```
seq[-1]
seq. (press tab key)
seq.find?
seq.find('ATG')
seq[42]
seq[42:45]
seq.count('ATG')
seq[::-1]
```

Python uses object oriented programming.

25 Example (Object Oriented Programming)
For example, consider the python command:

```
marker = MARKER(color = blue)
```

The function `MARKER(color = blue)` is a factory function which manufactures objects, in this case markers. The `color=blue` argument specifies that a blue marker should be manufactured.

Objects have attributes. For example, the color attribute `marker.color` should equal blue.

Objects also have methods. For example, the marker method `marker.change_color(red)` changes the color attribute of the marker from blue to red.

In IPython, typing `marker.` followed by the tab key will list all the attributes and methods associated with the marker object. Typing `marker?` will provide information about the marker object.

26 Example (Biopython)
Explain what the following Biopython commands do:

```
file = open('insulin_cDNA.txt')
seq = handle.readline().strip()
print seq

from Bio.Seq import Seq
from Bio.Alphabet import IUPAC
DNA = Seq(seq,IUPAC.unambiguous_dna)
DNA
print DNA
DNA?
DNA. (press tab key)
print DNA.reverse_complement()
mRNA = DNA.transcribe()
print protein
protein.find('M')
protein.find('*')
print protein[14:125]
```

9 Lesson (Translating DNA)
Download the following files:

```
insulin_human_DNA.txt
insulin_human_cDNA.txt
```

(a) How many start codons are there in a the complete gene for human DNA? Make sure you check all six reading frames.

Solution:

(b) Translate the cDNA sequence for insulin to a protein sequence. Check your answer using Uniprot.

27 Example (Sequence Objects)
The first 10 letters of the human insulin protein sequence are: `MALWMRLLPL`. Lets use these letters to create a short sequence object using Biopython.

```
from Bio.Seq import Seq
from Bio.Alphabet import IUPAC
seq = Seq('MALWMRLLPL',IUPAC.protein)

seq
print seq
seq?
```

```
seq. (press tab)
seq.alphabet (sequence attribute)
seq.count('L') (sequence method)
```

28 Example (Fasta File Format)
The fasta file format is a common format for sequence data.

Single Sequence

```
>sp|P01308|INS_HUMAN Insulin OS=Homo sapiens GN=INS PE=1 SV=1
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED
LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
```

Multiple Sequences

```
>sp|P01308|INS_HUMAN Insulin OS=Homo sapiens GN=INS PE=1 SV=1
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED
LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
>sp|P67970|INS_CHICK Insulin OS=Gallus gallus GN=INS PE=1 SV=1
MALWIRSLPLLALLVFSGPGTSYAAANQHLCGSHLVEALYLVCGERGFFYSPKARRDVEQ
PLVSSPLRGEAGVLPFQQEEYEKVKRGIVEQCCHNTCSLYQLENYCN
>sp|P01310|INS_HORSE Insulin OS=Equus caballus GN=INS PE=1 SV=1
FVNQHLCGSHLVEALYLVCGERGFFYTPKAXXEAEDPQVGEVELGGGPGLGGLQPLALAG
PQQXXGIVEQCCTGICSLYQLENYCN
```

29 Example (Reading Single Sequence Fasta File)
Biopython can read fasta files.

```
from Bio import SeqIO
file = open('insulin_human.fasta')
seqrec = SeqIO.read(file,'fasta')
seqrec
print seqrec
seqrec?
seqrec. (press tab key)
print seqrec.id
print seqrec.seq
```

Biopython can also read fasta files containing multiple sequences. Instead of using the `SeqIO.read()` method, we use the `SeqIO.parse()` method for fasta files containing multiple sequences. The python `for` loop command, list comphrehension and the python `list` command are used with the `SeqIO.parse()` command to read fasta files with muliple sequences.

30 Example (Python For Loop)

```
animals = ['cow','sheep','goat']
for animal in animals:
    print animal
```

31 Example (Reading Multiple Sequence Fasta File)

```
from Bio import SeqIO
file = open('insulin_human_horse_chicken.fasta')
for seqrec in SeqIO.parse(file,'fasta'):
    print seqrec.id
    print seqrec.seq
```

32 Example (Python List Comprehension)

```
animals = ['cow','sheep','goat']
len('cow')
[len(animal) for animal in animals]
'cow'.count('e')
[animal.count('e') for animal in animals]
```

10 Lesson (Fasta Files)
Download the fasta file `insulin_76.fasta`.

(a) Print the sequence identifiers for all the sequences contained in this fasta file. (Hint: use a for loop.)

(b) Create a list of all the sequence identifies contained in this fasta file. Name the list `ids` for sequence ids. (Hint: use list comprehension.)

(c) How many sequences are in the `insulin_76.fasta` file. (Hint: use the `len()` command.)

33 Example (List of Sequence Records from Fasta File)
We can store all the sequences from a fasta file in a list using the `list` command.

```
from Bio import SeqIO
file = open('insulin_76.fasta')
seqrecs = list(SeqIO.parse(file,'fasta'))

seqrecs[0]
seqrecs[1]
seqrec[1].id
for i in range(len(seqrecs):
    print seqrecs[i]
    print
```

The SwissProt data format for protein sequence files contains more information than the fasta format.

34 Example (SwissProt Sequence Format)
Download the `insulin_76.txt` SwissProt sequence file and open it. Notice that it contains much more information than the corresponding `insulin_76.fasta` file.

```
from Bio import SwissProt
file = open('insulin_76.txt')
secrecs = list(SwissProt.parse(file))
rec0 = secrecs[0]
rec0. (press tab key)

for rec in seqrecs:
    print rec.accessions[0]
    print rec.organism
    print 'sequence length = %d' % rec.sequence_length
    print rec.sequence
    print
```

A small database of protein sequence information can be created and stored as a python dictionary

35 Example (Python Dictionary)
The words `'dog'`, `'cat'`, and `'mouse'` are the keys of the following Python dictionary:

```
amharic_dictionary = {'dog':'wosha','cat':'dimet','mouse':'eyet'}

amharic_dictionary['cat']
amharic_dictionary['mouse']
```

36 Example (Dictionary of Insulin Sequences)

```
from Bio import SeqIO
seqdict = SeqIO.to_dict(SeqIO.parse('insulin_76.txt','swiss')
seqdict.keys()
print seqdict['P01308']
```

4 Sequence Alignment

DNA is subject to mutations. We will only consider insertions, deletions and substitutions.

37 Definition (Mutations)

```
orignal sequence   ATTGCTCC

orignal sequence   ATTG_CTCC
       insertion   ATTGGCTCC

orignal sequence   ATTGCTCC
        deletion   ATT_CTCC

orignal sequence   ATTGCTCC
    substitution   ATTTCTCC
```

38 Example (Sequence Alignment)
Consider the sequences:

```
TAGTA
ATAT
```

Before we can determine how similar the sequences are to each other, we must first align the sequences. Two optimal alignments obtained using *dynamic programming* are:

```
TAGTA   _TAGTA
_A_TAT  ATA_T_
```

39 Example (Dot Plots)
Use a dot plot to compare the following sequences:

```
TAGTA
ATAT
```

	T	A	G	T	A
A		○			○
T	○			○	
A		○			○
T	○			○	

11 Lesson (Dot Plots)
How similar are human, horse and chicken insulin? Use Jemboss to create dot plots comparing the insulin sequence for each.

- Go to www.uniprot.org.
- In the search field click on advanced.
- Select Gene name [GN] and type INS (for the insulin gene).
- Scroll down the results and click on the check box in the left column for human, horse and chicken insulin.
- Select download and a new window will appear containing the insulin sequences for human, horse and chicken in `fasta` format.
- Open Jemboss.
- Select ALIGNMENT, Dot Plots, polyplots.
- Cut and paste the `fasta` sequence data into Jemboss.
- Select pdf format for the output.
- Go to the Jemboss folder to retrieve the results.
- Interpret the plots.

12 Lesson (Dot Plots)
Repeat the previous lesson except compare the following insulin sequences:

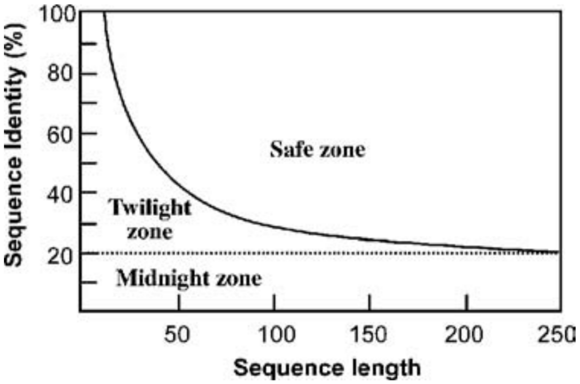
```
P01319 INS_CAPHI (Goat)
P01317 INS_BOVIN (Cow)
P01318 INS_SHEEP
```

(You may need to click in bottom right corner to display all insulin sequences at once.)

40 Definition (Homology)
Sequences which have evolved from a common ancestor are called **homologous**.

Similar sequences are likely to be homologous. However, we should keep in mind that sequences that have evolved from a distant ancestor may no longer be very similar to each other.

41 Definition (Sequence Alignment Zones)



Jin Xiong, Essential Bioinformatics, p. 33.

- safe zone: sequences are very likely to be homologous.
- twilight zone: sequences may be homologous.
- midnight zone: no reliable conclusion possible.

42 Definition (Percent Sequence Identity and Similarity)
After two sequences have been aligned, sequence identity and similarity is computed in one of two possible ways:

L_a is the length of the shorter sequence.

L_b is the length of the longer sequence.

N is either the number of identical or the number of similar letters in the alignment.

Sequence identity/similarity is computing using one of the two following formulas:

Formula 1

$$I = 100\,\frac{N}{L_a}$$

Formula 2

$$I = 100\,\frac{N}{\frac{L_a+L_b}{2}}$$

13 Lesson (Sequence Identity and Similarity)
Use `uniprot.org` to align cow insulin `P01317`, sheep insulin `P01318` and goat insulin `P01319`.

(a) In the `uniprot.org` search box type

```
P01317 or P01318 or P01319
```

Select the check boxes for these Ainsulin sequences and then select the alignment button. Wait a few seconds for the alignment to be computed by `uniprot.org`.

(b) Which sequences have a signal peptide attached? (Hint: check the box `signal peptide` in left column.)

(c) Which sequences have the propeptide attached? (Hint: check the box `propeptide` in left column.)

(d) Which sequences have the peptide segment? (Hint: check the box `peptide` in left column.)

(e) Complete the following tables *using only the peptide segment of each sequence*.

Sequence Identity:

	cow	sheep	goat
cow	100%		
sheep		100%	
goat			100%

Sequence Similarity:

	cow	sheep	goat
cow	100%		
sheep		100%	
goat			100%

Solution:

43 Definition (Paralogs)
If two sequences *from the same organism* are homologous, then the sequences are **paralogs**.

14 Lesson (Paralogs)
Use `uniprot.org` to align the insulin protein sequences: `P01325, P01326, P01322, P01323`.

(a) Which pairs of sequences are homologs and which are paralogs? Explain.

(b) Look at just the peptide segment of each sequence. (Check the box `peptide` in the left column.) Did the insulin gene duplicate before or after mouse and rat become separate species? Justify your answer.

44 Definition (Local vs Global Alignment)
Two basic types of sequence alignments are possible:

- local alignment (also called Smith-Waterman alignment)
- global alignment (also called Needleman-Wunsch alignment)

If sequence lengths are very different, we should consider using a local alignment. If the sequences are of similar lengths and likely to be closely related, we should use a global alignment. (Local alignments are used more often than global alignments.)

15 Lesson (Local Alignment)
Use Jemboss to align the cDNA and DNA sequences for human insulin. Identify where the exons and introns in the DNA sequence for human insulin are located.