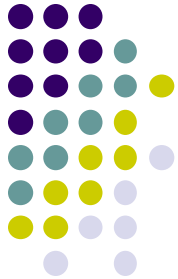
FastQC

Quality Assurance tool for FASTQ sequences

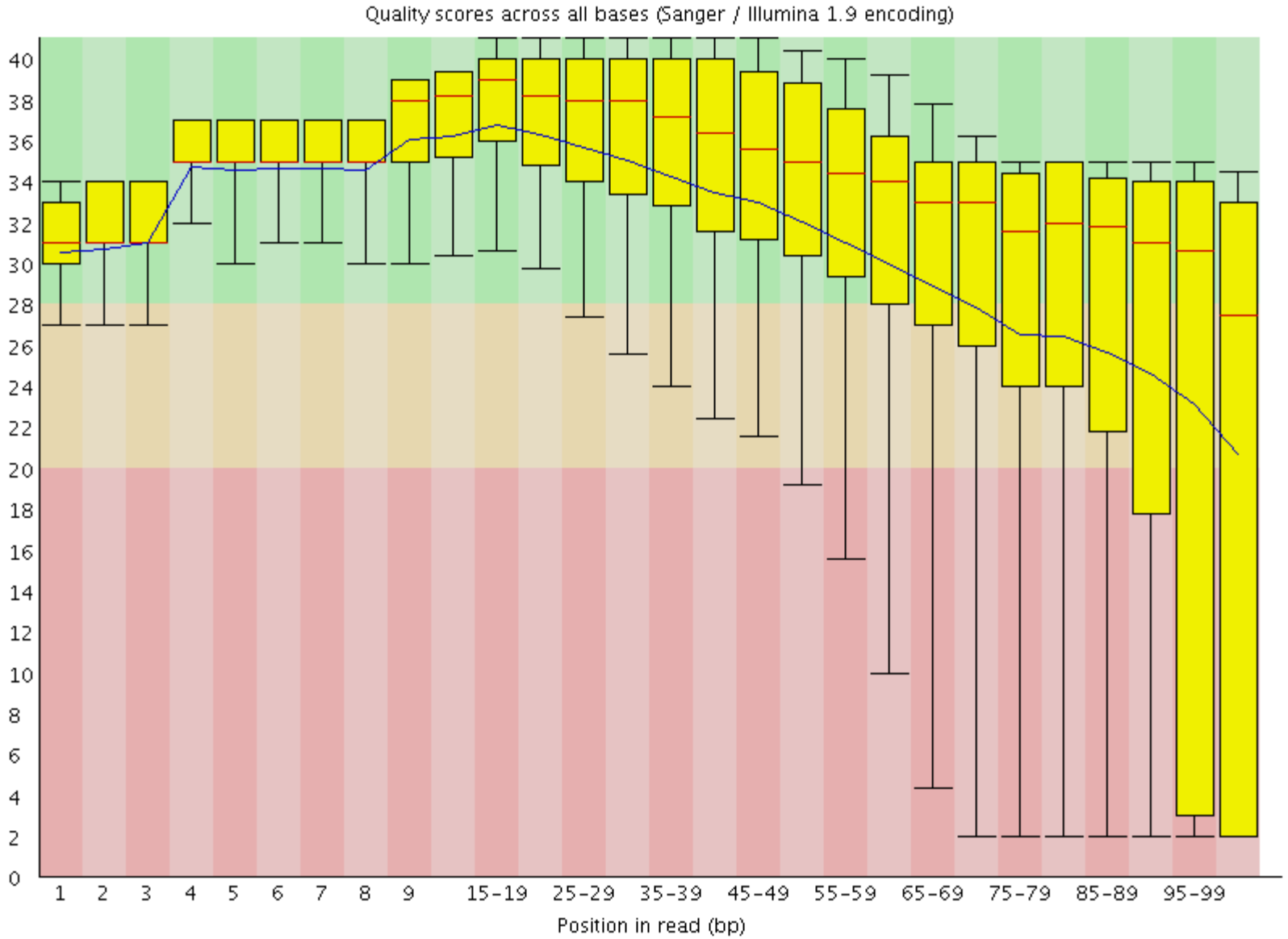


- FastQC website:
  http://www.bioinformatics.babraham.ac.uk
- FastQC report documentation:
  http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/
- Good Illumina dataset:
  http://www.bioinformatics.babraham.ac.uk/projects/fastqc/good_sequence_short_fastqc/fastqc_report.html
- Bad Illumina dataset:
  http://www.bioinformatics.babraham.ac.uk/projects/fastqc/bad_sequence_fastqc/fastqc_report.html
- Real Yeast ChIP-seq dataset:
  http://web.corral.tacc.utexas.edu/BioITeam/yeast_stuff/Sample_Yeast_L005_R1.cat_fastqc/fastqc_report.html

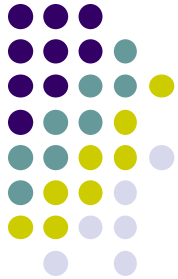
Most useful reports

- Should I trim low quality bases?
  - Per-base sequence quality Report
    - based on *all* sequences
- Do I need to remove adapter sequences?
  - Overrepresented sequences Report
    - based on *1st 200,000* sequences
- How complex is my library?
  - Sequence duplication levels Report
    - estimate based on *1st 200,000* sequences

Per-base sequence quality



Overrepresented Sequences

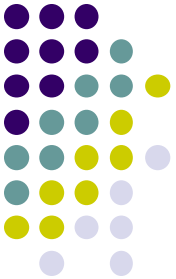


Sequence	Count	Percentage	Possible Source
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCAGAATCTCGTATG	60030	5.01369306977828	TruSeq Adapter, Index 1 (97% over 37bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCAGAATCTCGTATGC	42955	3.5875926338884896	TruSeq Adapter, Index 1 (97% over 37bp)
CACACGTCTGAACTCCAGTCACCTCAGAATCTCGTATGCCGTCTTCTGCT	3574	0.29849973398946483	RNA PCR Primer, Index 40 (100% over 41bp)
CAGATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCAGAATCTCGTAT	2519	0.2103863542024236	TruSeq Adapter, Index 1 (97% over 37bp)
GAGATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCAGAATCTCGTAT	1251	0.10448325887543942	TruSeq Adapter, Index 1 (97% over 37bp)

Overrepresented Sequences

Sequence	Count	Percentage	Possible Source
AACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTA	102020	1.0707851766890004	No Hit
AATTCTAGAGCTAATACGTGCAACAAACCCCGACTTATGGAAGGGACGCA	89437	0.9387160737848865	No Hit
AAAGGATTGGCTCTGAGGGCTGGGCTCGGGGGTCCCAGTTCCGAACCCGT	89427	0.9386111154260659	No Hit
TACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCC	87604	0.9194772066130483	No Hit
ATTGGCTCTGAGGGCTGGGCTCGGGGGTCCCAGTTCCGAACCCGTCGGCT	65829	0.6909303802809273	No Hit
TCTAGAGCTAATACGTGCAACAAACCCCGACTTATGGAAGGGACGCATTT	65212	0.6844544495416888	No Hit
TAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAAC	61582	0.646354565289767	No Hit
CTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCAACAAACCCCGAC	59180	0.6211435675010296	No Hit
ATGGATCCGTAACTTCGGGAAAAGGATTGGCTCTGAGGGCTGGGCTCGGG	56982	0.598073720232235	No Hit
AAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACG	54813	0.5753082522040206	No Hit
ATTCTAGAGCTAATACGTGCAACAAACCCCGACTTATGGAAGGGACGCAT	52688	0.5530046009546172	No Hit
GCGACCCCAGGTCAGGCGGGATTACCCGCTGAGTTTAAGCATATCAATAA	41363	0.4341392595901502	No Hit
CTAGAGCTAATACGTGCAACAAACCCCGACTTATGGAAGGGACGCATTTA	40019	0.4200328561646452	No Hit
AGAACTCCGCAGTTAAGCGTGCTTGGGCGAGAGTAGTACTAGGATGGGTG	39753	0.4172409638200141	No Hit
ACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCAACAAACCCCGA	38867	0.4079416532284981	No Hit
ACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAG	38438	0.40343893963508914	No Hit
ACTTCGGGAAAAGGATTGGCTCTGAGGGCTGGGCTCGGGGGTCCCAGTTC	37406	0.3926072370047907	No Hit
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATG	34199	0.35894709133098535	TruSeq Adapter, Index 4 (100% over 49bp)
GAACCTTGGGATGGGTCGGCCGGTCCGCCTTTGGTGTGCATTGGTCGGCT	34099	0.3578975077427782	No Hit

Overrepresented Sequences



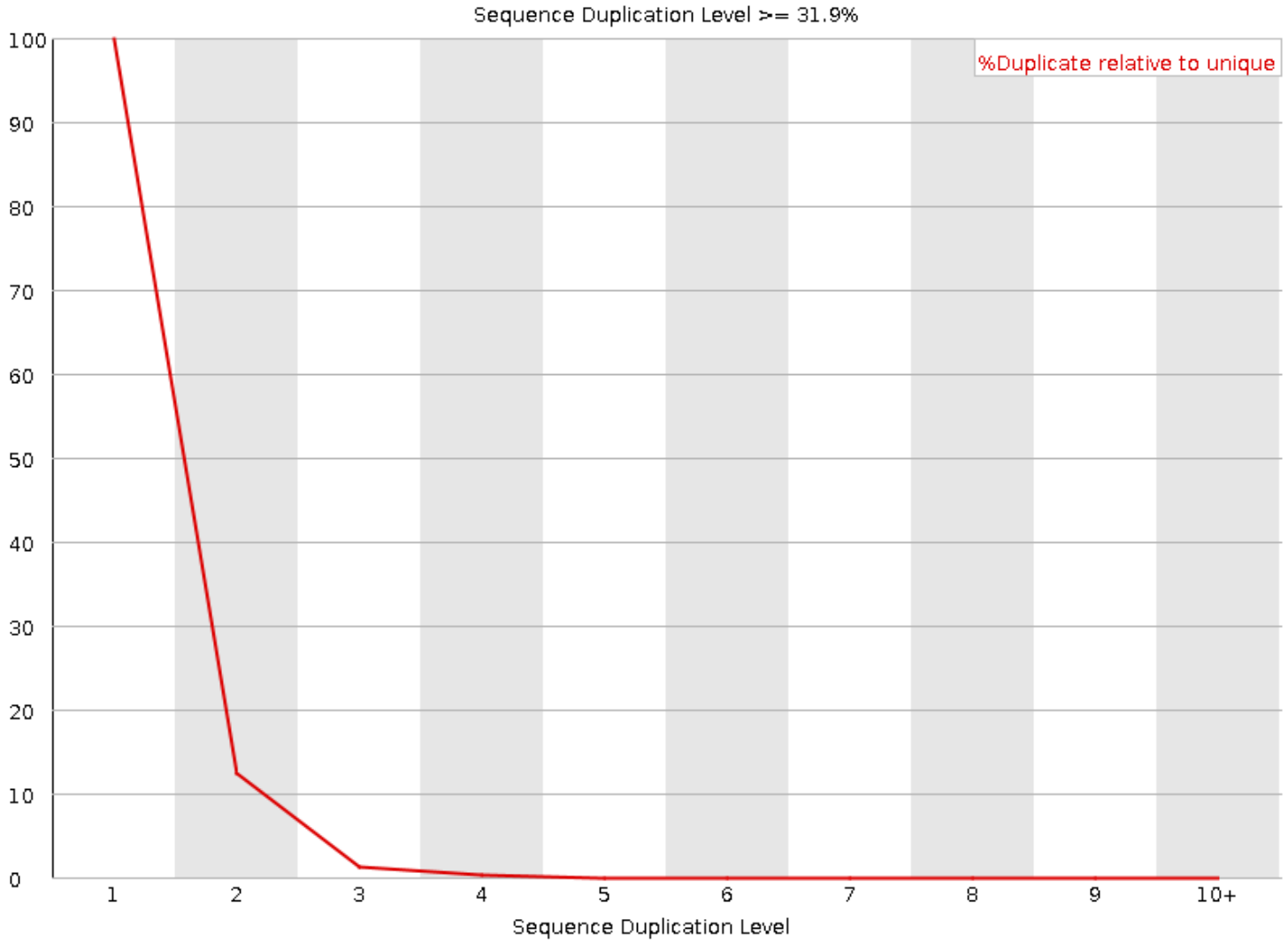
Sequence	Count	Percentage	Possible Source
GAAGGTCACGGCGAGACGAGCCGTTTATCATTACGATAGGTGTCAAGTGG	5632816	32.03026785752871	No Hit
TATTCTGGTGTCCTAGGCGTAGAGGAACAACACCAATCCATCCCGAACTT	494014	2.8091456822607364	No Hit
TCAAACGAGGAAAGGCTTACGGTGGATACCTAGGCACCCAGAGACGAGGA	446641	2.539765344040083	No Hit
TAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAAC	179252	1.0192929387357474	No Hit
GAAGGTCACGGCGAGACGAGCCGTTTATCATTACGATAGGGGTCAAGTGG	171681	0.9762414422996221	No Hit
AACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTA	143415	0.8155105483274229	No Hit
AGAACATGAAACCGTAAGCTCCCAAGCAGTGGGAGGAGCCCTGGGCTCTG	111584	0.6345077504066322	No Hit
AAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACG	111255	0.6326369351474214	No Hit
ATTACGATAGGTGTCAAGTGGAAGTGCAGTGATGTATGCAGCTGAGGCAT	73682	0.41898300890326096	No Hit
GAAGGTCACGGCGAGACGAGCCGTTTATCATTACGATAGGTGTCAAGGGG	71661	0.4074908580252516	No Hit
GGATGCGATCATACCAGCACTAATGCACCGGATCCCATCAGAACTCCGCA	69548	0.3954755612388914	No Hit
ATATTCTGGTGTCCTAGGCGTAGAGGAACAACACCAATCCATCCCGAACT	54017	0.30716057099328803	No Hit

Duplication levels

Yeast ChIP-seq

for every 100 unique sequences there are

- ~12 sequences w/2 copies
- ~1-2 with 3 copies

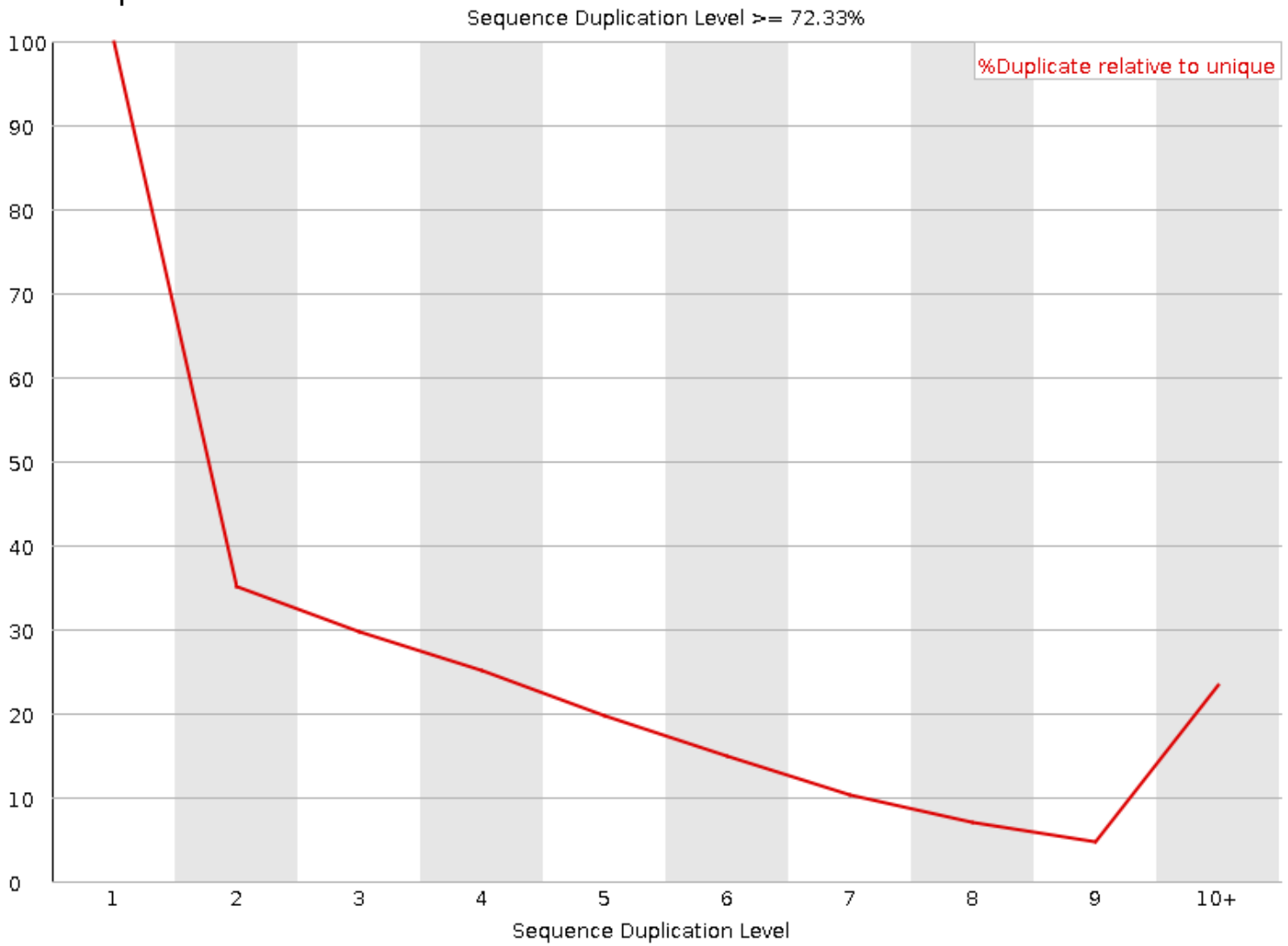


Duplication levels

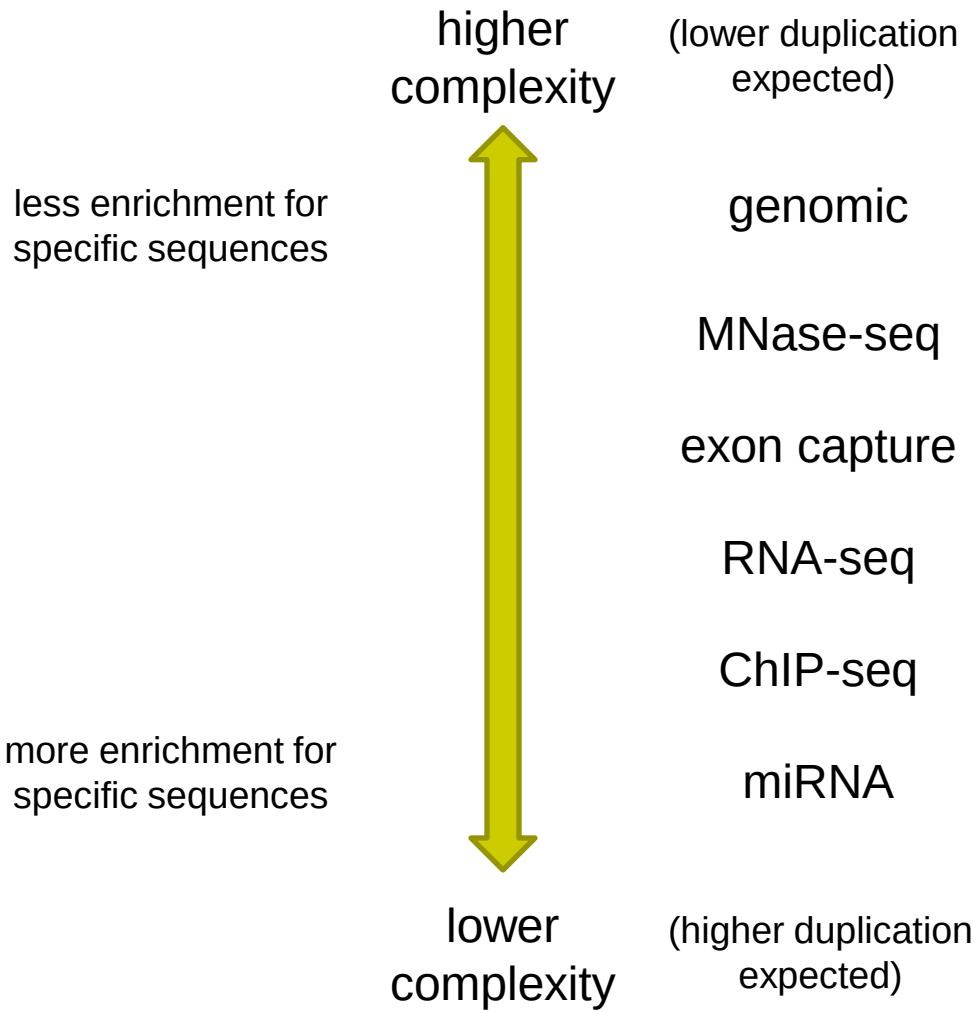
Yeast ChIP-exo

for every 100 unique sequences there are

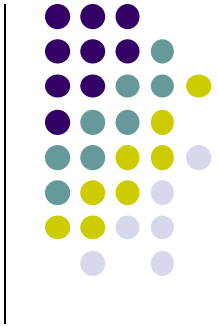
- ~35 sequences w/2 copies
- ~22 with 10+ copies



Library complexity is a function of experiment type (& sequencing depth)



Running FastQC



- Can run as interactive tool or command line
- Input:
  - fastq files (R1, R2 separately)
- Output:
  - directory with html & text reports
    - fastqc_report.html
    - fastqc_data.txt