Introduction to NGS data analysis

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1. Rigour and reproducibility

- Bioinformatics is *science*
- Keep a (digital) lab book
- Diligently record protocols:
    - software versions
    - commands
    - settings
    - file manipulations
    - numerical conversions
- Publish your code
- Data driven discovery is valid science, but requires self control

2. Getting your read data

- FTP or HTTP transfer (**wget** or **curl**)
- Might require a special program or plugin (e.g. Aspera)
- Files are compressed FASTQ, separate files for left and right pairs
- Verify file integrity after download (**md5sum / shasum**)
- Pay attention to download expiry dates
- Backup

Subject: Re: Re: F14FTSEUHT01[redacted] data release
Date: 2014-07-24 11:01
From: shirley xiaoxi guo <shirley.guo@bgitechsolutions.com>
To: [redacted]@cam.ac.uk>

Dear [redacted],

Please download data as below:

Link: http://cdts.genomics.hk/customerSupport/login.xhtml [1]
Account: 20140723F14FTSEUHT01[redacted]
Password: CHLhtbT201[redacted]

3. What your data looks like: the FASTQ format

```
@HWUSI-EAS100R:6:73:941:1973#0/1
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

sequence identifier

HWUSI-EAS100R	the unique instrument name
6	flowcell lane
73	tile number within the flowcell lane
941	'x'-coordinate of the cluster within the tile
1973	'y'-coordinate of the cluster within the tile
#0	index number for a multiplexed sample (0 for no indexing)
/1	the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

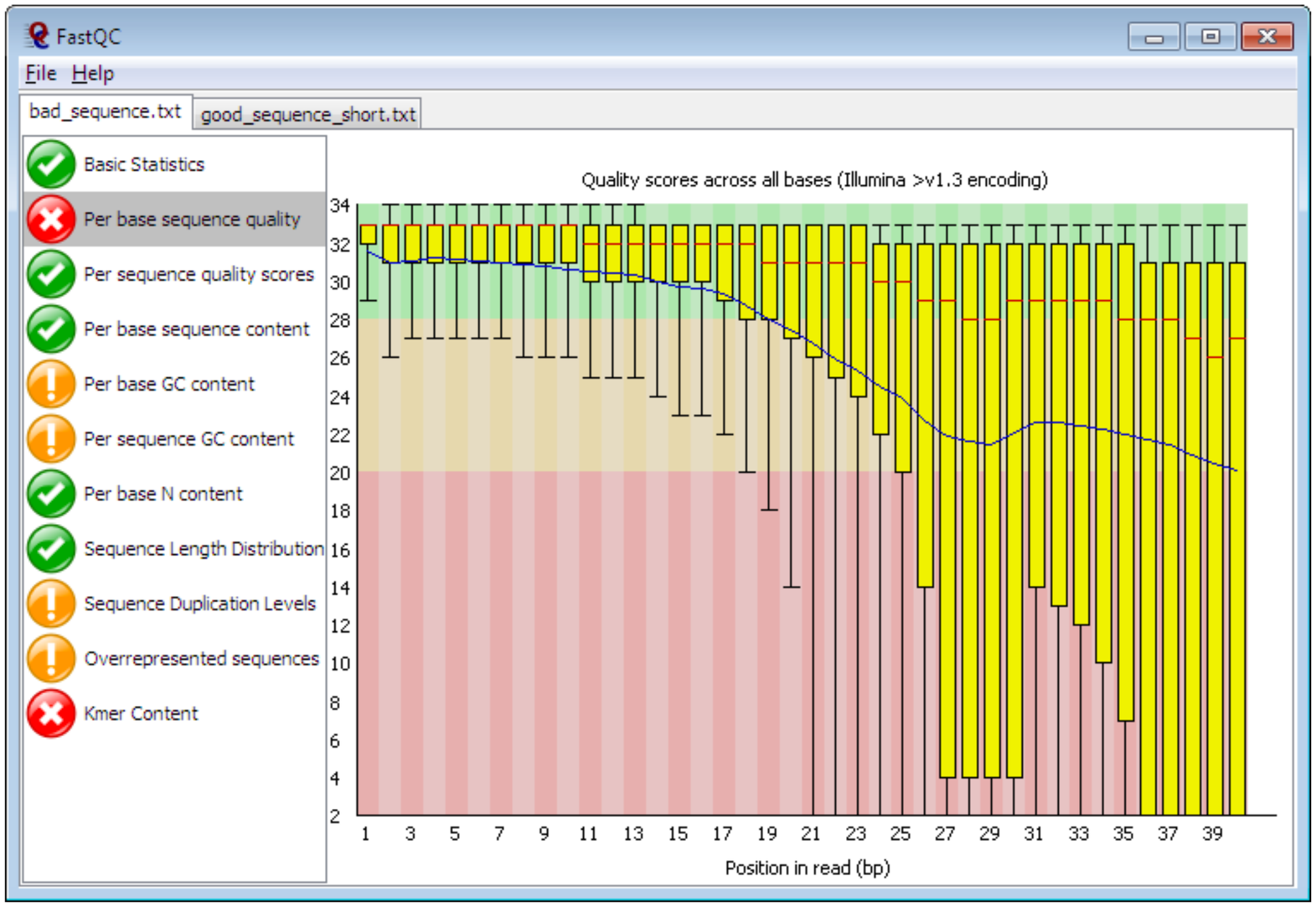
base quality

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
```

4. Organising the raw data

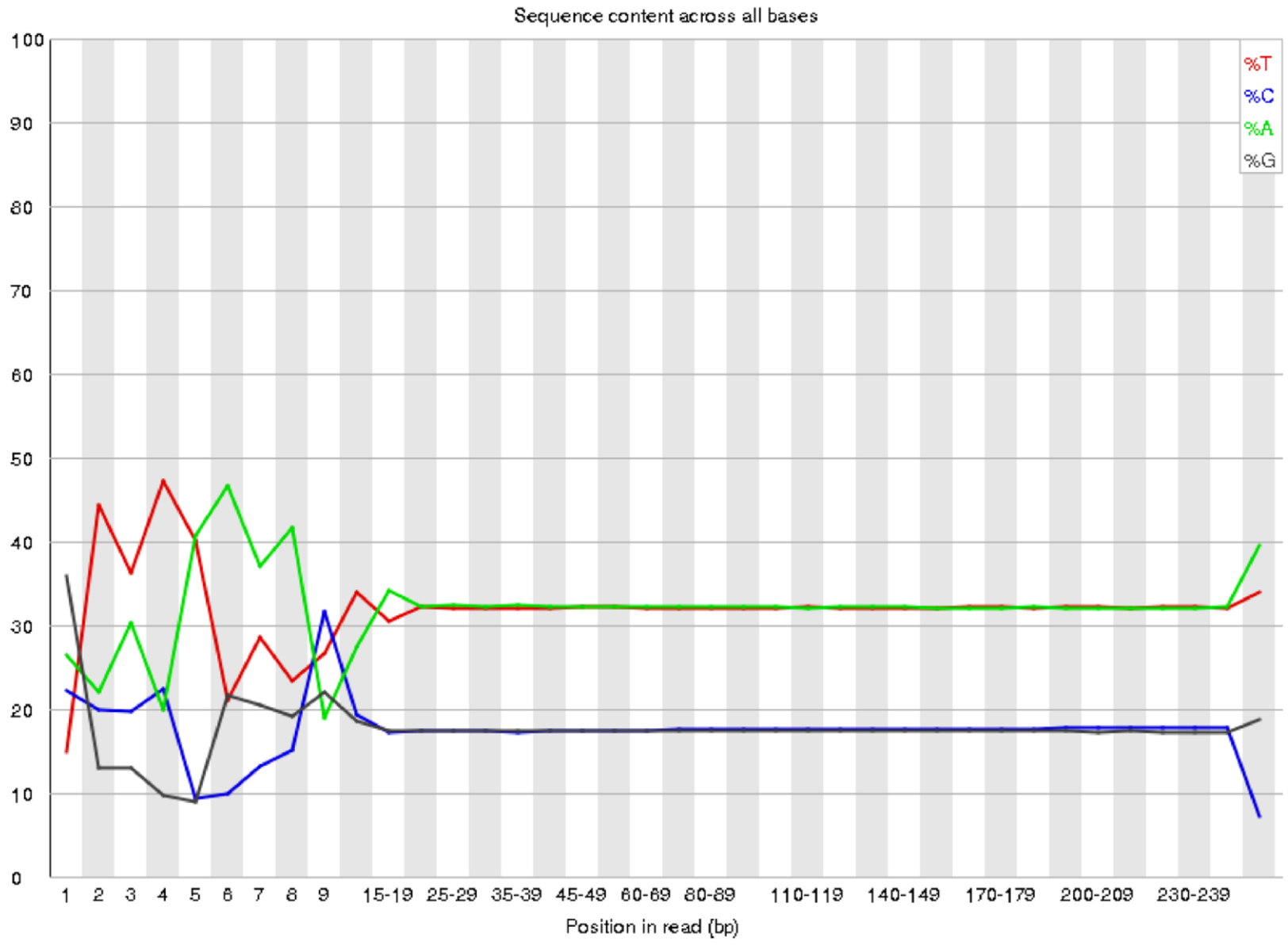
- if samples are multiplexed, map barcodes to samples
- may require splitting, e.g. with **fastx-toolkit barcode splitter**
- organise according to condition and sample
- give files machine-parseable names
    - rice_wet_rep1_left.fq.gz
    - rice_wet_rep1_right.fq.gz
    - rice_wet_rep2_left.fq.gz
    - rice_wet_rep2_right.fq.gz
    - rice_dry_rep1_left.fq.gz
    - rice_dry_rep1_right.fq.gz
    - rice_dry_rep2_left.fq.gz
    - rice_dry_rep2_rightt.fq.gz

5. Read data quality checking



http://www.bioinformatics.babraham.ac.uk/projects/fastqc

5. Read data quality checking



Sequence content (%)

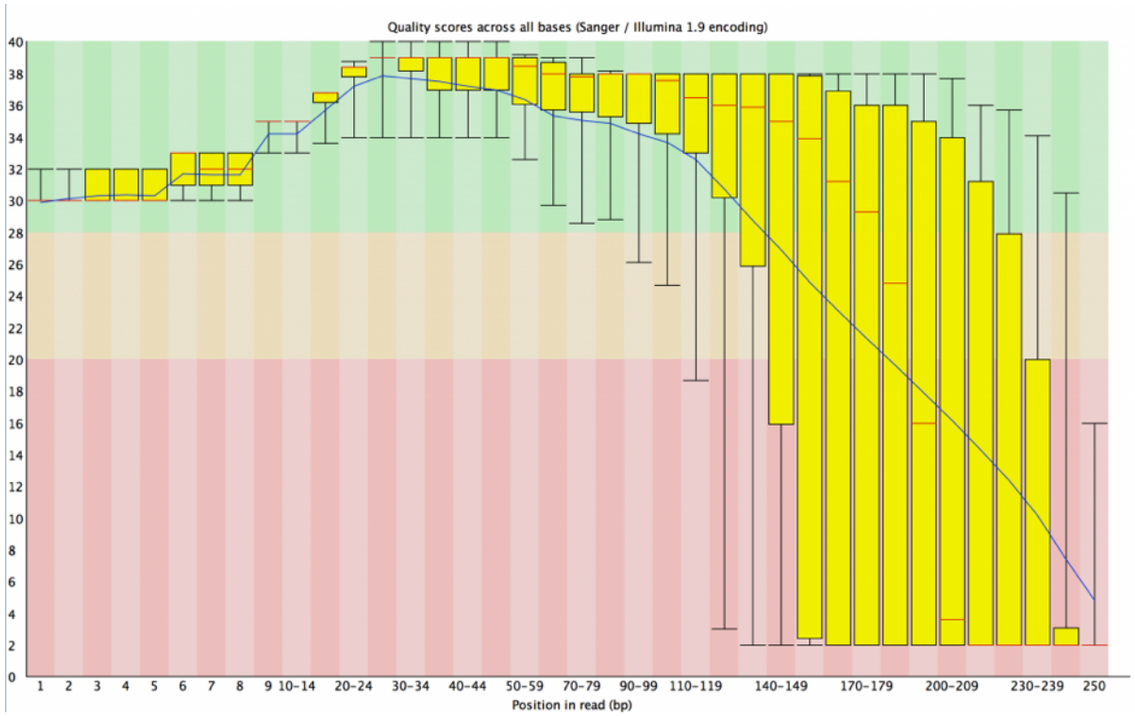
6. Quality improvement

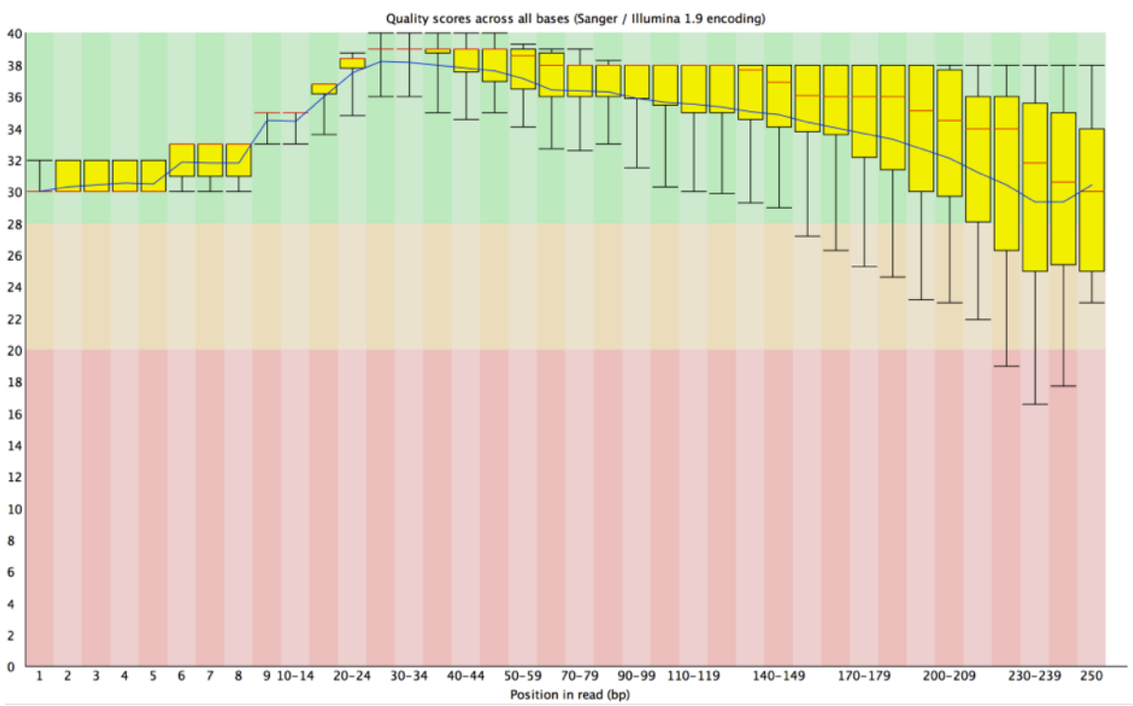
common approaches:

- trimming adapters
- trimming low-quality bases
- trimming to a fixed length
- discarding low-quality reads

tools:

- trimmomatic
- fastx-toolkit





7. Getting hold of a reference

genome or transcriptome?

- genome for:
    - variant calling
        - DNase-seq
        - CHiP-seq
- transcriptome for:
    - RNAseq
- FASTA format

>Seq1 [organism=Carpodacus mexicanus] actin (act) mRNA, partial cds
CCTTTATCTAATCTTTGGAGCATGAGCTGGCATAGTTGGAACCGCCCTCAGCCT
CCTCATCCGTGCAGAACTTGGACAACCTGGAACTCTTCTAGGAGACGACCAAAT
TTACAATGTAATCGTCACTGCCCACGCCTTCGTAATAATTTTCTTTATAGTAATAC
CAATCATGATCGGTGGTTTCGGAAACTGACTAGTCCCACTCATAATCGGCGCCC
CCGACATAGCATTCCCCCGTATAAACAACATAAGCTTCTGACTACTTCCCCCATC
ATTTCTTTTACTTCTAGCATCCTCCACAGTAGAAGCTGGAGCAGGAACAGGGTG
AACAGTATATCCCCCTCTCGCTGGTAACCTAGCCCATGCCGGTGCTTCAGTAGA
CCTAGCCATCTTCTCCCTCCACTTAGCAGGTGTTTCCTCTATCCTAGGTGCTATT
AACTTTATTACAACCGCCATCAACATAAAACCCCCAACCCTCTCCCAATACCAAA
CCCCCCTATTCGTATGATCAGTCCTTATTACCGCCGTCCTTCTCCTACTCTCTCTC
CCAGTCCTCGCTGCTGGCATTACTATACTACTAACAGACCGAAACCTAAACACTA
CGTTCTTTGACCCAGCTGGAGGA

7. Getting hold of a reference

- sources of existing references:
    - **ENSEMBL**
      http://ensembl.org
    - **JGI**
      http://genome.jgi.doe.gov
    - **EBI genomes**
      http://www.ebi.ac.uk/genomes/bacteria.html
- assembling your own:
    - covered tomorrow

8. Comparing reads to the reference

- Estimate the original source location of each read
- **alignment** finds the precise location of each base in the read
- **mapping** finds the approximate location of each read
- sometimes we don't need the location, only the source molecule (e.g. which chromosome/transcript
- **multi-mapping** is when a read matches multiple locations. Resolved by **assignment**.

9. Application-specific workflows

- Usually starts by converting read alignments into some numerical summary data (e.g. expression counts, peak calls)
- Running programs from the command-line
- Using specialist packages in a programming language like R (http://bioconductor.org), Python or Ruby (http://biogems.info)
- Writing your own analysis code
- Performing statistical analysis on alignment summary data
- Normalisation: accounting for different sample sizes and compositions
- Storytelling and plotting