



# Project – Sequence Alignment with Bowtie2









## Background

- Sequence alignment
  - Compare and align sequence data (reads) to reference to identify regions of similarity (e.g. genes)
- Bowtie2
  - A multi-threaded tool for aligning sequencing reads to long reference sequences

Read: GACTGGGCGATCTCGACTTCG

Reference: GACTG--CGATCTCGACATCG









#### **Tasks**

- Run bowtie2 with 1, 2, 4 and 8 threads
  - Record the wall clock time and memory usage
- Use Python (or any method you prefer) to estimate wall clock time and memory usage for the cases with 16 and 32 threads
- Run bowtie2 again with 16 and 32 threads
  - Again, record the wall clock time and memory usage
  - Compare them to the estimated values









### Step 1: Locate Files

cd to the directory where all files are located

```
cd /work/<user name>/bowtie2_yeast
```

– <user name> should be replace by your training account "hpctrnxx"

```
[hpctrn60@mike004 bowtie2_yeast]$ ls -al
total 2069360
drwxr-xr-x 2 hpctrn60 Users
                                  4096 May 28 13:33 .
drwx----- 3 hpctrn60 Users
                                  4096 May 28 13:33 ...
-rw-r--r-- 1 hpctrn60 Users 2080972366 May 28 11:53 read1.fastq
                               8247244 May 28 13:27 yeast ref.1.bt2
-rw-r--r-- 1 hpctrn60 Users
                               3039284 May 28 13:27 yeast_ref.2.bt2
rw-r--r-- 1 hpctrn60 Users
rw-r--r-- 1 hpctrn60 Users
                                   161 May 28 13:27 yeast ref.3.bt2
                               3039277 May 28 13:27 yeast_ref.4.bt2
 rw-r--r-- 1 hpctrn60 Users
rw-r--r-- 1 hpctrn60 Users
                              12400379 May 28 13:27 yeast_ref.fa
rw-r--r-- 1 hpctrn60 Users
                               8247244 May 28 13:27 yeast_ref.rev.1.bt2
rw-r--r-- 1 hpctrn60 Users
                               3039284 May 28 13:27 yeast ref.rev.2.bt2
```

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#### Step 2: Run Bowtie2

- Run bowtie2 with 1, 2, 4 and 8 threads
  - Command:

time bowtie2 --threads <number of threads> -x yeast\_ref -U read1.fastq -S yeast1.sam

Ex: with 2 threads

time bowtie2 --threads 2 -x yeast\_ref -U read1.fastq -S yeast1.sam

Time (seconds)	Memory (MB)
	Time (seconds)



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#### Step 3: Estimate

 Use Python (or any method you prefer) to estimate wall clock time and memory usage for the cases with 16 and 32 threads

Thread	Time (seconds)	Memory (MB)
1		
2		
4		
8		
16		
32		









### Step 4: Run Bowtie2 Again

- Run bowtie2 again with 16 and 32 threads
- Compare the observed data with your estimates

Thread	Time (seconds)		Memory (MB)	
1				
2				
4				
8				
16	Estimate	Measured	Estimate	Measured
32	Estimate	Measured	Estimate	Measured









# Step 5: What Do You Learn?



