  1176    1304    1351
7428   4560     250     296
```

REDUNDANCY REMOVED!

```
GRanges with 6 ranges and 3 metadata columns:
     seqnames                 ranges strand |  gene_id
        <Rle>              <IRanges>  <Rle> |    <...>
55845    chr3 [10157333, 10157503]      + |    55845
55845    chr3 [10167310, 10167392]      + |    55845
55845    chr3 [10167953, 10168874]      + |    55845
7428     chr3 [10183319, 10183871]      + |     7428
7428     chr3 [10188198, 10188320]      + |     7428
7428     chr3 [10191471, 10195354]      + |     7428
```

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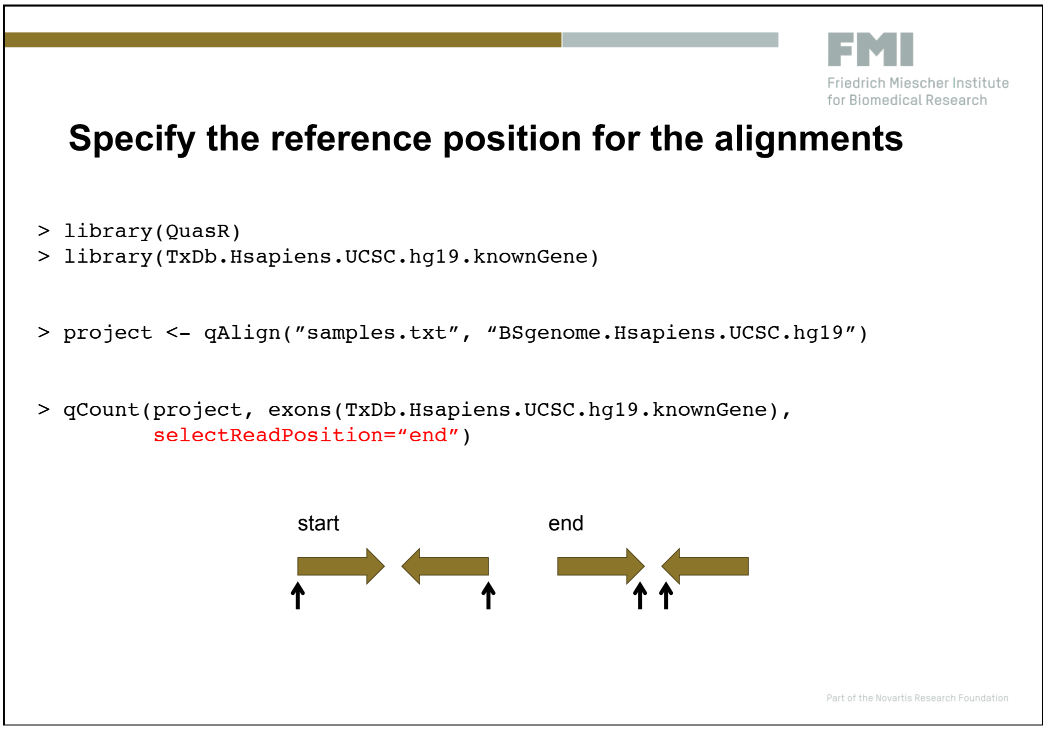

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Specify the reference position for the alignments

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)


> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)


> qCount(project, exons(TxDb.Hsapiens.UCSC.hg19.knownGene),
         selectReadPosition=“end”)
```

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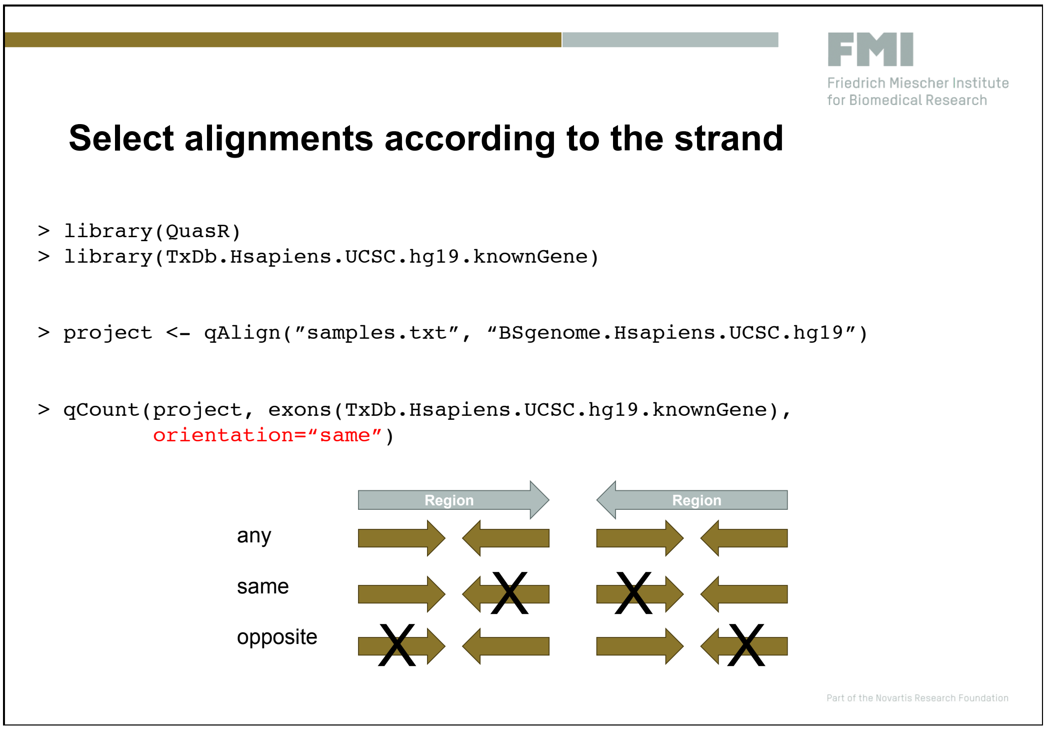

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Select alignments according to the strand

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)


> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)


> qCount(project, exons(TxDb.Hsapiens.UCSC.hg19.knownGene),
         orientation=“same”)
```

Region
Region
any
same
opposite
X
X
X
X

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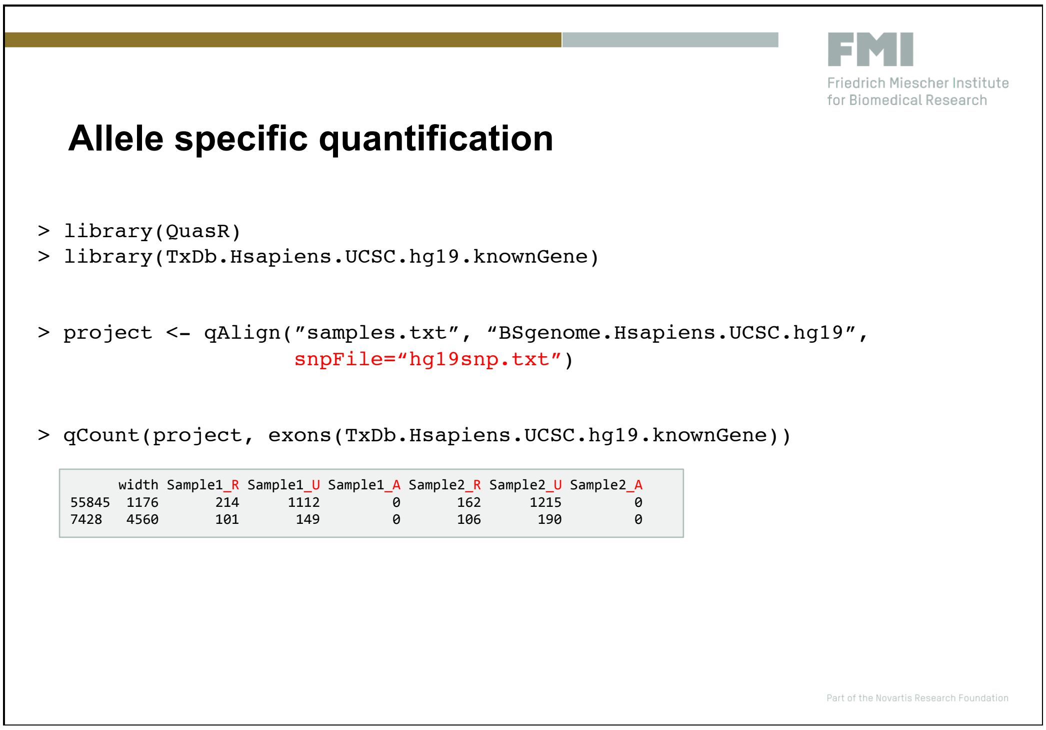

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Allele specific quantification

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)


> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”,
                    snpFile=“hg19snp.txt”)


> qCount(project, exons(TxDb.Hsapiens.UCSC.hg19.knownGene))
```

```
      width Sample1_R Sample1_U Sample1_A Sample2_R Sample2_U Sample2_A
55845  1176       214      1112         0       162      1215         0
7428   4560       101       149         0       106       190         0
```

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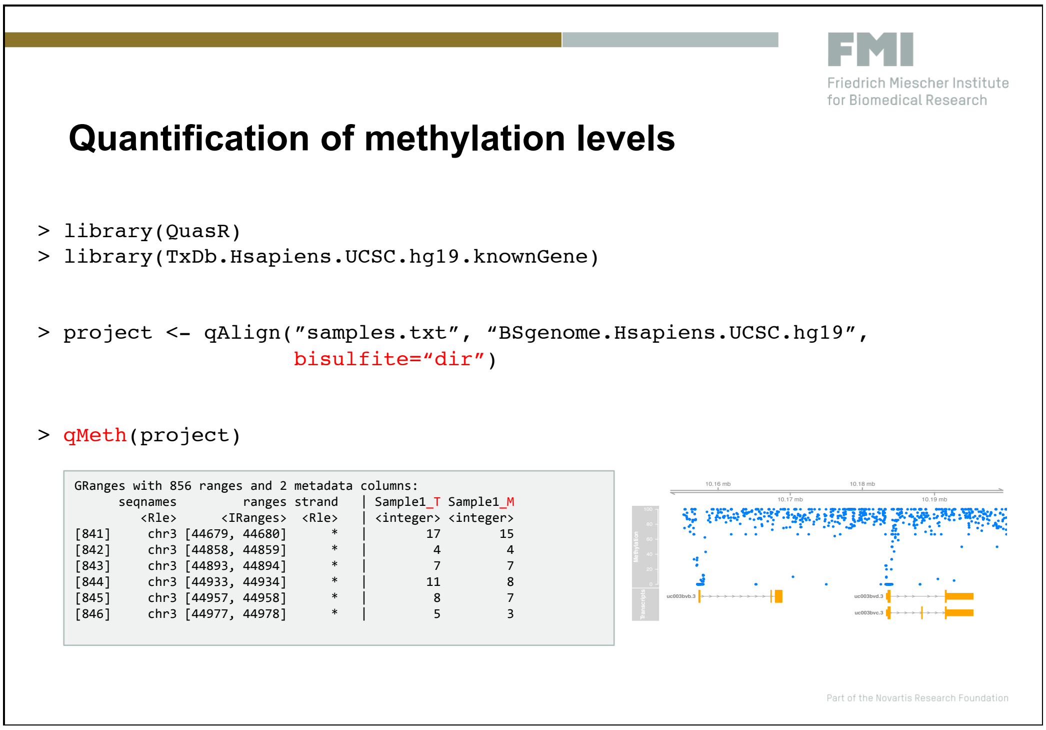

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Quantification of methylation levels

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign("samples.txt", "BSgenome.Hsapiens.UCSC.hg19",
                    bisulfite="dir")

> qMeth(project)
```

```
GRanges with 856 ranges and 2 metadata columns:
      seqnames          ranges strand   | Sample1_T Sample1_M
         <Rle>       <IRanges>  <Rle>   | <integer> <integer>
[841]     chr3 [44679, 44680]       *   |        17        15
[842]     chr3 [44858, 44859]       *   |         4         4
[843]     chr3 [44893, 44894]       *   |         7         7
[844]     chr3 [44933, 44934]       *   |        11         8
[845]     chr3 [44957, 44958]       *   |         8         7
[846]     chr3 [44977, 44978]       *   |         5         3
```

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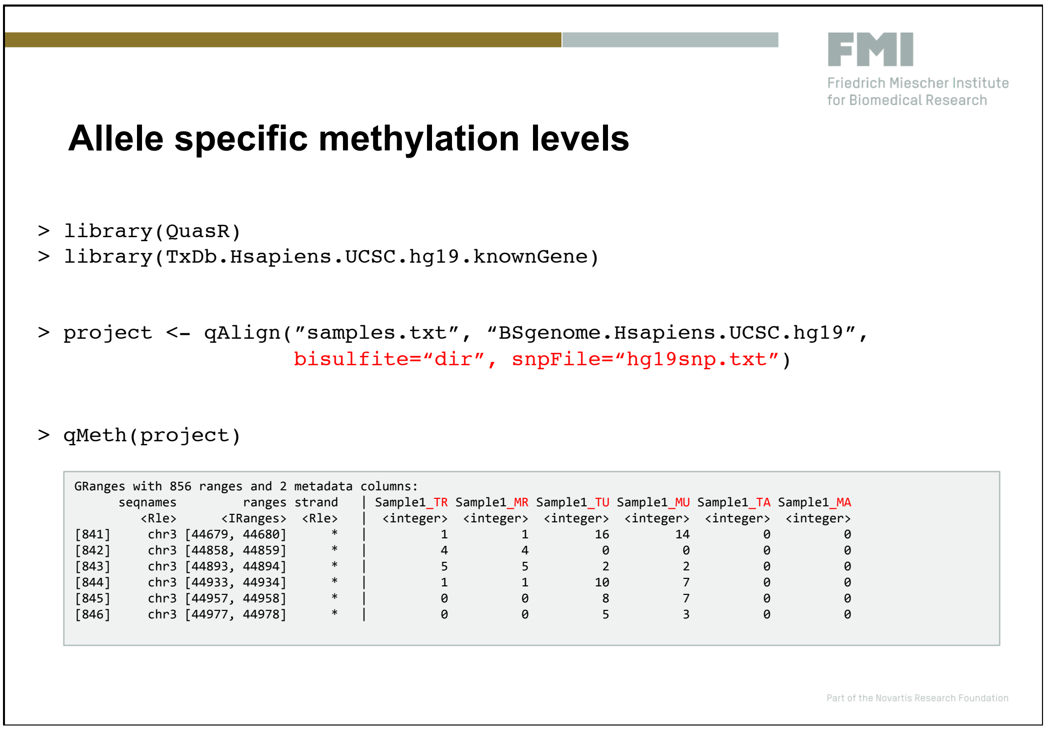

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Allele specific methylation levels

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign("samples.txt", "BSgenome.Hsapiens.UCSC.hg19",
                    bisulfite="dir", snpFile="hg19snp.txt")

> qMeth(project)
```

```
GRanges with 856 ranges and 2 metadata columns:
      seqnames          ranges strand   | Sample1_TR Sample1_MR Sample1_TU Sample1_MU Sample1_TA Sample1_MA
         <Rle>       <IRanges>  <Rle>   |  <integer>  <integer>  <integer>  <integer>  <integer>  <integer>
[841]     chr3 [44679, 44680]       *   |          1          1         16         14          0          0
[842]     chr3 [44858, 44859]       *   |          4          4          0          0          0          0
[843]     chr3 [44893, 44894]       *   |          5          5          2          2          0          0
[844]     chr3 [44933, 44934]       *   |          1          1         10          7          0          0
[845]     chr3 [44957, 44958]       *   |          0          0          8          7          0          0
[846]     chr3 [44977, 44978]       *   |          0          0          5          3          0          0
```

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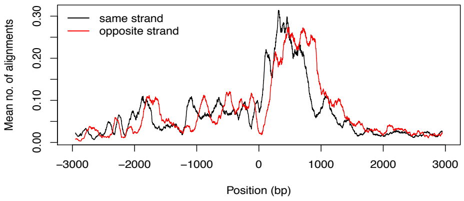
Genomic profiles

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)

> query <- cds(TxDb.Hsapiens.UCSC.hg19.knownGene, columns=“gene_id”)

> qProfile(project, query, upstream=3000, downstream=3000)
```

```
$coverage
      -3000 -2999 -2998 -2997 -2996 ...
query     8     8     8     8     8 ...

$Sample1
      -3000 -2999 -2998 -2997 -2996 ...
query     1     0     0     0     0 ...

$Sample2
      -3000 -2999 -2998 -2997 -2996 ...
query     0     0     0     2     0 ...
```



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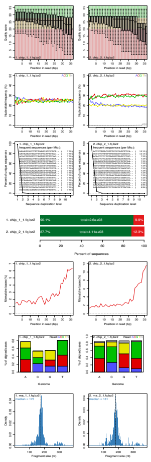
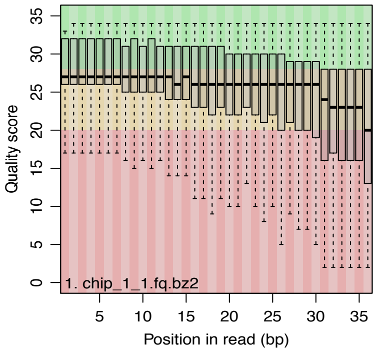
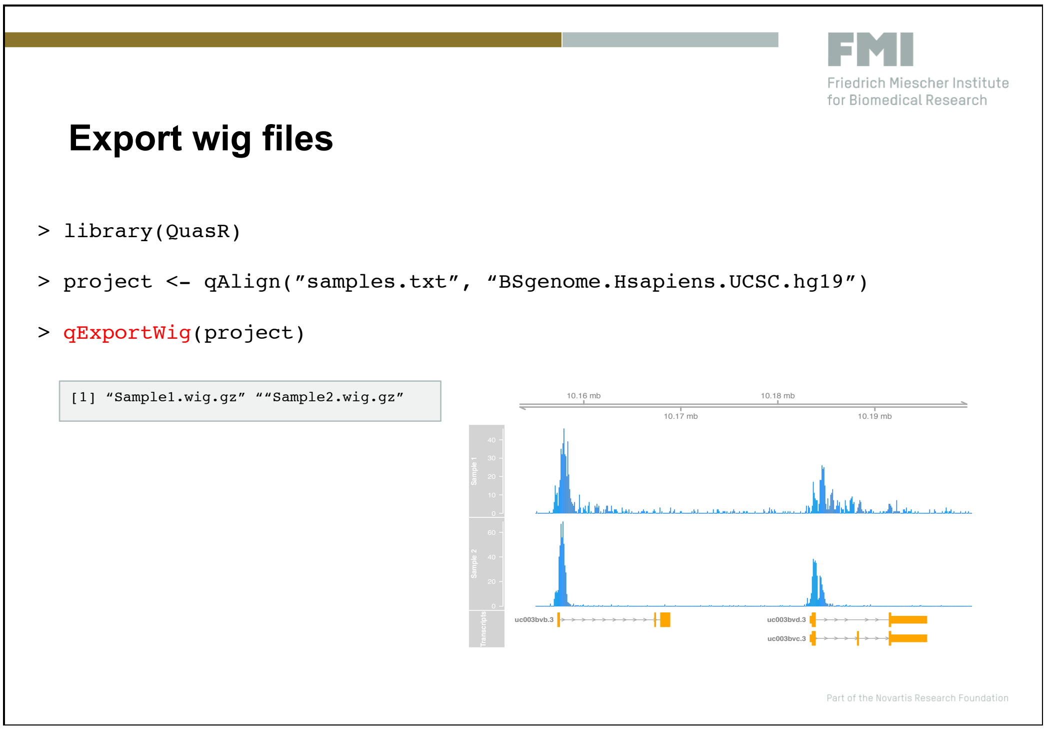
Quality control plots

```
> qQCReport(project, "qc_plots.pdf”)
```







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Export wig files

```
> library(QuasR)

> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”)

> qExportWig(project)
```

```
[1] “Sample1.wig.gz” ““Sample2.wig.gz”
```

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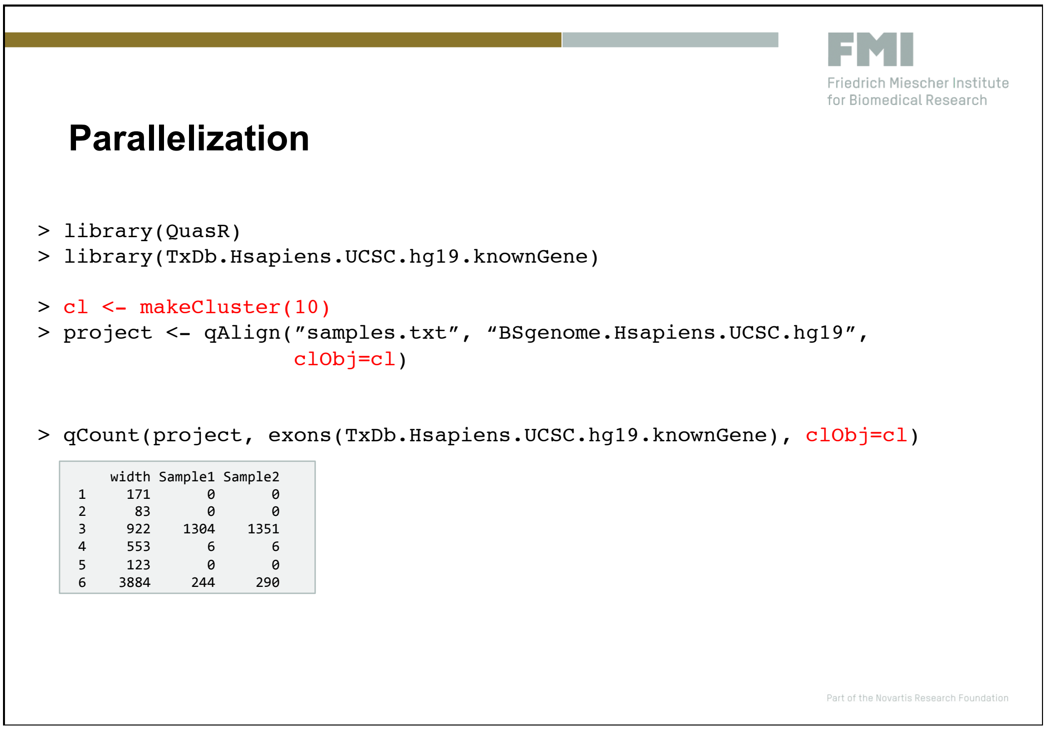

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Parallelization

```
> library(QuasR)
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)

> cl <- makeCluster(10)
> project <- qAlign(”samples.txt”, “BSgenome.Hsapiens.UCSC.hg19”,
                    clObj=cl)


> qCount(project, exons(TxDb.Hsapiens.UCSC.hg19.knownGene), clObj=cl)
```

```
   width Sample1 Sample2
1    171       0       0
2     83       0       0
3    922    1304    1351
4    553       6       6
5    123       0       0
6   3884     244     290
```

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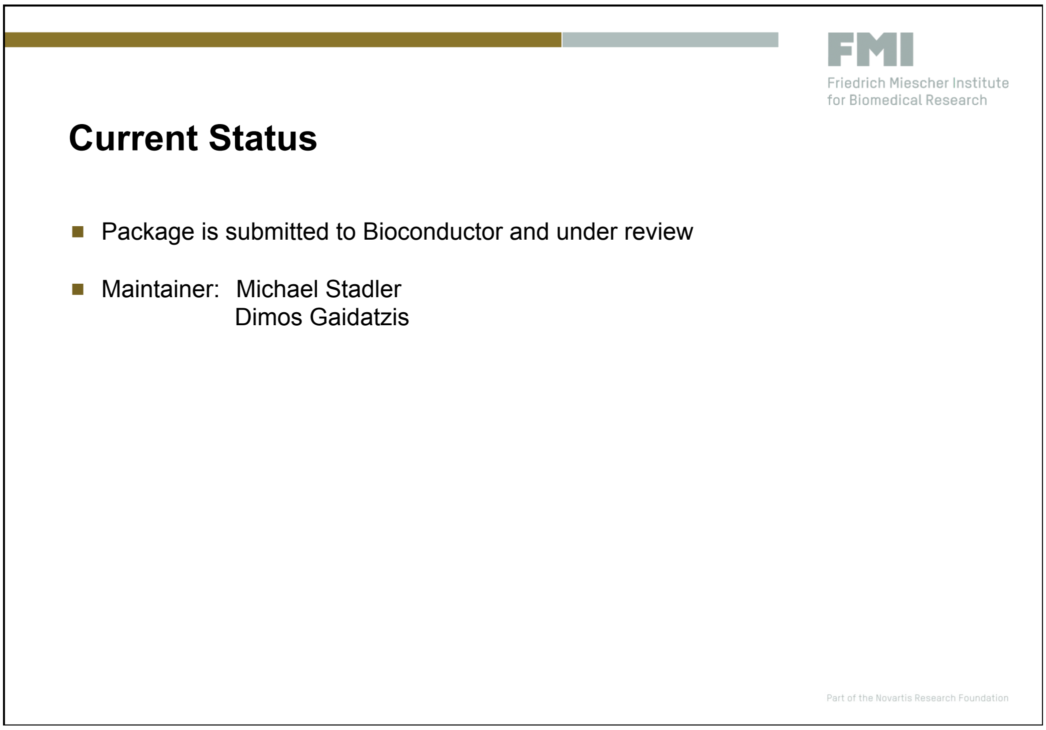

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Current Status

- Package is submitted to Bioconductor and under review
- Maintainer: Michael Stadler
  Dimos Gaidatzis

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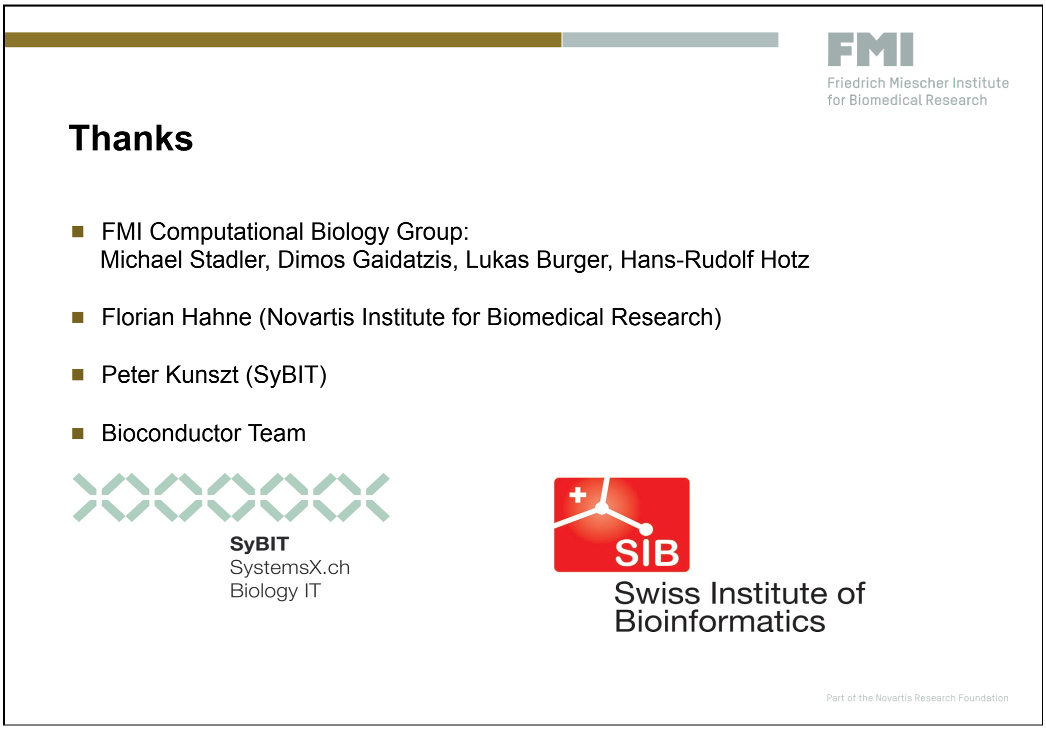

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- Florian Hahne (Novartis Institute for Biomedical Research)
- Peter Kunszt (SyBIT)
- Bioconductor Team

SyBIT
SystemsX.ch
Biology IT

SIB
Swiss Institute of
Bioinformatics

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