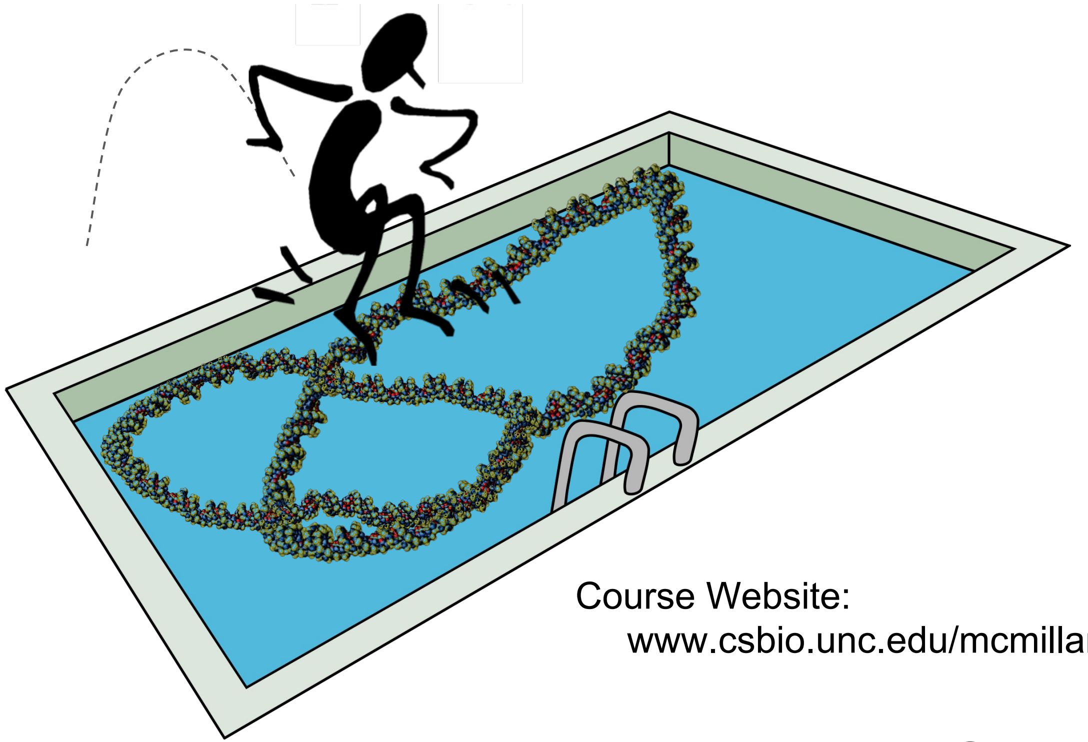
Comp 555 - BioAlgorithms - Spring 2018

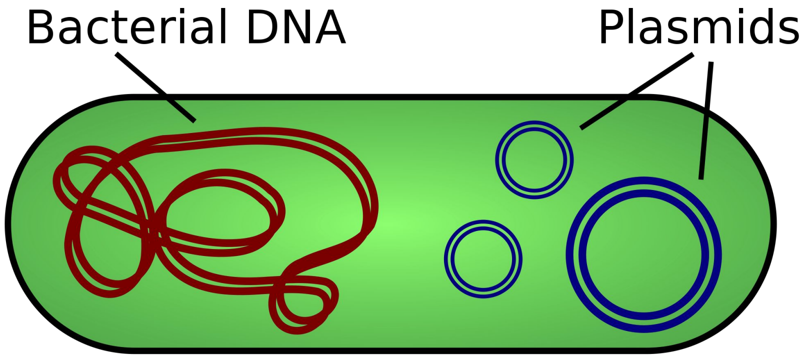


Course Website:
www.csbio.unc.edu/mcmillan/index.py?run=Courses.Comp555S19

Jumping into Genomes

A simple genome

Let’s first consider a Bacterial genome.



Characteristics of Bacterial DNA

- A “circular” primary chromosome (a few million bases) with essential genes
- Smaller chromosomes or circular plasmids (10-100K bases) with a few additional genes
- There can be multiple plasmid sequences with varible numbers of copies

FASTA file format

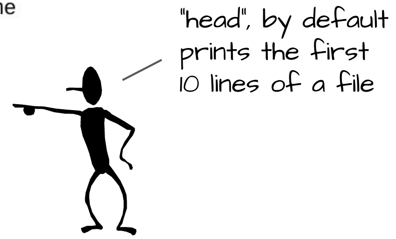
FASTA is a common format for biological sequences

- **Each sequence is preceeded by a header line that starts with '>'**
- **Followed by multiple lines of sequence data from a standard alphabet**
  - For DNA, alphabet = "ACGT"
  - For Proteins, alphabet = "ACDEFGHIKLMNOPQRSTUVWY"
- **A sequence ends when either another header line is reached or the end-of-file**
- **Multiple sequences per file are allowed**
- **Sequences are 1-indexed rather than 0-indexed!**

An Example

```
In [1]:  !head data/VibrioCholerae.fa
```

```
>gi|146313784|gb|CP000626.1| Vibrio cholerae O395 chromosome 1, complete genome
ACAATGAGGTCACTATGTTCGAGCTCTTCAAACCGGCTGCGCATACGCAGCGGCTGCCATCCGATAAGGT
GGACAGCGTCTATTCACGCCTTCGTTGGCAACTTTTCATCGGTATTTTTGTTGGCTATGCAGGCTACTAT
TTGGTTCGTAAGAACTTTAGCTTGGCAATGCCTTACCTGATTGAACAAGGCTTTAGTCGTGGCGATCTGG
GTGTGGCTCTCGGTGCGGTTTCAATCGCGTATGGTCTGTCTAAATTTTTGATGGGGAACGTCTCTGACCG
TTCTAACCCGCGCTACTTTCTGAGTGCAGGTCTACTCCTTTCGGCACTAGTGATGTTCTGCTTCGGCTTT
ATGCCATGGGCAACGGGCAGCATTACTGCGATGTTTATTCTGCTGTTCTTAAACGGCTGGTTCCAAGGCA
TGGGTTGGCCTGCTTGTGGCCGTACTATGGTGCACTGGTGGTCACGCAAAGAGCGTGGTGAGATTGTTTC
GGTCTGGAACGTCGCTCACAACGTCGGTGGTGGTTTGATTGGCCCCATTTTCCTGCTCGGCCTATGGATG
TTTAACGATGATTGGCGCACGGCCTTCTATGTCCCCGCTTTCTTTGCGGTGCTGGTTGCCGTATTTACTT
```



```
In [2]:  !tail data/VibrioCholerae.fa
```

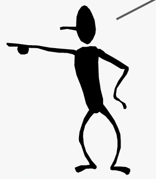
```
AAGTGGTGCCGGCTGCCGGAATCGAACTGGCGACCTACTGATTACAAGTCAGTTGCTCTACCTACTGAGC
TAAGCCGGCACACGTAACCTTTGCTGTTTGTGTCTTACACCAACAATCTAAAATTCGTGGTGCCCGGAGG
CGGAATCGAACCACCGACACGAGGATTTTCAATCCTCTGCTCTACCGACTGAGCTATCCGGGCAACGGAG
CGCTATTAAACGGATTTTCCCTTTCCCCGTCAACCTGTTTTTTGAAATATTTCGAAAAATCAGTTTGATT
GCCGTTATTTTCAGCAAACGGCGGGCTTTTTGTTATCCCGCGTTAAATTCCTTCTTAAATTTGGTCACTT
TTTCCAGATAACGACGCGCTTCCGCATTCGGATGTTTTTTGGTTAACGCCCAATACACTTGGTTAGGTTG
CAGGGCATTAAGGTCACGCATGGCGCGTTGACGATCACTGCTAAAGGTACTCAACACTCCGCCAGTGCCG
CCGTTATAGGCAGAAATCATGCTGTATTCGAGAGATGTGGGGTGGCGAACCTCTTTCAAATAGCGATTTT
TCAGGATGTAAAAATAGGCCGTACCCGTATCAATGTTGTTTTCTGGGTTAAACAGATACTCGGGGCTGG
```

```
In [3]:  !wc data/VibrioCholerae.fa
```

```
   59038   59050 4191517 data/VibrioCholerae.fa
```

A little code for reading FASTA

```
In [5]:  import gzip

         def loadFasta(filename):
             """ Parses a classically formatted and possibly
                 compressed FASTA file into two lists. One of
                 headers and a second list of sequences.
                 The ith index of each list correspond."""
             if (filename.endswith(".gz")):
                 fp = gzip.open(filename, 'r')
             else:
                 fp = open(filename, 'r')
             # split at headers
             data = fp.read().split('>')
             fp.close()
             # ignore whatever appears before the 1st header
             data.pop(0)
             headers = []
             sequences = []
             for sequence in data:
                 lines = sequence.split('\n')
                 headers.append(lines.pop(0))
                 # add an extra "+" to make string "1-referenced"
                 sequences.append('+' + ''.join(lines))
             return (headers, sequences)
```



"splits" the file at every header line. Then each of those sections is split at each return '\n'. "pop()" is used to remove the header line. The sequence is formed by joining together the remaining lines of sequences. A "+" is added to the front to give the string an offset of 1.

```
In [6]:  header, seq = loadFasta("data/VibrioCholerae.fa")

         for i in range(len(header)):
             print(header[i])
             print(len(seq[i])-1, "bases", seq[i][:30], "...", seq[i][-30:])
             print()
```

```
gi|146313784|gb|CP000626.1| Vibrio cholerae O395 chromosome 1, complete genome
1108250 bases +ACAATGAGGTCACTATGTTCGAGCTCTTC ... CCGATAGTAGAGGTTTATACCATCGCAAAA

gi|147673035|ref|NC_009457.1| Vibrio cholerae O395 chromosome 2, complete genome
3024069 bases +GTTCGCCAGAGCGGTTTTTGACTAGCTTG ... TTTCTGGGTTAAACAGATACTCGGGGCTGG
```

Vibrio Cholerae

Aquatic microorganism that causes Cholera

An abundant marine and freshwater bacterium that causes Cholera. Vibrio can affect shellfish, finfish, and other marine animals and a number of species are pathogenic for humans. Vibrio cholerae colonizes the mucosal surface of the small intestines of humans where it causes, a severe and sudden onset diarrheal disease.

One famous outbreak was traced to a contaminated well in London in 1854 by John Snow. Epidemics, which can occur with extreme rapidity, are often associated with conditions of poor sanitation. The disease is highly lethal if untreated. Millions have died over the centuries incuding seven major pandemics between 1817 and today. Six were attributed to the classical biotype, while the 7th, which started in 1961, is associated with this El Tor biotype.

Let’s take a minute to explore

Genome sequences are best understood by examining subsequences

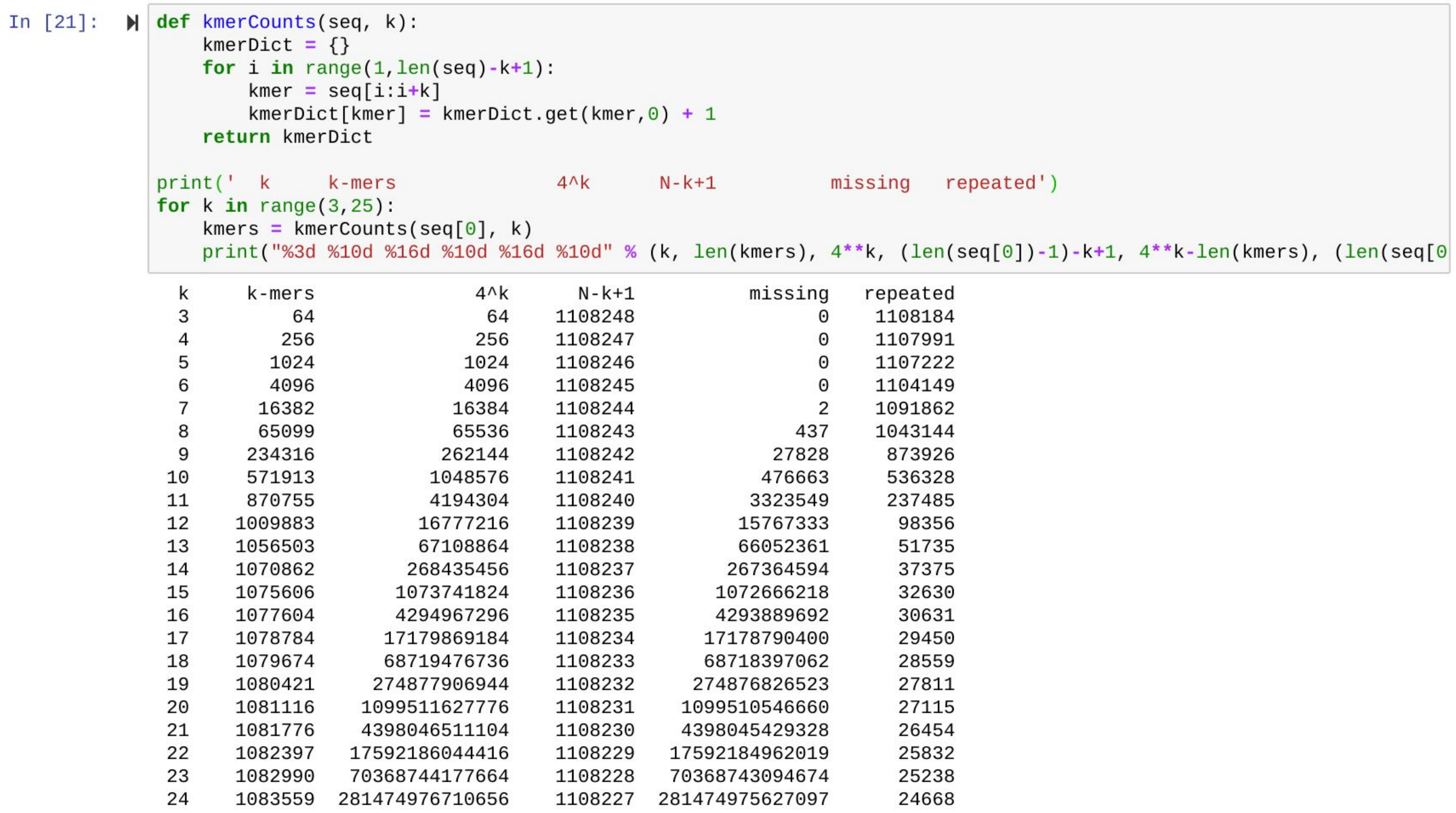
Often we examine subsequences of length *k*, called *k-mers*.

The statististics and patterns of k-mers can shed light on a genome’s organization and local function.

Two simple rules to consider:

1) There are 4^k possible DNA k-mers
2) A linear sequence of length N has *N - k + 1* k-mers
   A circular sequence of length N has *N* k-mers

Genome “k-mer” statistics



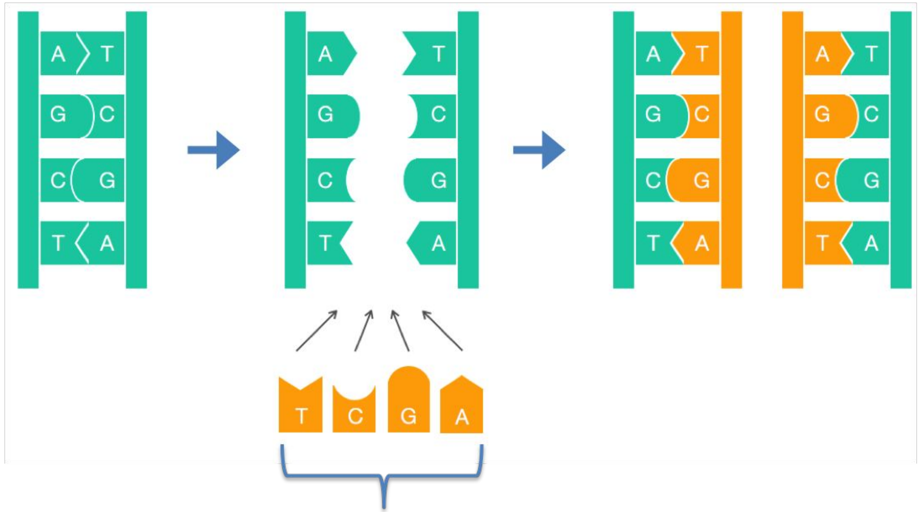
```
In [21]:  def kmerCounts(seq, k):
              kmerDict = {}
              for i in range(1,len(seq)-k+1):
                  kmer = seq[i:i+k]
                  kmerDict[kmer] = kmerDict.get(kmer,0) + 1
              return kmerDict

          print('  k      k-mers              4^k       N-k+1          missing   repeated')
          for k in range(3,25):
              kmers = kmerCounts(seq[0], k)
              print("%3d %10d %16d %10d %16d %10d" % (k, len(kmers), 4**k, (len(seq[0])-1)-k+1, 4**k-len(kmers), (len(seq[0
```

```
  k      k-mers              4^k       N-k+1          missing   repeated
  3          64               64     1108248                0    1108184
  4         256              256     1108247                0    1107991
  5        1024             1024     1108246                0    1107222
  6        4096             4096     1108245                0    1104149
  7       16382            16384     1108244                2    1091862
  8       65099            65536     1108243              437    1043144
  9      234316           262144     1108242            27828     873926
 10      571913          1048576     1108241           476663     536328
 11      870755          4194304     1108240          3323549     237485
 12     1009883         16777216     1108239         15767333      98356
 13     1056503         67108864     1108238         66052361      51735
 14     1070862        268435456     1108237        267364594      37375
 15     1075606       1073741824     1108236       1072666218      32630
 16     1077604       4294967296     1108235       4293889692      30631
 17     1078784      17179869184     1108234      17178790400      29450
 18     1079674      68719476736     1108233      68718397062      28559
 19     1080421     274877906944     1108232     274876826523      27811
 20     1081116    1099511627776     1108231    1099510546660      27115
 21     1081776    4398046511104     1108230    4398045429328      26454
 22     1082397   17592186044416     1108229   17592184962019      25832
 23     1082990   70368744177664     1108228   70368743094674      25238
 24     1083559  281474976710656     1108227  281474975627097      24668
```

"Functional" Genome Sequences

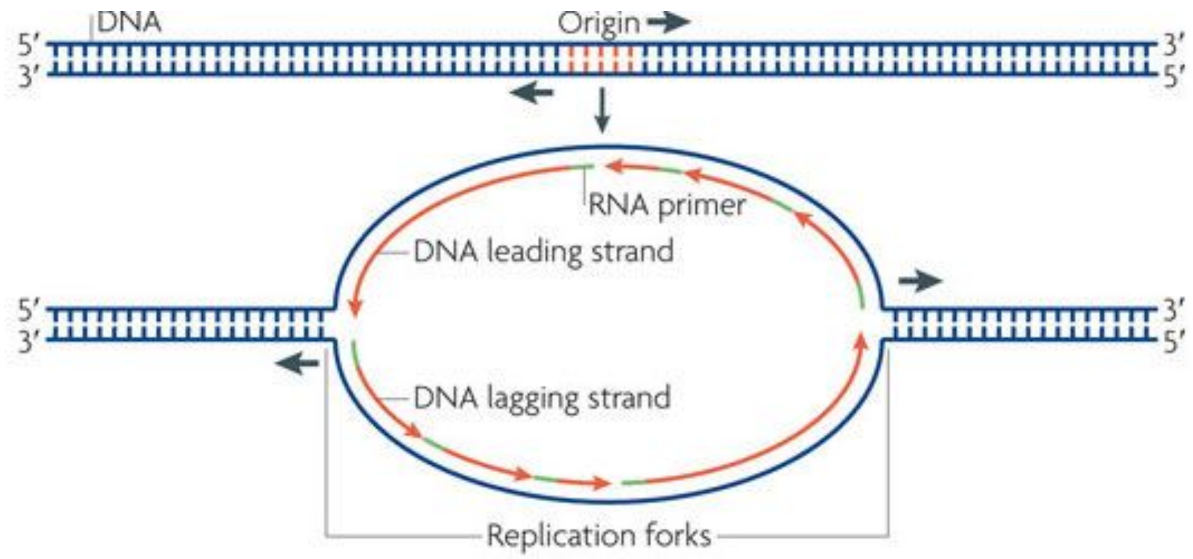
Life ≡ Reproduction ≡ Replicating a Genome

One of the most incredible things about DNA is that it provides instructions for replicating itself. Today, we consider how the replication process initiates.



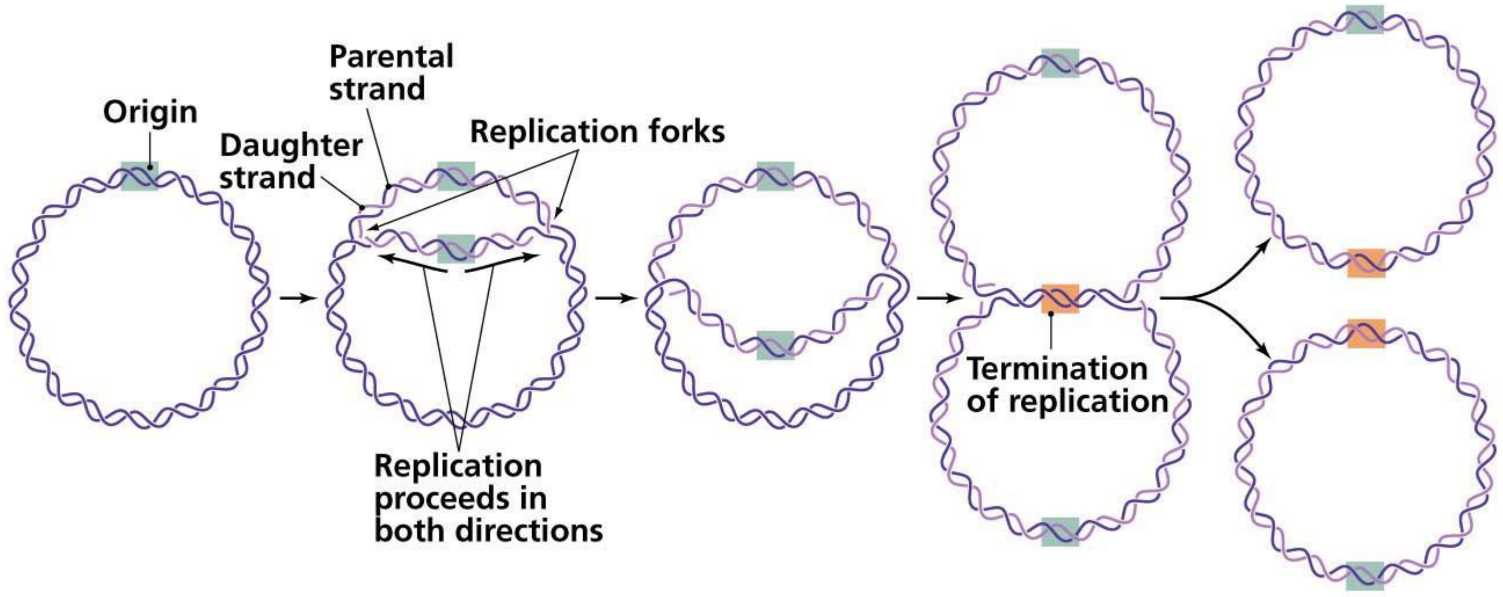
Free Nucleotides

Where Does Replication Begin?



The DNA replication process begins reliably at a regions of the genome called the *origins of replication* or *oriC*. Today we explore the sequence properties of these regions to gain insight into how they might be identified?

A cartoon of the DNA replication process

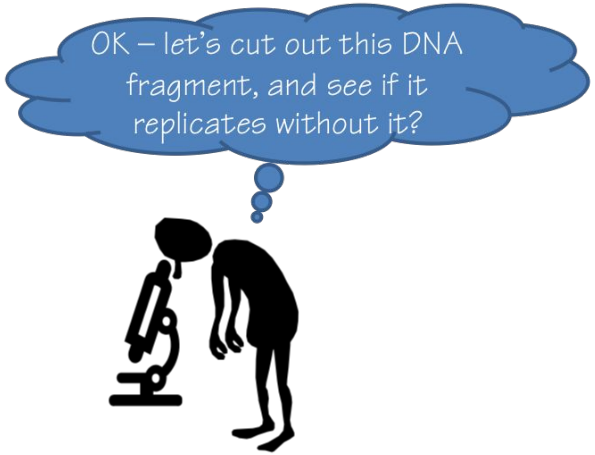

Origin
Parental strand
Daughter strand
Replication forks
Replication proceeds in both directions
Termination of replication

We seek to find the DNA sequence pattern at the point of origin, which is consistent.

The *oriC* finding Problem

Given a genome, find its oriC region or regions

Wet lab Approach:


OK – let's cut out this DNA fragment, and see if it replicates without it?

Advantage: You can start immediately
Disadvantage: It can take a long time

Computational Approach:

Maybe there is a pattern I could look for, but I have no idea where to start
?

Advantage: It can be fast, and general
Disadvantage: Problem is not adequately specified

Let's look at an example *oriC*

The replication origin of Vibrio Cholerae:

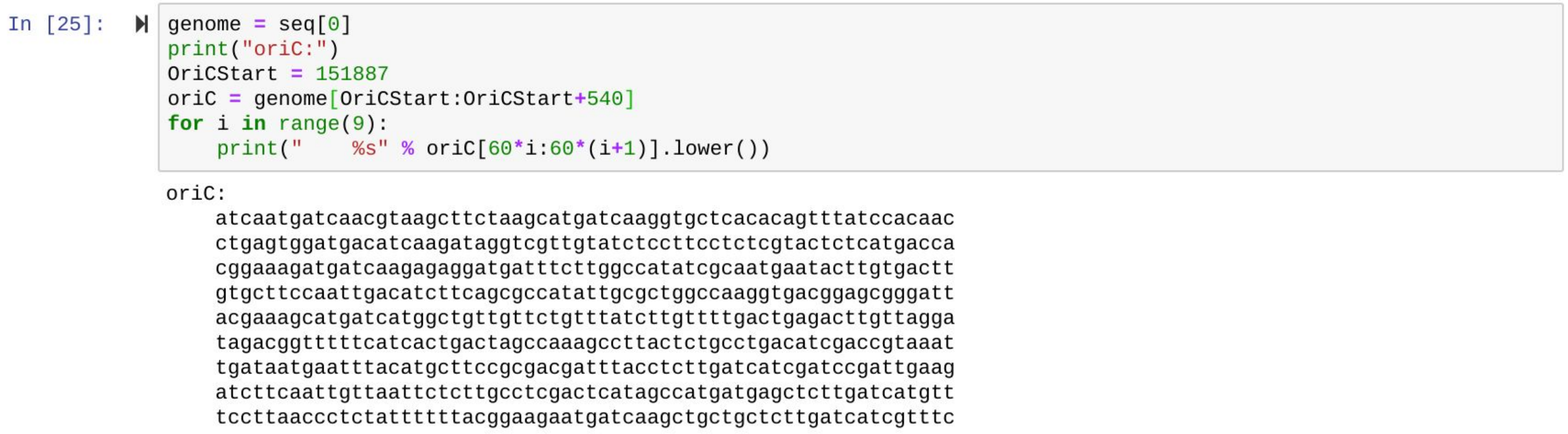
```
atcaatgatcaacgtaagcttctaagcatgatcaaggtgctcacacagtttatccacaac
ctgagtggatgacatcaagataggtcgttgtatctccttcctctcgtactctcatgacca
cggaaagatgatcaagagaggatgatttcttggccatatcgcaatgaatacttgtgactt
gtgcttccaattgacatcttcagcgccatattgcgctggccaaggtgacggagcgggatt
acgaaagcatgatcatggctgttgttctgtttatcttgttttgactgagacttgttagga
tagacggtttttcatcactgactagccaaagccttactctgcctgacatcgaccgtaaat
tgataatgaatttacatgcttccgcgacgatttacctcttgatcatcgatccgattgaag
atcttcaattgttaattctcttgcctcgactcatagccatgatgagctcttgatcatgtt
tccttaaccctctattttttacggaagaatgatcaagctgctgctcttgatcatcgtttc
```

Is there some pattern which might help us to develop an algorithm?

Where is it?

From before, seq[0], is chromosome 1 from our FASTA file.

Here we print a 540 base region of the genome after 151,887, known to be near *oriR*. See any patterns?



```
In [25]:  genome = seq[0]
          print("oriC:")
          OriCStart = 151887
          oriC = genome[OriCStart:OriCStart+540]
          for i in range(9):
              print("    %s" % oriC[60*i:60*(i+1)].lower())
```

```
oriC:
    atcaatgatcaacgtaagcttctaagcatgatcaaggtgctcacacagtttatccacaac
    ctgagtggatgacatcaagataggtcgttgtatctccttcctctcgtactctcatgacca
    cggaaagatgatcaagagaggatgatttcttggccatatcgcaatgaatacttgtgactt
    gtgcttccaattgacatcttcagcgccatattgcgctggccaaggtgacggagcgggatt
    acgaaagcatgatcatggctgttgttctgtttatcttgttttgactgagacttgttagga
    tagacggttttcatcactgactagccaaagccttactctgcctgacatcgaccgtaaat
    tgataatgaatttacatgcttccgcgacgatttacctcttgatcatcgatccgattgaag
    atcttcaattgttaattctcttgcctcgactcatagccatgatgagctcttgatcatgtt
    tccttaaccctctatttttacggaagaatgatcaagctgctgctcttgatcatcgtttc
```

How to Look for Interesting Patterns

- So let's look at our example *oriC* region to see if we can find any interesting patterns
- Still not sure what "interesting" means yet
- So let's consider every pattern of a given length, k

A new *well-specified* problem: Find the frequency of all subsequences of length k, k-mers

```
atcaatgatcaacgtaagcttctaagcatgatcaaggtgctcacacagtttatccacaac
atca     caacg     ttctaa     atcaagg     acacagtt
 tcaa     aacgt     tctaag     tcaaggt     cacagttt
  caat     acgta     ctaagc     caaggtg     acagttta
   aatg     cgtaa     taagca     aaggtgc     cagtttat
    atga     gtaag     aagcat     aggtgct     agtttatc
     tgat     taagc     agcatg     ggtgctc     gtttatcc
   4-mers    5-mers     6-mers      7-mers       8-mers
```

- Let's count the occurence of every k-mer in the sequence, given a value for k.

Example k-mer counts

This genome example from before was a little unwieldy. Let’s look at some smaller examples.

```
In [26]:  print(kmerCounts("TAGACAT",3))
          print(kmerCounts("missmississippi",3))

          {'AGA': 1, 'GAC': 1, 'ACA': 1, 'CAT': 1}
          {'iss': 3, 'ssm': 1, 'smi': 1, 'mis': 1, 'ssi': 2, 'sis': 1, 'sip': 1, 'ipp': 1, 'ppi': 1}
```

Now lets look at a k-mer counts for a range of k-mers sizes in the given oriC region

```
In [32]:  def mostFreqKmer(start, end, sequence):
              for k in range(start,end):
                  kmerStats = kmerCounts(sequence,k)
                  kmerOrder = sorted(kmerStats, reverse=True, key=kmerStats.get)
                  mostFreq = [(kmer, kmerStats[kmer]) for kmer in kmerOrder[0:6]]
                  print(k, mostFreq)

          mostFreqKmer(1,10,oriC)

          1 [('T', 174), ('A', 135), ('C', 122), ('G', 108)]
          2 [('TT', 55), ('AT', 53), ('TC', 48), ('TG', 47), ('GA', 47), ('CT', 44)]
          3 [('TGA', 25), ('GAT', 21), ('ATC', 20), ('TCA', 17), ('CTT', 17), ('TTG', 17)]
          4 [('ATGA', 12), ('TGAT', 11), ('GATC', 10), ('ATCA', 10), ('CTTG', 9), ('TGAC', 8)]
          5 [('TGATC', 8), ('GATCA', 8), ('ATGAT', 7), ('TCTTG', 6), ('ATCAA', 5), ('AATGA', 4)]
          6 [('TGATCA', 8), ('ATGATC', 5), ('GATCAA', 4), ('ATCAAG', 4), ('GATCAT', 4), ('CTCTTG', 4)]
          7 [('ATGATCA', 5), ('TGATCAA', 4), ('TGATCAT', 4), ('GATCAAG', 3), ('TGACATC', 3), ('CTCTTGA', 3)]
          8 [('ATGATCAA', 4), ('TGATCAAG', 3), ('CTCTTGAT', 3), ('TCTTGATC', 3), ('CTTGATCA', 3), ('TTGATCAT', 3)]
          9 [('ATGATCAAG', 3), ('CTCTTGATC', 3), ('TCTTGATCA', 3), ('CTTGATCAT', 3), ('AATGATCAA', 2), ('AAGCATGAT', 2)]
```

k-mer Likelihoods

Are two 5-mers repeated 8 times interesting? Surprizing? How about four 9-mers repeated 3 times?

Under the assumption that all k-mers are equally likely, we'd expect a given k-mer to occur:

$$p(k)=1/4^k$$

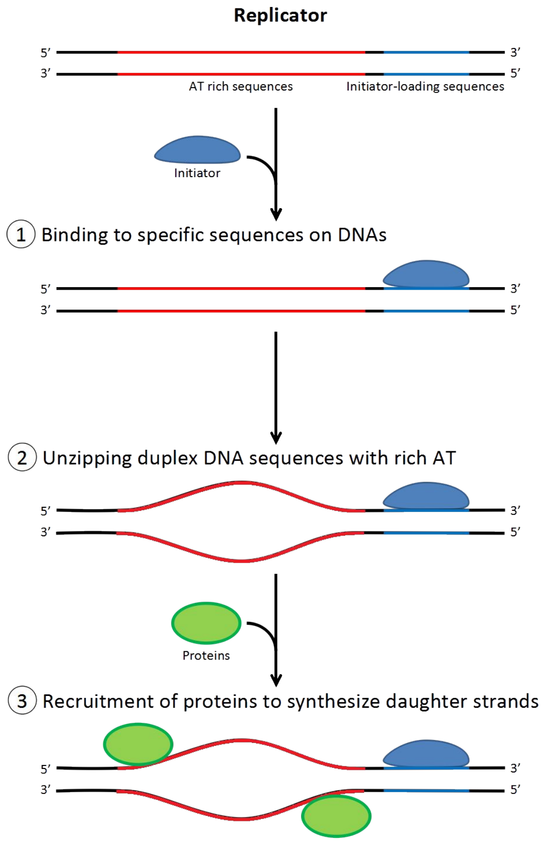
So we expect a specific 5-mer once per 1024 bases, so having 8 in 535 (540 - 5) bases is more likely than expected. We also expect a specific 9-mer once per 262,144 bases, so having 3 in 531 (540 - 9) is much more than expected.

Moreover, is their any relationship between the 9-mers ATGATCAAG and CTTGATCAT?

```
atcaatgatcaacgtaagcttctaagcATGATCAAGgtgctcacacagtttatccacaac
ctgagtggatgacatcaagataggtcgttgtatctccttcctctcgtactctcatgacca
cggaaagATGATCAAGagaggatgatttcttggccatatcgcaatgaatacttgtgactt
gtgcttccaattgacatcttcagcgccatattgcgctggccaaggtgacggagcgggatt
acgaaagcatgatcatggctgttgttctgtttatcttgttttgactgagacttgttagga
tagacggtttttcatcactgactagccaaagccttactctgcctgacatcgaccgtaaat
tgataatgaatttacatgcttccgcgacgatttacctCTTGATCATcgatccgattgaag
atcttcaattgttaattctcttgcctcgactcatagccatgatgagctCTTGATCATgtt
tccttaaccctctattttttacggaagaATGATCAAGctgctgctCTTGATCATcgtttc
```

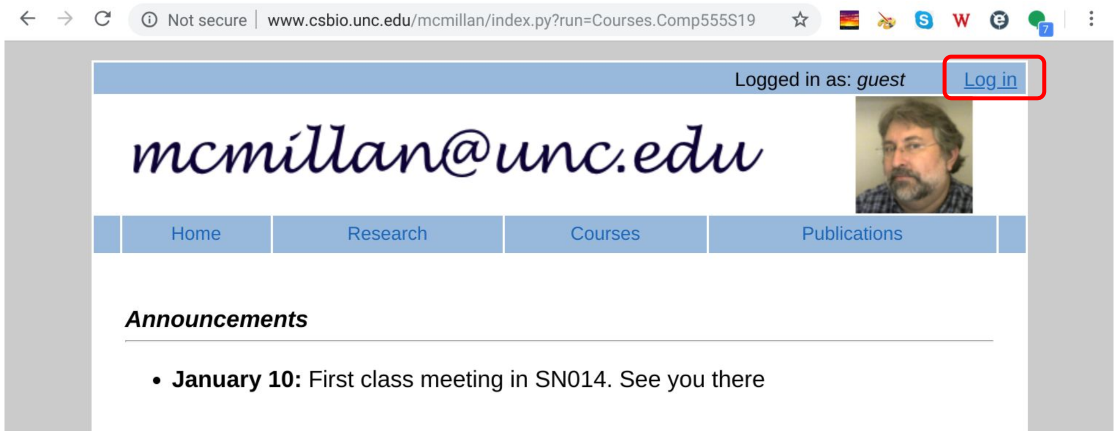
Biological Insights

- Replication is performed by a DNA polymerase, and the initiation of replication is mediated by a protein called *DnaA*.
- DnaA binds to short (≈ 9 nucleotides long) segments within the replication origin known as a *DnaA* box (≈ 500 bases).
- A DnaA box is a signal telling DnaA to “bind here!”
- DnaA can bind to either strand. Thus, both the DnaA box and its reverse-complement are equal targets.
- For reliablity “Life” wants to see multiple nearby DnaA boxes.
- Sequences used by DnaA tend to be "AT-rich" (rich in adenine and thymine bases), because AT base pairs have two hydrogen bonds (rather than the three formed in a CG pair) which makes them easier to unzip. (Recall A and T are the most common bases with 174 and 135)
- Once the origin has been located, these initiators recruit other proteins and form the pre-replication complex, which unzips the double-stranded DNA.


Replicator
5′
3′
3′
5′
AT rich sequences
Initiator-loading sequences
Initiator
1 Binding to specific sequences on DNAs
2 Unzipping duplex DNA sequences with rich AT
Proteins
3 Recruitment of proteins to synthesize daughter strands

Comptational Deductions

1) **Login to your Comp555 account**



Not secure | www.csbio.unc.edu/mcmillan/index.py?run=Courses.Comp555S19

Logged in as: *guest* Log in

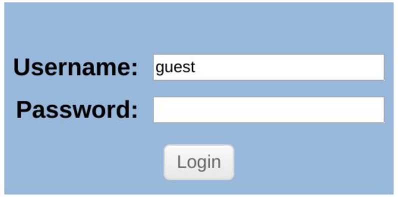
mcmillan@unc.edu

Home | Research | Courses | Publications

Announcements

- **January 10:** First class meeting in SN014. See you there

2) **Your username is your UNC ONYEN and password is your PID**



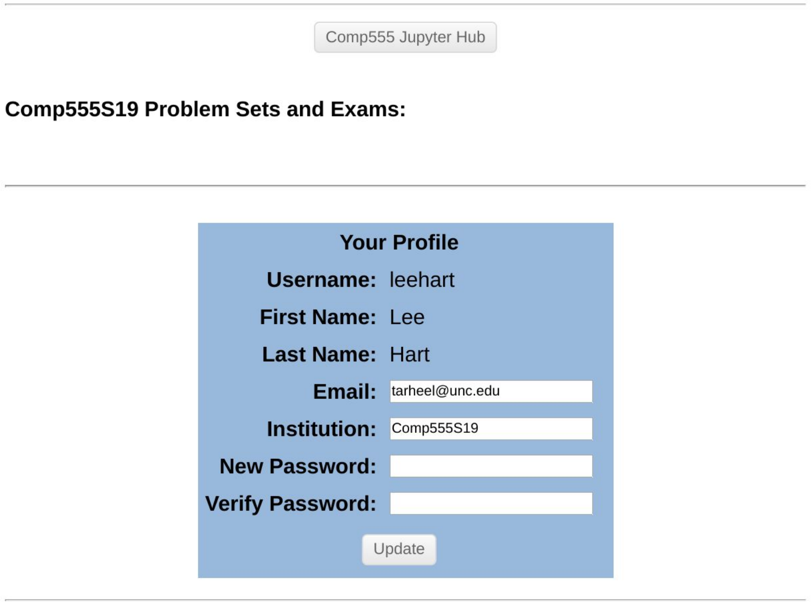
Username: guest

Password:

Login

Next Steps

3) Once you are logged in, press “Setup” and you should see something like:



Comp555S19 Problem Sets and Exams:

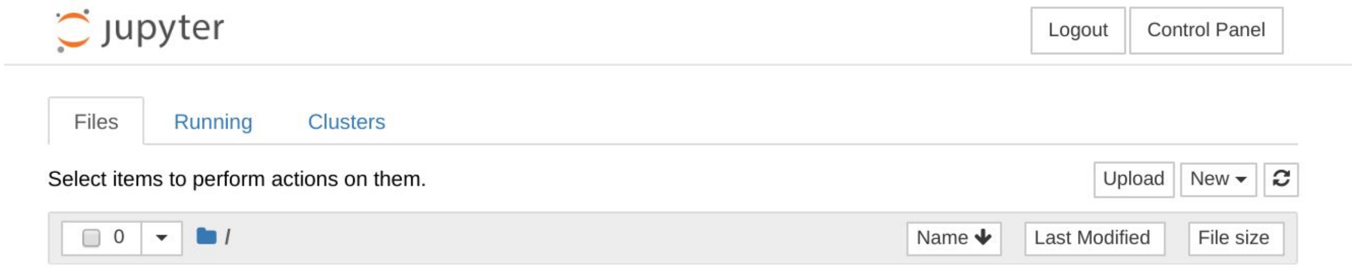
Your Profile

Username:	leehart
First Name:	Lee
Last Name:	Hart
Email:	tarheel@unc.edu
Institution:	Comp555S19
New Password:	
Verify Password:	

4) Now press the [Comp555 Jupyter Hub] button.
(BTW, you can also change your password here if you want).

Your Own Notebook

5) You should eventually get to a page like:



Select items to perform actions on them.

6) At this point you should download the Lecture02 notebook from the course website and upload it to your notebook. Run it. And be ready to try things next class meeting.