Discovering Origins of Replication

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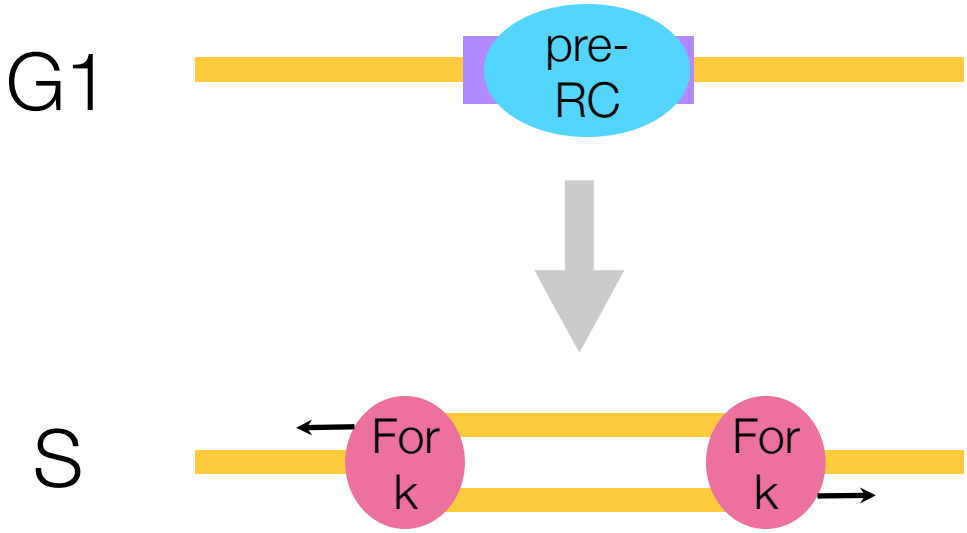
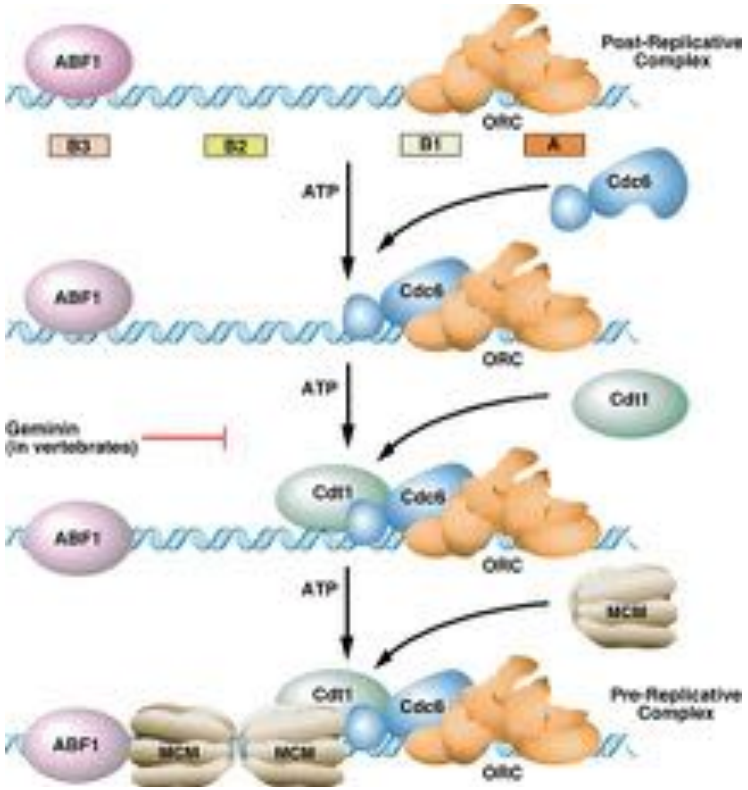
QB Bootcamp Lecture 3

~300 separate loci direct DNA replication initiation in Saccharomyces cerevisiae

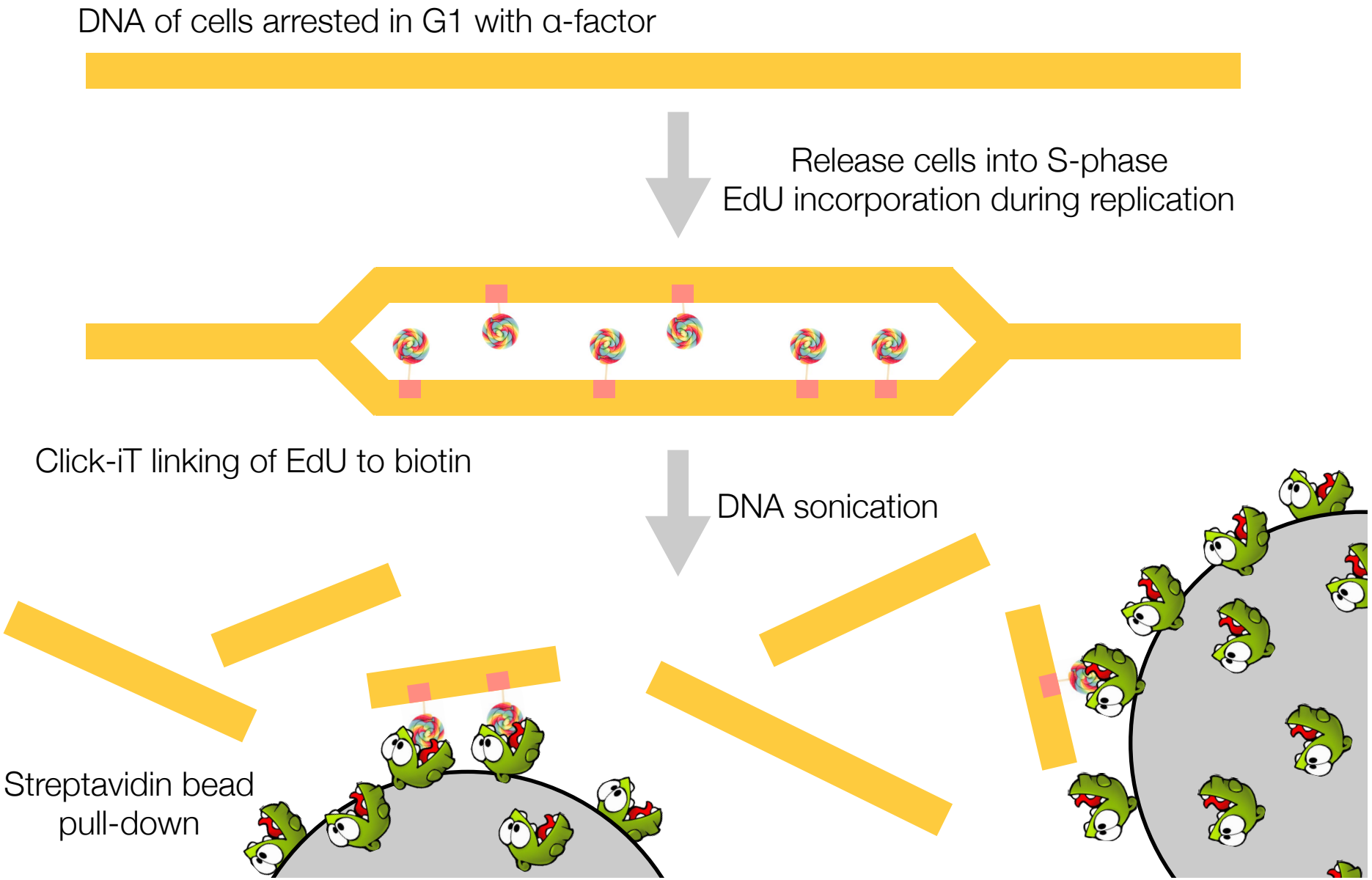
ARS: autonomously replicating sequence



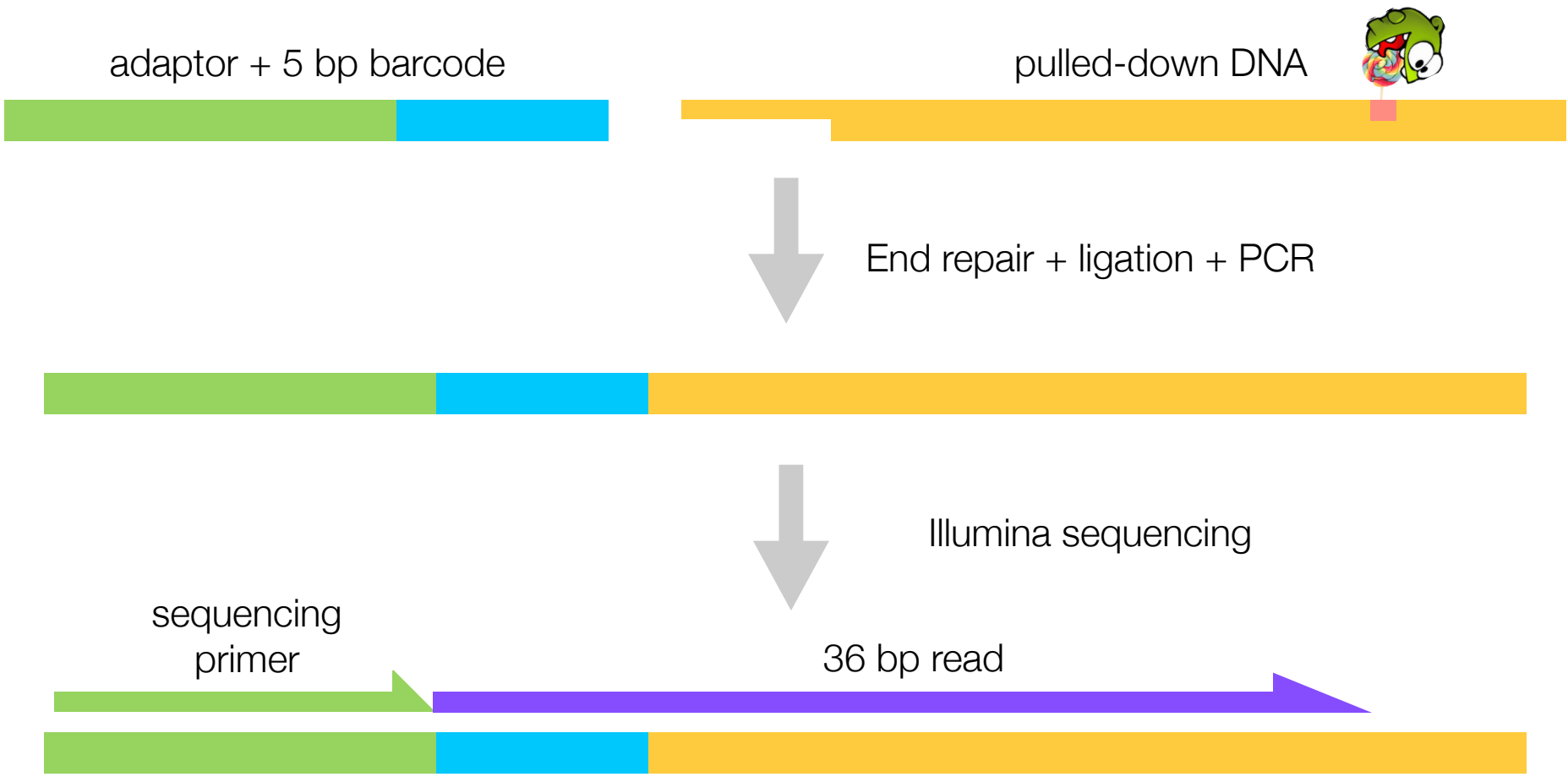


The Stillman lab is interested, in part, in the signaling mechanisms governing pre-RC firing -> genome-wide replication tracking

Tracking replication with EdU pulldown + sequencing



Barcoding samples for sequencing



~15 M reads for 14 barcoded samples

Thanks Yi-Jun!

What we will do

- Today
  - Map reads to the yeast genome
  - Compute “replication profiles”: # of reads covering each genomic position
  - View these data using the UCSC genome browser; compare to known ARSs
- Tomorrow
  - Python tutorial (cont)
  - Load replication profiles into Python
  - Smooth and plot replication profiles

Analysis Pipeline

- No single application available that will let us analyze these data
  - Just 4 steps to go from raw observations to biological discovery
- Each step requires selection, tuning, and debugging
  - Analogous to a wetlab protocol for running an experiment
- The components of the pipeline can be used in many other assays
  - Reads => Comparative Genomics, Transcriptome Analysis, de novo sequencing, Protein binding sites, Chromatin regulation…
  - Alignment => Forms the basis for almost every assay
  - SAMTools => Filtering, selection, interpretation of alignments

Analysis Pipeline

- Get the files (curl dash Capital-O)

```
$ curl -O http://schatzlab.cshl.edu/data/challenges/replication_exercise.tgz
```

- Unpack the files

```
$ tar xzvf replication_exercise.tgz
```

- Check out the files

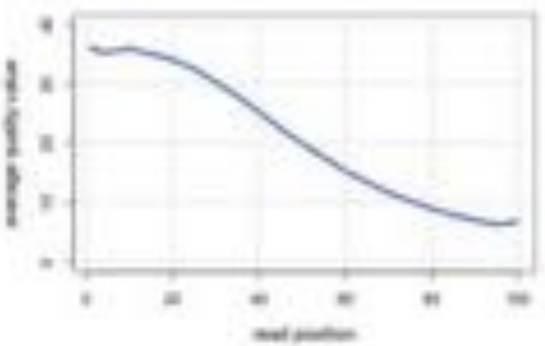
```
$ cd replication_exercise/
$ ls -R
$ less *.txt
$ less reads/A1.fastq
```

[What is the secret phrase?]

Illumina Quality

QV	p_{error}
40	1/10000
30	1/1000
20	1/100
10	1/10

$$Q_{\text{sanger}} = -10 \log_{10} p$$



```
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                     |
 33                        59   64       73                            104                   126

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

http://en.wikipedia.org/wiki/FASTQ_format

Paired-end and Mate-pairs

Paired-end sequencing

- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

Mate-pair sequencing

- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads

Analysis Pipeline

- Check out the analysis script

```
$ cat course_pipeline.sh
```

- We have done already done the first steps to partition reads into batches

```
# Quality filter reads
# fastq_quality_filter -q 10 -p 90 -i /data/kinney/data/illumina_sequencing/
11.01.24_sheu_edu/reads.fastq -o reads/reads_qual.fastq

# Split reads by batch
# cat reads/reads_qual.fastq | fastx_barcode_splitter.pl --bcfile /data/
kinney/data/illumina_sequencing/11.01.24_sheu_edu/barcodes.txt --prefix reads/
tmp1_ --suffix .fastq --mismatches 0 –bol
```

- You can embed comments into scripts with '#'

Analysis Pipeline

- Now that the reads are prepared, next step is to align

```
# Create bwa index for genome
# bwa index genome/genome.fasta

# Align reads using bwa
bwa aln genome/genome.fasta reads/A1.fastq > mappings/A1.sai
bwa samse genome/genome.fasta mappings/A1.sai reads/A1.fastq > mappings/A1.sam
```

- BWA (Li & Durbin, 2009) is one of the most popular tools for aligning short reads to a reference genome. It is used in almost every sequencing assay that start from short reads. It takes a few steps to run because it uses the special BWT index of the genome for making the alignments fast.

Analysis Pipeline

- Now that the reads are aligned, need to transform and sort them

```
# Create pileup using samtools
samtools view -bS mappings/A1.sam > mappings/A1.bam
samtools sort mappings/A1.bam mappings/A1.sorted
samtools index mappings/A1.sorted.bam
samtools pileup -c -f genome/genome.fasta mappings/A1.sorted.bam > pileups/A1.pileup
```

- The pileup file encodes how many reads align to each position in the genome

```
$ less pileups/A1.pileup
```

- Run a quick command to find positions with deep coverage

```
$ awk '{if ($8>50){print}}' A1.pileup | less
```

[AWK is a really powerful, if arcane filter]

Analysis Pipeline

- Now run a custom script to summarize the depth information

```
$ ./pileup2bedfile.py pileups/A1.pileup 31
$ less pileups/A1.pileup.bed
```

- This file can then be loaded into the UCSC Genome Brower for inspection, and relate it to known annotations

See http://genome.ucsc.edu/

Exercise & Homework

- Replication Analysis
  - Use Galaxy to identify any SNPs in A1, B1, C1, or D1 files

  - Modify course_pipeline.sh to analyze B1, C1, D1
  - Load the bed files into the UCSC genome browser
  - See if you can spot and interesting variations between the data sets

- Need more help?
  - http://www.codecademy.com/tracks/python
  - http://docs.python.org/3/tutorial/index.html
  - http://www.ee.surrey.ac.uk/Teaching/Unix/