TEQC
An R-package for Quality Control in Target Capture Experiments

Manuela Hummel
manuela.hummel@crg.eu
Microarrays Unit, 4th floor, office 439.01
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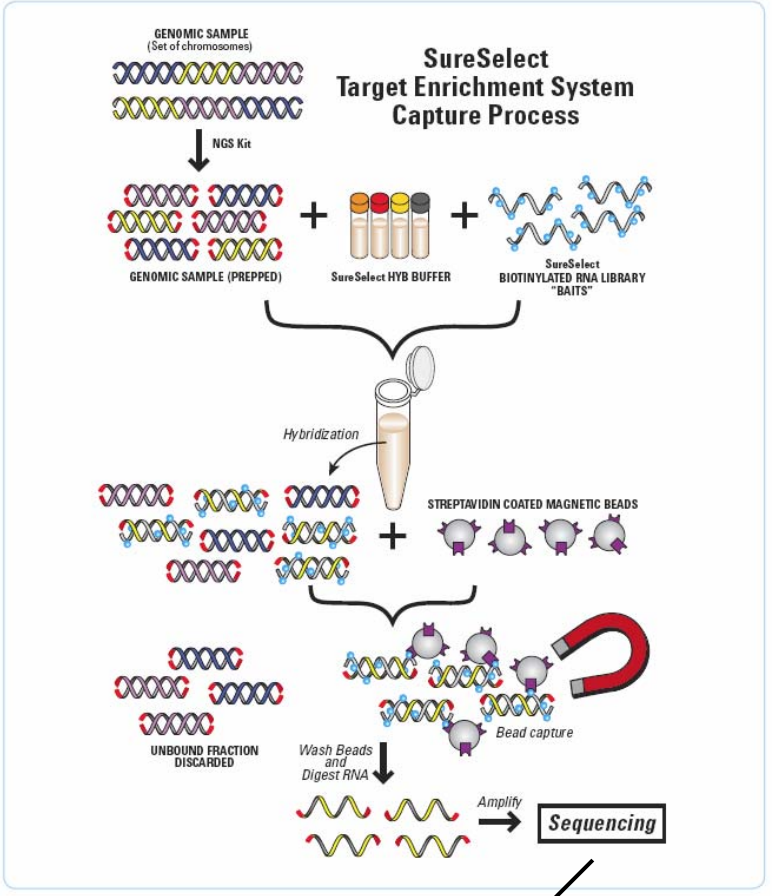
Target Capture Experiments

- Sequencing complete genomes at **high coverage** is still expensive
- Targeted sequencing is **cost-efficient** approach for variant detection in genomic **regions of interest**
- Genomic DNA within regions of interest is **captured** (on microarrays or in solution) by pre-designed **hybridization probes**, and **enriched** previous to high-throughput sequencing
- Target regions can be commercial solutions (e.g. **whole exome** kits by Agilent, NimbleGen, Illumina) or customized genomic regions (e.g. **linkage regions**)

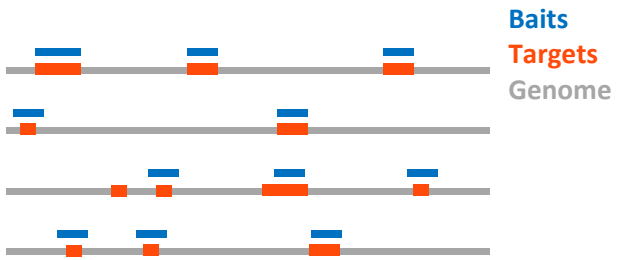


Target Capture Designs

- **Many small targets** (e.g. exons)
  -> hybridization probes ("baits") will cover more or less all targeted bases

vs

Few large targets (genomic regions)
-> for repetitive regions usually no hybridization probes are designed



- **Large total target size** (e.g. whole exome ~50Mb)
  -> probe design without or little tiling

vs

Small total target size (e.g. ~5Mb)
-> probe design with higher tiling

Quality of Enrichment Process

- Besides standard sequencing quality control, the quality of the target enrichment process should be checked
- Main quality issues in target capture experiments
  - **Capture Specificity**
    What fraction of sequenced reads fall on targeted regions?
  - **Capture Sensitivity**
    Do the targets have good coverage?
  - **Reproducibility**
    Are coverage distribution and coverage uniformity similar across replicates?
- The R/Bioconductor package *TEQC* was developed to address these and other issues

Hummel M, Bonnin S, Lowy E, Roma G. TEQC: an R-package for quality control in target capture experiments. Bioinformatics 2011 doi: 10.1093/bioinformatics/btr122

Availability, Installation, Documentation

- *TEQC* is **available** from Bioconductor (from Release 2.8)
  http://www.bioconductor.org/packages/release/bioc/html/TEQC.html

- and can be **installed** inside R by

```
> source("http://www.bioconductor.org/biocLite.R")
> biocLite("TEQC")
```

- The package has to be **loaded** to the current session before usage

```
> library(TEQC)
```

- See the package “vignette” for complete **documentation**

```
> vignette("TEQC")
```

Or
http://www.bioconductor.org/packages/2.8/bioc/vignettes/TEQC/inst/doc/TEQC.pdf

Input Files

1. A file containing genomic positions of the **target regions**

```
chr18 39629471    39629591    ccds|CCDS11920.1,ens|ENST00000262039,ens|ENST00000398870,ref|NM_002647,ref|PI
chr13 76395288    76395768    ccds|CCDS9454.1,ens|ENST00000321797,ens|ENST00000341547,ens|ENST00000357063,e
chr7  144345875   144345995   ccds|CCDS5888.1,ens|ENST00000360057,ens|ENST00000378098,ens|ENST00000378099,e
chr22 30738143    30738263    ccds|CCDS13875.1,ccds|CCDS46684.1,ens|ENST00000215793,ens|ENST00000411423,ens
chr19 40421033    40421753    ccds|CCDS12546.1,ens|ENST00000221347,ref|FCGBP,ref|NM_003890      1000  +
```

```
> targets <- get.targets(targetsfile="Targets.bed",
+ chrcol=1, startcol=2, endcol=3, skip=0)
```

2. A file containing genomic positions of the **aligned reads**

```
chr18 39629321    39629374    1_63_4568_5253
chr18 39629326    39629379    1_13_12498_6781
chr18 39629332    39629385    1_47_15232_10978
chr18 39629334    39629387    1_97_7517_2849
chr18 39629340    39629393    1_21_13390_12100
```

```
> reads <- get.reads("Reads.bed", chrcol=1,
+ startcol=2, endcol=3, idcol=4, zerobased=F, skip=0)
```

3. (Optionally) a file containing genomic positions and sequences of the **hybridization capture probes** (baits)

```
BaitID  Sequence        Chr     Start   End     Strand
A_36_B256315    CTTTGTTTTCATTTCTTTTCACTAACACAAGAAAACAAAGTACAGTACTTACCACTGAAGCAACCTGTCTCCCTGTGTTTCGCAAAATGCTTGGAAGCCAGGAAAACTTCGGTAAAAATC        chr1    11158024        11158144
A_36_B256316    ATACTCATCGCCAAACTGTGGTAGGCCCCCAGATGCCTTGGTGACTGCCACCACGGACCCAAGAGCAAACTGTCAAAAACAAGAAAAGATGGCATGTTTACACAGGGATCCCCATTCATT        chr1    11158144        11158264
A_36_B235510    GCGGCCGGAGCGGGCCATGGACGCGCTCAAGTCGGCGGGGCGGGCGCTGATCCGGAGCCCCAGCTTGGCCAAGCAGAGCTGGGGGGGCGGTGGCCGGCACCGCAGTGAGTGTGCGCGCGT        chr1    25870173        25870293
A_36_B245529    GTAAGCTTTGTGTTTGGGAAATTTTCTTGAATACCTCTTTTTTTCTTGTCTTTTTAATAGAAGTTGGTGAGATGGCAAAGCAGTATATAGAGAAAAGTCTTTTGGTTCCAGACCATGTGA        chr1    65656332        65656452
A_36_B245530    TCACACGCCTAATGATGTCCGAGTTGGAGAACAGGCGTGGCCAGCACTGGCTCCTTGATGGTGAGTTGAAACTGTGGGTAAACCGAATTTCTGGGAATAGTCAGTTAAGGTCTTGCACAC        chr1    65656452        65656572
A_36_B257183    AAGATCAAGCAAGCACCAGTTCCAGAACGAGCCAAGAAATCTGCCCACCTCATAGTGTAGCCGCCGGACTTTGCTCATAGCTGGCAGGCTGGACTGCTTCCCACTGATGTTCCGAAACAC        chr1    68611503        68611623
```

```
> baits <- get.baits("Baits.txt", chrcol=3, startcol=4,
+ endcol=5, seqcol=2)
```

Note on Genomic Coordiantes

- Bed files, as defined by UCSC (see *http://genome.ucsc.edu/FAQ/FAQformat*), follow the **0-based start / 1-based end** coordinate system

–> E.g. the first 100 bases on chromosome X have coordinates

chr	*start*	*end*
chrX	**0**	100

- BUT: bed files might also follow the **1-based** coordinate system!

(e.g. results from Bowtie mapper)

–> The same region from above would have coordinates

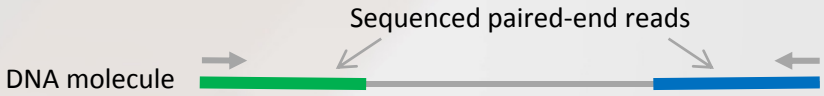
chr	*start*	*end*
chrX	**1**	100

- *TEQC* **assumes** the "official" **0-based start / 1-based end** system and then **shifts** start coordinates **to the 1-based** system

- In case your files are already in 1-based system, set parameter `zerobased` to FALSE in order to avoid the shifting (as shown in previous example in `get.reads()`)

Paired-End Data

- In case of paired-end data, some quality checks might rather be considered for read pairs instead of single reads
- Reads can be "**merged**" by pairs



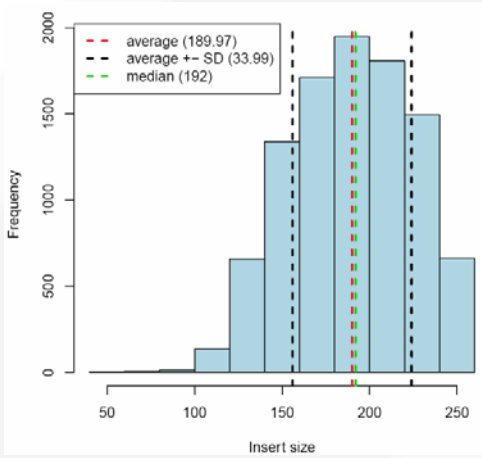
	chr	*start*	*end*
read1	chrX	135	185
read2	chrX	285	335

	chr	*start*	*end*
read pair	chrX	135	335

```
> readpairs <- reads2pairs(reads)
```

- Reads without matching pair and pairs where both reads align to different chromosomes or too far apart from each other are not included
- **Insert size** (= length from start of first read to end of second read) **distribution**

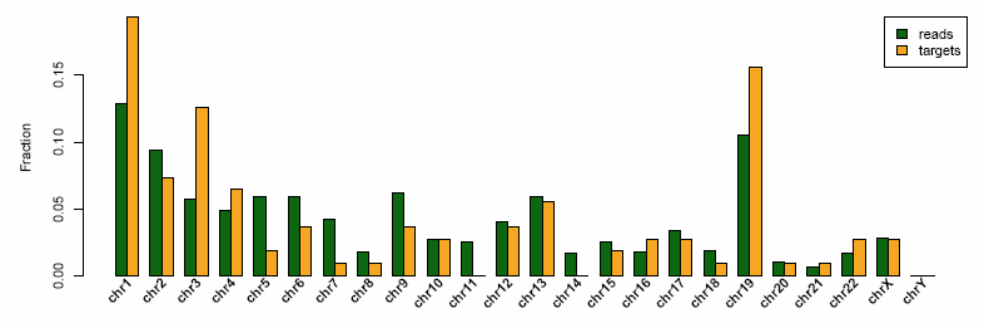
```
> insert.size.hist(readpairs)
```



Specificity

- Is the number of **reads mapping to each chromosome proportional to the amount of targeted** bases?

```
> chrom.barplot(reads, targets)
```



- **Fraction** of aligned **reads** that **overlap target** regions (by at least 1 base)

```
> fraction.reads.target(reads, targets)
```

- Consider also e.g. 50 bases on both **sides of each target** in the calculation

```
> fraction.reads.target(reads, targets, Offset=50)
```

- For paired-end data, fraction of **read pairs** that overlap target regions (i.e. where at least one of the two reads overlaps a target)

```
> fraction.reads.target(readpairs, targets)
```

Enrichment

- Measure for the **enrichment of sequences within the targeted** region
- Depends on fraction of reads mapping to the target and total target size relative to the respective genome size

$$\frac{\text{\# reads on target / \# aligned reads}}{\text{target size / genome size}}$$

```
> fr <- fraction.reads.target(reads, targets)
> ft <- fraction.target(targets, genome="hg19")
> enrichment <- fr / ft
```

- In case of paired-end data, enrichment can also be calculated for read pairs instead for single reads

Coverage

```
> Coverage <- coverage.target(reads, targets)
```

- **Overall on-target** coverage average, standard deviation, quantiles

```
> Coverage$avgTargetCoverage
> Coverage$targetCoverageSD
> Coverage$targetCoverageQuantiles
```

- Coverage average and standard deviation **per target**

```
> Coverage$targetCoverages
```

- Coverage **per targeted base**

```
> Coverage$coverageTarget
```

- Coverage per **sequenced and/or targeted base**

```
> Coverage$coverageAll
```

- **Number of reads** overlapping each **target**

```
> readsPerTarget(reads, targets)
```

- All coverage calculations are based on single reads!

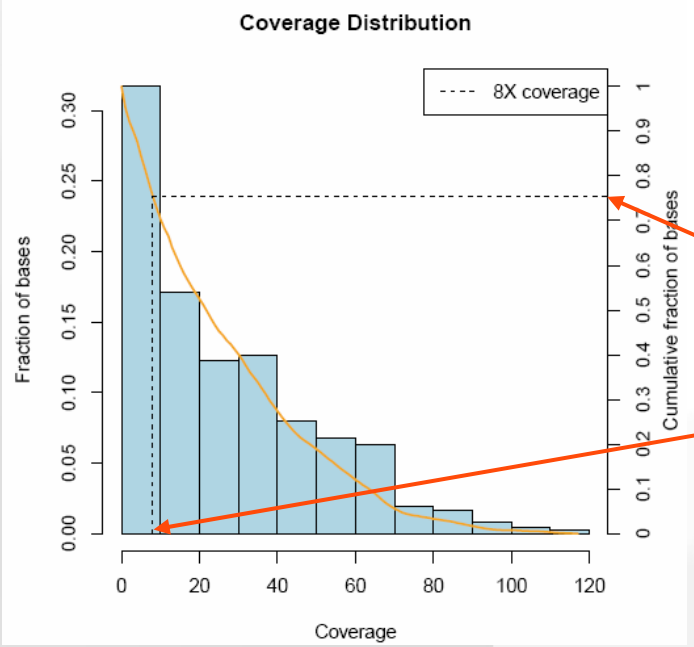
Sensitivity

- What fraction of target bases is covered by at least 1, 2, 5, 10, … reads?

```
> covered.k(Coverage$coverageTarget)
```

- Coverage histogram: graphical presentation of per-target-base coverage

```
> coverage.hist(Coverage$coverageTarget,
+ covthreshold=8)
```



~75% of targeted bases have at least 8X coverage

Coverage Uniformity

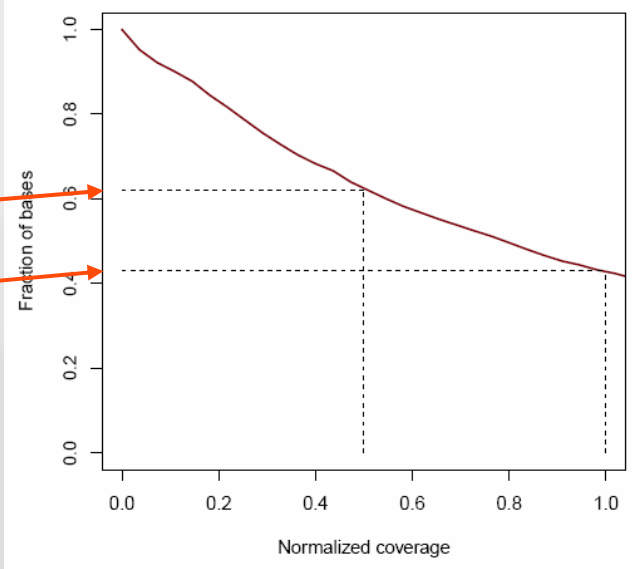
- Is the coverage uniform across targeted bases?
- Figure is based on **normalized coverage**

$$\frac{\text{Per-base coverage}}{\text{Average coverage over all target bases}}$$

- Normalized coverage is not dependent on absolute quantity of sequenced reads and therefore better comparable between samples / experiments

```
> coverage.uniformity(Coverage)
```

Fraction of targeted bases that reach at least
- **half the average** normalized coverage (=0.5)
- **average** normalized coverage (=1)



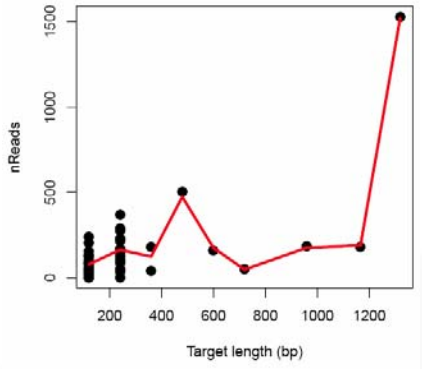
The steeper the curve is falling, the less uniform is the coverage

Coverage vs Target Length

- Is the **number of reads** mapping to a **target** proportional to its **size**?

–> Expected, since for larger targets there should be more capture probes

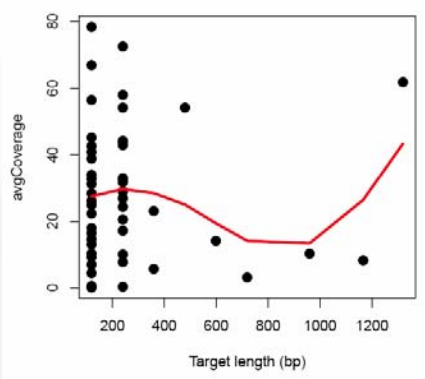
```
> RpT <- readsPerTarget(reads, targets)
> coverage.targetlength.plot(RpT,
+ plotcolumn="nReads", pch=16, cex=1.5)
```



- Does the **average coverage** depend on **target size**?

–> E.g. small targets might have smaller coverage due to worse bait tiling as compared to larger targets

```
> avgC <- Coverage$targetCoverages
> coverage.targetlength.plot(avgC,
+ plotcolumn="avgCoverage", pch=16, cex=1.5)
```



Coverage vs GC Content

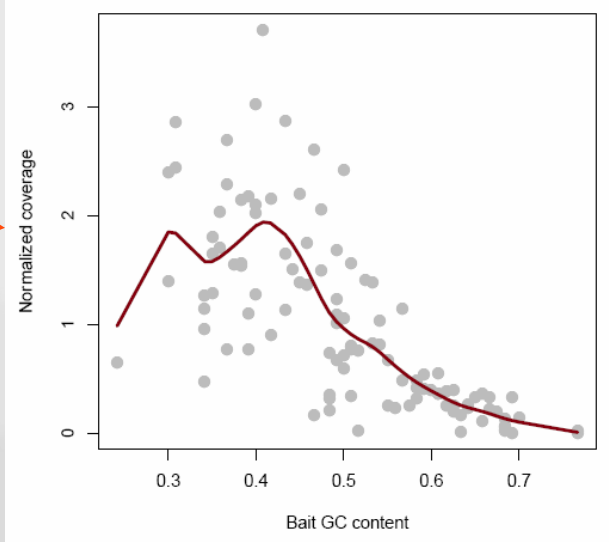
- Does **coverage** depend on **GC content** of the capture probes

–> Expected, since capture probes with very high or very low GC content have worse hybridization properties

```
> baits <- get.baits("Baits.txt", chrcol=3,
+ startcol=4, endcol=5, seqcol=2)

> coverage.GC(Coverage$coverageAll, baits, pch=16,
+ cex=1.5)
```

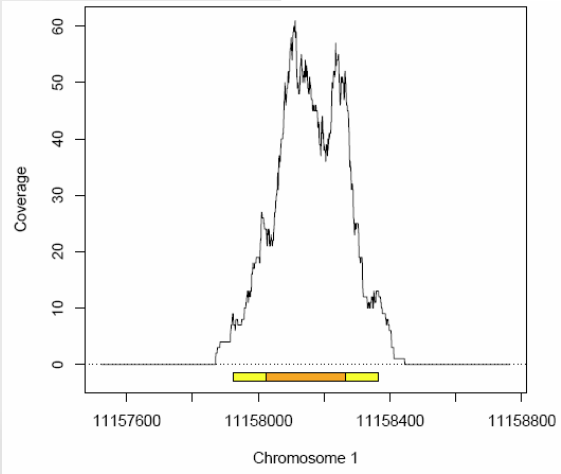
Average (normalized) coverage **per bait**



Coverage along Genome

- **Display per-base coverage** along a genomic interval
- Highlight target regions

```
> coverage.plot(Coverage$coverageAll, targets,
+ Offset=100, chr="chr1", Start=11157524,
+ End=11158764)
```



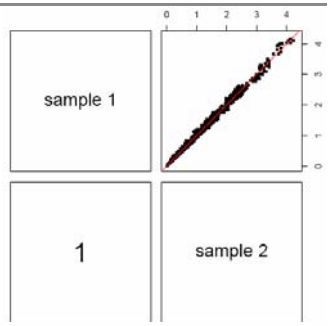
- … or export per-base coverage to **wiggle files** for use of genome browsers

```
> make.wigfiles(Coverage$coverageAll)
```

Reproducibility

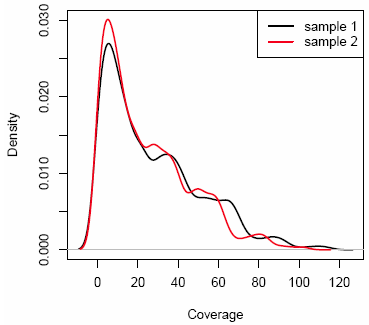
- Target coverage of (technical) replicates should be similar

```
> coverage.correlation(covlist,
+ plotfrac=0.1, cex.pch=4)
```



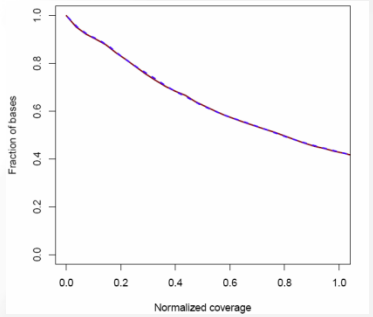
- Coverage densities

```
> covlist <- list(Coverage, Coverage2)
> coverage.density(covlist, normalized=F)
```



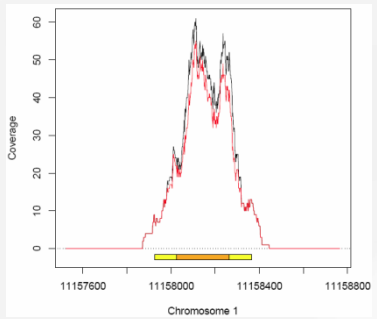
- Coverage uniformity

```
> coverage.uniformity(Coverage,
+ addlines=F)
> coverage.uniformity(Coverage2,
+ addlines=F, add=T, col="blue", lty=2)
```



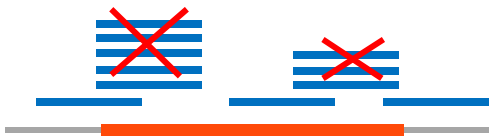
- Coverage along genomic region

```
> coverage.plot(Coverage$coverageAll, …
> coverage.plot(Coverage2$coverageAll,
+ add=T, col.line=2, …
```



Duplicate Analysis

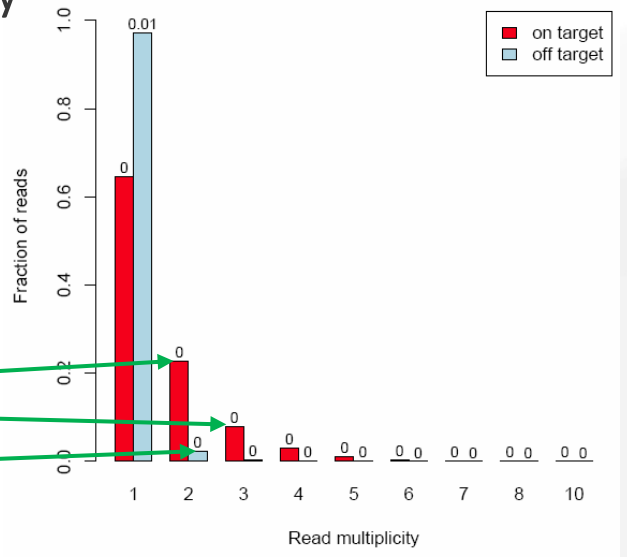
- Read duplicates, i.e. reads with same start and end positions, are usually **removed** before follow-up analysis because they are supposed to be **PCR artefacts**



- Problematic for Target Capture experiments, because many **"real" duplicates**, i.e. reads derived from actually different DNA molecules, are expected due to the **enrichment** process!

```
> duplicates.barplot(reads, targets)
```

>20% of **on-target** reads are present in 2 copies
almost 10% in 3 copies
in **off-target** reads a much lower fraction is duplicated



- After removing duplicates the per-base **coverage is limited** by the read length!
E.g. if the read length is 30, a base can only be covered by *at most* 30 reads starting at *different* positions

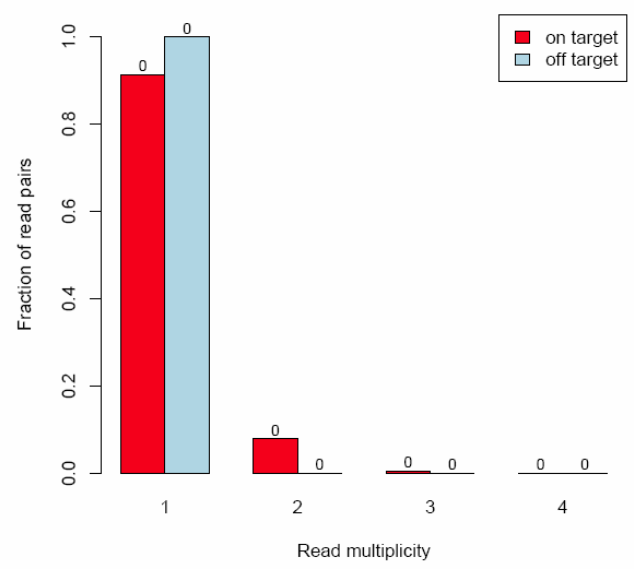
Duplicate Analysis

“Solutions”

- Remove duplicates only if also read **sequences** are identical (outside of TEQC)
- Use **long reads**
- Use **paired-end** sequencing

–> a read pair is only considered a duplicate if start and end positions of *both* reads are identical to those of another read pair
–> less likely to occur for different DNA fragments

```
> duplicates.barplot(readpairs,
+ targets,
+ ylab="Fraction of read pairs")
```



Future Work

- Provide automated HTML or PDF **report** that can be evoked by just one command line
- Allow **BAM** file format for the input files
- Improvements in terms of required computing **time** and **memory**
- Further **functionalities**, e.g. functions to retrieve easily average / per-base coverage for a selected region

References

- Hummel M, Bonnin S, Lowy E, Roma G (2011) *TEQC: an R-package for quality control in target capture experiments.* Bioinformatics. *doi: 10.1093/bioinformatics/btr122*

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- Tewhey R, Nakano M, Wang X, Pabón-Peña C, Novak B, Giuffre A, Lin E, Happe S, Roberts DN, LeProust EM, Topol EJ, Harismendy O, Frazer KA (2009) *Enrichment of sequencing targets from the human genome by solution hybridization*. Genome Biol. 10(10): R116.

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