Homework 7

yyin@glu:~/work/class\$ less gene2pubmed | awk '\$1==9606' | head -5 9606 1 2591067 9606 1 3458201 9606 1 3610142 9606 1 8889549 9606 1 12477932

How many homo sapiens (using human taxid) genes are there?
yyin@glu:~/work/class\$ less gene2pubmed | awk '\$1==9606' | cut -f2 | sort -u
| wc -l
31386

```
The top 10 genes with the largest number of pubmed paper

yyin@glu:~/work/class$ less gene2pubmed | awk '$1==9606' | cut -f2 | sort |

uniq -c | sort -k 1,1nr | head -5

5662 7157

3900 7124

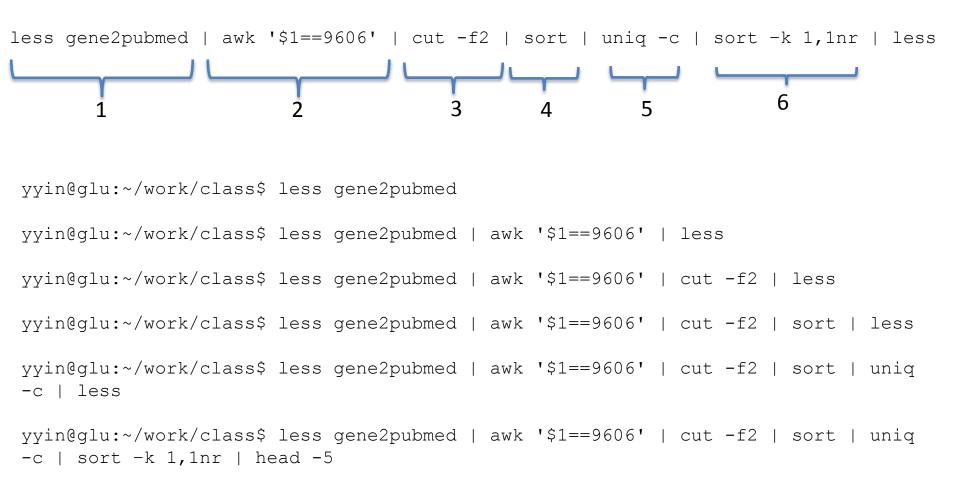
3074 348

2736 1956

2723 7316
```

A good way to understand a long command line:

Run each step and less to see what happened and then add the next step and less



Homework #10

1. In homework #9, you got fasta sequences of GenBank GH5 proteins.

- Design command line to extract sequence IDs and save as separate file.
- Using this ID file as input, write a simple perl script to generate a color definition file with 1st col to be the protein ID and the 2nd col to be the hex blue color code (ref to slide 14 of this class).

2. In homework #9, you also got the output files of searching GH5 homologous proteins against cow rumen metagenome using three search tools (blast, fasta and hmmer).

- Modify the command line in slide #9 of http://cys.bios.niu.edu/yyin/teach/PBB/Apr02-2013.pdf to extract the hit IDs (use the shown e-value and coverage cutoffs in the slide) and save as separate file.
- Use the example perl script that I have in this class (slide 24) to extract the fasta sequences from cow rumen metagenome database (two input files: ID file and fasta seq file).

3. Similar to 1, prepare a color definition file for metagenome hit IDs using hex red color. Combine the two definition files into one using cat.

4. Combine the fasta sequences from GenBank (step 1) and metagenome (step 2).

5. For combined fasta sequences from 4, MAFFT alignment and then FASTTREE to build phylogeny.

6. Upload newick tree file in 5 and the combined color definition file in 3 to iTOL to generate colored phylogeny and save the radiation view as a PDF figure.

Report due April 23 (send by email)

Office hour: Tue, Thu and Fri 2-4pm, MO325A Or email: yyin@niu.edu

```
Create a file called math.pl
perl math.pl
If use vi
Press i to the edit mode
Esc then :x to save and exit
Esc then :q! to exit without save
```

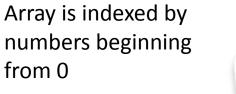
```
#!/usr/bin/perl -w
$x=3; # assign value to scalar
$y=2;
print "$x plus $y is equal to ", x+, x', "\n";
$sum=$x+$y;
print "\sum is equal to sum \n'';
print "$x minus $y is equal to ", x-, "\n";
$minus=$x-$v;
print "\$minus is equal to $minus n'';
print "$x times $y is equal to ", x*, x*, "\n";
print "$x divided by $y is equal to ", x/$y ,"\n";
```

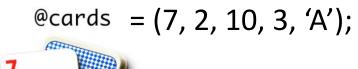
Array and hash are VERY useful to hold text data in the memory and are <u>often created using loops</u>

<u>Creating Array:</u>
@fruit_list = ('apple', 'orange', 'banana'); ____

<u>Creating Hash:</u>
%ip2hostname = (
"glu" => "131.156.41.196",
"gly" => "131.156.41.193",
"cys" => "131.156.41.195"
);

\$ip2hostname{"glu"}='131.156.41.196'; \$ip2hostname{"gly"}='131.156.41.193'; \$ip2hostname{"cys"}='131.156.41.195';







Function	Meaning
push(@array, "some value")	add a value to the end of the list
<pre>\$popped_value = pop(@array)</pre>	remove a value from the end of the list
\$shifted_value = shift (@array)	remove a value from the front of the list
unshift(@array, "some value")	add a value to the front of the list
splice()	everything above and more!

http://korflab.ucdavis.edu/Unix_and_Perl/unix_and_perl_v3.1.1.html

% ip2hostname

glu	131.156.41.196
gly	131.156.41.193
cys	131.156.41.195

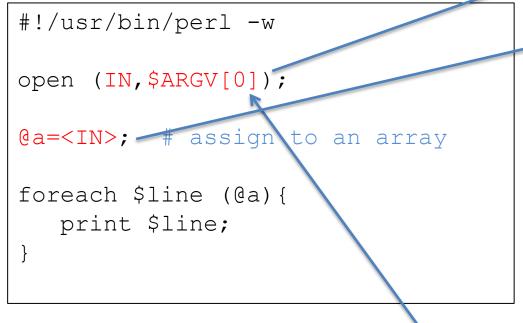
@server = keys %ip2hostname

@server will have three server names ('glu', 'gly', 'cys')

Function	Meaning
keys %hash	returns an array of keys
values %hash	returns an array of values
exists \$hash{key}	returns true if the key exists
delete \$hash{key}	removes the key and value from the hash

Create an array from a tabular format file using perl

vi array-from-file.pl



Save and exit vi

What happened?

File name is captured by an internal special variable and passed to a file handle

Then file content is written to an array and stored in the memory

File handle: IN, a temporary name assigned to a file.

@ARGV: special variable to capture command line arguments

\$ARGV[0]: the first element

- less /home/yyin/work/class/cosmicRaw.txt.head10.6col
- perl array-from-file.pl /home/yyin/work/class/cosmicRaw.txt.head10.6col

```
#!/usr/bin/perl -w
open (IN,$ARGV[0]);
@a=<IN>; # assign to an array
print $a[0];
print $a[-1];
```

Including the new line character

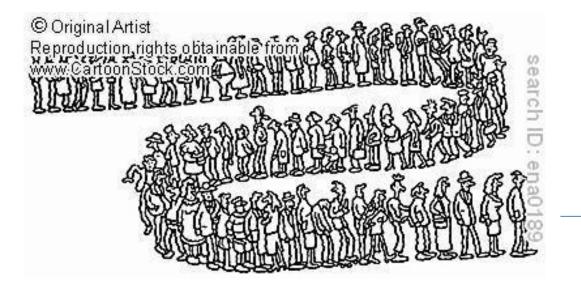
The entire file content is now in @a

COSMIC v63	COSM383711 fqmc, 39340	ENST00000401030 Substitution - Nonsense 1 lung
COSMIC v63	COSM568223 A1BG	ENST00000263100 Substitution - coding silent 19 lung
COSMIC v63	COSM226401 A1BG	ENST00000263100 Substitution - Missense 19 NS
COSMIC v63	COSM568222 A1BG	ENST00000263100 Unknown 19 lung
COSMIC v63	COSM395741 A1BG	ENST00000263100 Substitution - Missense 19 lung
COSMIC v63	COSM568221 meetirA1BGers	ENST00000263100 Substitution - coding silent 19 lung
COSMIC v63	COSM1002703 Aliso A1BG	ENST00000263100 Substitution - coding silent 19 endometrium
COSMIC v63	COSM1129683 A1BG	ENST00000263100 Substitution - Missense 19 prostate
COSMIC v63	COSM339965 A1BG	ENST00000263100 Substitution - Missense 19 lung
COSMIC v63	COSM308725 A1BG	ENST00000263100 Substitution - coding silent 🔪 19 lung

less /home/yyin/work/class/cosmicRaw.txt.head10.6col

\$a[9] or \$a[-1]

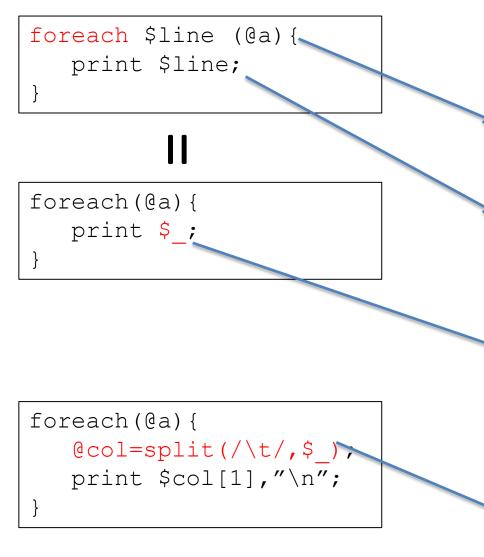
_\$a[0]



Something need to be
 repeated done for each element in the array

foreach \$line (@a) {

print \$line;



Loop: read one element from an array at a time and process it

The element is renamed as \$line and passed into the loop

Inside the loop, print the element, which is each of the rows in the file

Instead of renaming each element using a new variable, use the internal special variable \$_ to capture each element. This makes the code easy to write but not easy to read

Create a new array @col:

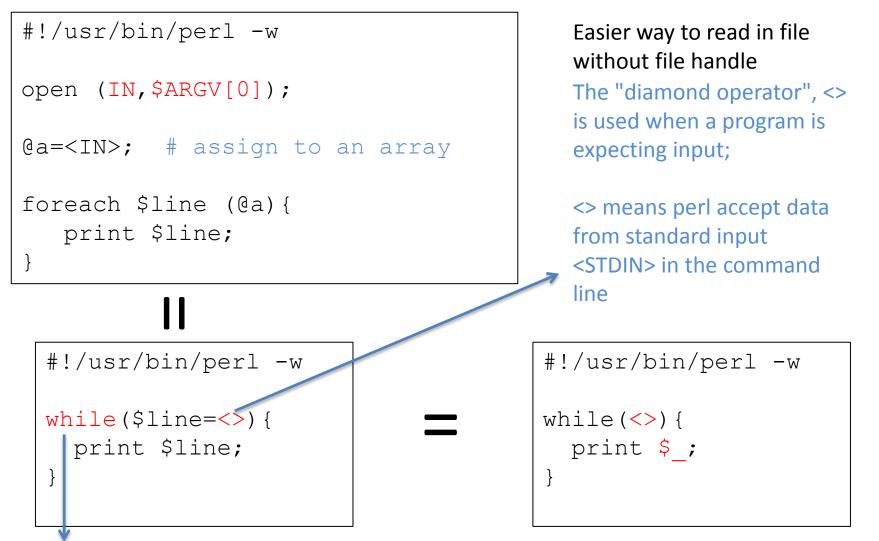
Split is a new function, here to split a string into pieces given a field delimiter. "\t" is tabular space.

There are a lot of built-in perl functions that are useful for text processing, just like functions for numeric calculations (sqrt, log, abs, sin, cos etc.) 11

Perl has many predefined special variables that contain default values designed to **make life easier for programmers**. Most special variables are a combination of punctuation marks and obscure characters, and a programmer following the good coding practice of creating meaning variable names will never accidentally run into them.

Variable	Function
\$_ \$/ and \$\ \$, @ARGV	default input and regexp search space input and output record separator output field separator array with the command line arguments for the current script

Table 2.4Special variables



while loop check a condition first (here if there are data coming in from a file handle, again one line at a time) and then get into the loop

perl array-from-file2.pl /home/yyin/work/class/cosmicRaw.txt.head10.6col

cat /home/yyin/work/class/cosmicRaw.txt.head10.6col | perl array-from-file2.pl

```
#!/usr/bin/perl
Open (IN,$ARGV[0]);
There's More T
```

```
@a=<IN>;
```

}

```
foreach(@a) {
    @col=split(/\t/,$_);
    print $col[1],"\tmutation\n";
}
```

```
#!/usr/bin/perl
```

```
while (<>) {
    @col=split(/\t/,$_);
    print $col[1],"\tmutation\n";
```

There's More Than One Way To Do It

<u>The pro of while:</u> No need to load all data into memory; process data on a line by line basis

<u>The con of while:</u> Can not reference other lines and can only work once

```
cat cosmicRaw.txt.head10.6col |
cut -f2 | awk `{print
$1,"mutation"}' | sed `s/ /\t/'
```

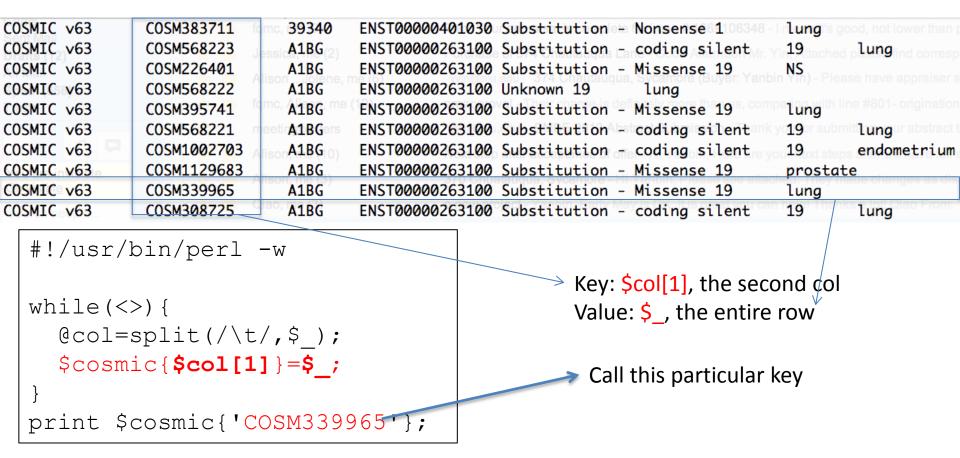
perl array-from-file3.pl /home/yyin/work/class/cosmicRaw.txt.head10.6col

hash: name-value pair, name also called key

\$hash{'key'}='value';

Like array, key and value of a hash can be assigned in a loop

Key and value could have strict one-to-one correspondence or not

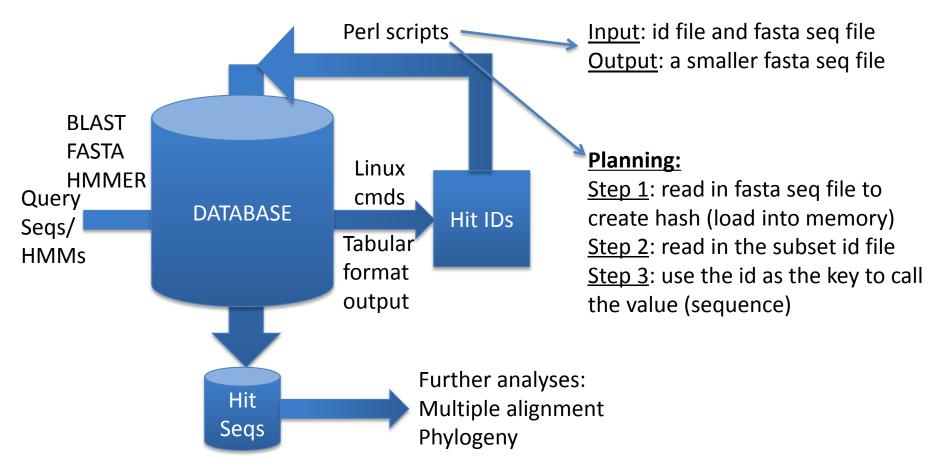


perl hash-from-file.pl cosmicRaw.txt.head10.6col

This could be VERY useful for holding fasta sequences: id as the key and sequence as the value

We can store a large fasta file in the memory and call any specific ids anytime

e.g. read in a list of subset IDs to extract the sequences



do the following in /media/DATAPART1/z1576493/class/mar19/ formatdb - # see the options, for nt db, also use -p F less ecoli-all.faa # select the 3rd protein sequence(YP_488309.1) vi test-query.fa # create a file to store this protein seq

[now blast, which is in your path already]
blastall -p blastp -i test-query.fa -d ecoli-all.faa
blastall -p blastp -i test-query.fa -d ecoli-all.faa > test-query.fa.out

[-m 9, the tabular format output without alignment, easy to parse] blastall -p blastp -i test-query.fa -d ecoli-all.faa -m 9 blastall -p blastp -i test-query.fa -d ecoli-all.faa -m 9 > testquery.fa.out.m9

[-e 1e-2, showing only hits with evalue < 1e-2]
blastall -p blastp -i test-query.fa -d ecoli-all.faa -m 9 -e 1e-2</pre>

[Now try something big (and slow)]
time blastall -p blastp -i test-query.fa -d
/home/yyin/work/class/metagenemark_predictions.faa -m 9 -e 1e-2 > testqery.fa.cowrumen.out.m9 &

[Do some parsing]

```
less test-query.fa.cowrument.out.m9 | cut -f1,2,3,7- | less
less test-query.fa.cowrument.out.m9 | cut -f1,2,3,7- | grep -v '^#' |
cut -f2 | sort -u | head
```

Save a test id file

less test-query.fa.cowrument.out.m9 | cut -f1,2,3,7- | grep -v '^#' | cut f2 | sort -u | head > test-query.fa.cowrument.out.m9.head10.id

Check a smaller fasta seq file

less test-query.fa.cowrument.out.m9.head10.fa

>NODE_27_length_1627_cov_2.424708_orf_00100 2..1597 SLPSMRADSFTKELMEKISSVRTSTLTFAPEAGTPRLRDIINKNITEEEILRACR VAYEAGKNQIKLYFMDGLPGETYEDIAGIAALASHVVDEYYRTPGRNKARQP QVTLSVACFIPKPHTPFQWERQNAPEELADKQAFLSGKITDRKVRHNYHDA KVSRIEAVFARGDRRLGRALEEAARRHVRFDAWEDCFDYDGWMDIFETVG IDPAFYANRTIPDDEILPWDMISCGVTKSFLLSERHKAQQAIATPACRDQCSG CGVNRLVDKRYCRWCPGHPESSDSAGRITSDREIRKKPEETSAQKGNVKPAR QIRIRFRKYGAMLYISHLDLAKTVMRSIVRSGLPVYYSEGFNPKPKLVFGTPLS VGCGGEAEVLDIRLMKAVSNAEITEKLKAVMPNGVEVTQVYEQKGKLTDVK WAENVIEWRNTDVSPELAEKTEALFQSPVVMMKKSKSGEKEVDITSYIRSLR AEALDGGLRITAVTAAEQENYLNPEYIVQAAERAFGISGENGWHVITRTRLLL ADGETDFA*

>NODE_33_length_1571_cov_1.473584_orf_00110 1..186 GVVTAKDADVTSAPNNKSQTLNTLSEGTTFEVLSEQGGFVEIRLGEKIRGFVK TSDVGIVK*

>NODE_33_length_1571_cov_1.473584_orf_00120 complement(218..991)

MVKRGENQLSLMQKFLCALLLALCCNAFATESSGDDSSSYDDQAWRNSKK YKTWKKYSERDVHAPKALEFRVAGMYPTAFDASVLAFRAVNLVEINDRWRF YVGYDPFHVTYNEKGFSDESLMLVGAVLAISPFTLIYSAIKGSGSRDPAEEMN DYYKEASIPKIIFFYIPAYIWCGNLYFPLVEGSWLGLNDQSHVVTHIIEEGGFYL RSFTYTNDVSLRFSKSGYFVDAGVRLEKNFADDFKARIILQIGVFGSG* Description line does not allow to break into multiple lines (have multi- newline character)

Sometimes there are multiple lines (newline)

<u>Step 1</u>: process fasta seq file to create hash (load into memory)

We want to get ride of newline character inside the sequences

vi get-seq.pl	Here is where the second entry started	The tabular space
#!/usr/bin/perl -w		if/else statement to control the flow
while (<>) {		
<pre>chomp \$_; # get ride if (\$_=~/>/) { # =~ is \$_=~s/>//; # =~s is print \$_,"\t"; # inso } else{</pre>	used to match regex used to substitute	p { action;
print \$_; # print the	e sequence line (no	}
<pre># newline }</pre>	character anymore)	else{ action; }

perl get-seq.pl test-query.fa.cowrument.out.m9.head10.fa

gil3884761261ref1YP_488309.11 homoserine kinase [Escherichia coli str. K-12 substr. W3110] WVKVYAPASSANMSVGFDVLGAAVT PVDGALLGDVVTVEAAETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGSGLGSSACSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPC FLGGMQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARALLPAQYRRQDCIAHGRHLAGFIHACYSRQPELAAKLMKDVIAEPYRERLLPGFRQARQAVAEIGAVASGISGSGPTLF ALCDKPETAQRVADWLGKNYLQNQEGFVHICRLDTAGARVLENNODE_3573325_length_256363_cov_8.500103_orf_03140 complement(202076..203146) MKKIKVFAPASIANLGCGFDIMGMALDEVGDVLEMSLDEDSSGISIVNETDVPLPEDIDQNVITPVIRKFFEMTGHSGRVDVRVLKKIYPGSGIGSSAASSAAAFGINELFGAP LSEEDVVVCAMEGENLASGGYHADNAAPAVMGGIILIRGYEPLDVIKLPVPGNLYCPVIHPHLMVSTKAARSILPKEIPMHTAITQWGNVGGLVAGLCTGNIELVGRAMRDAVAEPYRKGF IPGFDELRAKLLGAGALAMNISGSGPSVFALANRGDIAQRVGAIMERHFAQQGILSETYVVKVXXXAAPQARGGRQALALRRQDGGRKVFRLPAGRREPAFLLCTGRXXXSNKGARLIA* NODE_3641687_length_21494_cov_1.063320_orf_137760 complement(19693..20634) MKVSVRVPATVANIGPGFDCLGMALPIYNTITIEETVLPGTGIEIN VLANEDVTDELSLEHIPMDENSIIYKAVELLYNSIGQTPSELKITIHSEIPIAKGLGSSASVIVGGLIAANELLGKPADEAALLSIATEVEGHPDNITPAIIGGLTLSSAEEDGSIVSRNL PWPEEWVLTVCVPEYELATEISRSVLPKEVPLTDAVYNAQRMAMFVQAIYTKDEELMKLALRDKLHQPYRMKLVPGFDKISENLKHEESVLGVVLSGAGPSILVVSLKTNLDKVKTIIKET WDELSINAQMYTLPIDKTGAVVIPE*NODE_3701631_length_68488_cov_2.143266_orf_51600 complement(15723..16385) MGVSAFAPASIGNVSVG FDILGAALKPIDGQILGDNVDVYAGDSEFDLSIEGWFASKLPADPKKNICYDAYVGFKALLEEKGIAVKPVKMVLKKNLPIGSGLGSSAASIVAAVEALNAFHDYPLSKDEALTLMGRLEG #!/usr/bin/perl -w while(<>) { **chomp** \$; # get ride of newline character if $(\$ = / /) \{ \# = ~ is used to match regexp$ \$ =~s/>//; # =~s is used to substitute print "\n",\$,"\t"; # insert a tabular space } else{ print \$; # print the sequence line (no # newline character anymore) }

yyın@glu:~/work/class\$ perl get-seq.pl test-query.fa.cowrument.out.m9.head10.fa | less

gil388476126lreflYP_488309.11 homoserine kinase [Escherichia coli str. K-12 substr. W3110] MVKVYAPASSANMSVGFDVLGAAVT PVDGALLGDVVTVEAAETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGSGLGSSACSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPC FLGGMQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARAILPAQYRRQDCIAHGRHLAGFIHACYSRQPELAAKLMKDVIAEPYRERLLPGFRQARQAVAEIGAVASGISGSGPTLF ALCDKPETAQRVADWLGKNYLQNQEGFVHICRLDTAGARVLEN

ALCOKPETAQRVADWLGKNYLQNQEGEVHICKLDTAGAKVLEN NODE_3573325_length_256363_cov_8.500103_orf_03140 Complement(202076..203146) MKKIKVFAPASIANLGCGFDIMGMALDEVGDVLEMSLDEDS SGISIVNETDVPLPEDIDQNVITPVIRKFFEMTGHSGRVDVRVLKKIYPGSGIGSSAASSAAAAFGINELFGAPLSEEDVVVCAMEGENLASGGYHADNAAPAVMGGIILIRGYEPLDVIK LPVPGNLYCPVIHPHLMVSTKAARSILPKEIPMHTAITQWGNVGGLVAGLCTGNIELVGRAMRDAVAEPYRKGFIPGFDELRAKLLGAGALAMNISGSGPSVFALANRGDIAQRVGAIMER HFAQQGILSETYVVKVXXXAAPQARGGRQALALRRQDGGRKVFRLPAGRREPAFLLCTGRXXXXSNKGARLIA*

NODE_3641687_length_21494_cov_1.063320_orf_137760 complement(19693..20634) MKVSVRVPATVANIGPGFDCLGMALPIYNTITIEETVLPGT GIEINVLANEDVTDELSLEHIPMDENSIIYKAVELLYNSIGQTPSELKITIHSEIPIAKGLGSSASVIVGGLIAANELLGKPADEAALLSIATEVEGHPDNITPAIIGGLTLSSAEEDGSI VSRNLPWPEEWVLTVCVPEYELATEISRSVLPKEVPLTDAVYNAQRMAMFVQAIYTKDEELMKLALRDKLHQPYRMKLVPGFDKISENLKHEESVLGVVLSGAGPSILVVSLKTNLDKVKT

```
#!/usr/bin/perl -w
while(<>) {
  chomp $ ; # get ride of newline character
  if ($ =\sim/>/) { # =\sim is used to match regexp
    $ =~s/>//; # =~s is used to substitute
    (\hat{d}) = (\hat{s} = \langle (\hat{s}) \rangle); \# regexp, match a portion of text
                      # and capture the matched by ()
    print "\n", $id, "\t"; # insert a tabular space
  else{
    print $ ; # print the sequence line (no
        # newline character anymore)
```

yyin@glu:~/work/class\$ perl get-seq.pl test-query.fa.cowrument.out.m9.head10.fa | less

gi13884761261ref1YP_488309.11 MVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKN MPIGSGLGSSACSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPCFLGGMQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARAILPAQYRRQDCIAHGRHLAGF IHACYSRQPELAAKLMKDVIAEPYRERLLPGFRQARQAVAEIGAVASGISGSGPTLFALCDKPETAQRVADWLGKNYLQNQEGFVHICRLDTAGARVLEN NODE_3573325_length_256363_cov_8.500103_orf_03140 MKKIKVFAPASIANLGCGFDIMGMALDEVGDVLEMSLDEDSSGISIVNETDVPLPEDIDQNVITP VIRKFFEMTGHSGRVDVRVLKKIYPGSGIGSSAASSAAAAFGINELFGAPLSEEDVVVCAMEGENLASGGYHADNAAPAVMGGIILIRGYEPLDVIKLPVPGNLYCPVIHPHLMVSTKAAR SILPKEIPMHTAITQWGNVGGLVAGLCTGNIELVGRAMRDAVAEPYRKGFIPGFDELRAKLLGAGALAMNISGSGPSVFALANRGDIAQRVGAIMERHFAQQGILSETYVVKVXXXAAPQA RGGRQALALRRQDGGRKVFRLPAGRREPAFLLCTGRXXXXSNKGARLIA* NODE_3641687_length_21494_cov_1.063320_orf_137760 MKVSVRVPATVANIGPGFDCLGMALPIYNTITIEETVLPGTGIEINVLANEDVTDELSLEHIPMD ENSIIYKAVELLYNSIGQTPSELKITIHSEIPIAKGLGSSASVIVGGLIAANELLGKPADEAALLSIATEVEGHPDNITPAIIGGLTLSSAEEDGSIVSRNLPWPEEWVLTVCVPEYELAT EISRSVLPKEVPLTDAVYNAQRMAMFVQAIYTKDEELMKLALRDKLHQPYRMKLVPGFDKISENLKHEESVLGVVLSGAGPSILVVSLKTNLDKVKTIIKETWDELSINAQMYTLPIDKTG AVVIPE*

regexp metacharacters

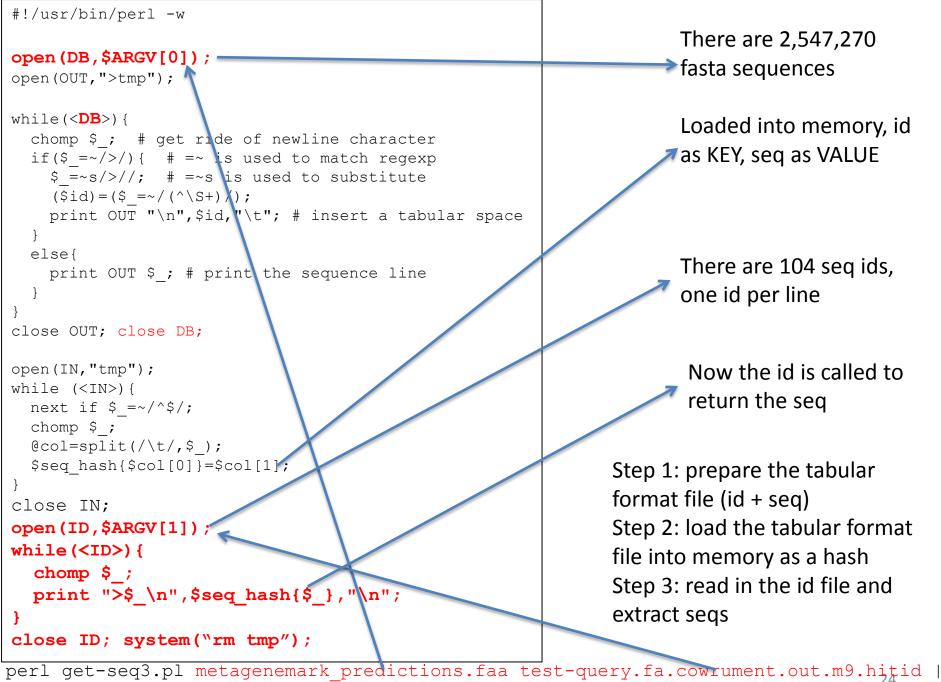
Symbol	Meaning
•	any character
\w	alphanumeric and _
\w	any non-word character
\s	any whitespace
\s	any non-whitespace 🧹
\d	any digit character
\D	any non-digit character
\t	tab
\n	newline
*	match 0 or more times
+	match 1 or more times
?	match 0 or 1 times
{n}	match exactly n times
{n,m}	match n to m times
^	match from start
\$	match to end

(\$id) = (\$_=~/(^\S+)/);

```
#!/usr/bin/perl -w
```

```
open(IN, $ARGV[0]);
while(<IN>) {
  chomp $ ; # get ride of newline character
  if (\$ = / / ) \{ \# = ~ is used to match regexp
    $ =~s/>//; # =~s is used to substitute
    ($id) = ($ = ~/(^{S+})/);
#
   print "\n",$id,"\t"; # insert a tabular space
    $seq hash{$id}=$seq; # creating hash id-seq pairs
    $seq=""; # empty the $seq variable
  else{
     $seq=$seq.$ ; # concatenate seq fragments that was
                     # separated by newlines
     print $ ; # print the sequence line (no
#
# newline character anymore)
print $seq hash{'NODE 3573325 length 256363 cov 8.500103 orf 03140'};
```

yyin@glu:~/work/class\$ perl get-seq.pl test-query.fa.cowrument.out.m9.head10.fa MVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGSGLGSSACSVVAALMAMNEHCGKPLNDT RLLALMGELEGRISGSIHYDNVAPCFLGGMQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARAILPAQYRRQDCIAHGRHLAGFIHACYSRQPELAAKLMKDVIAEPYRERLLPGF RQARQAVAEIGAVASGISGSGPTLFALCDKPETAQRVADWLGKNYLQNQEGFVHICRLDTAGARVLENyyin@glu:~/work/class\$



less