Perl for Biologists

Session 6

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Files, directories and I/O operations

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Reminder: What is a Hash?

Array

Index	Value
0	apple
1	banana
2	cranberry
3	daikon
4	eggplant
5	81

Hash

Key	Value
red fruit	apple
yellow fruit	banana
red berry	cranberry
white tuber	daikon
purple veggie	eggplant
nine squared	81

Reminder: Hash Syntax

- Declaring a variable to be a Hash, use “%”

```
%hash;
```

- Can initialize a hash using an Array

```
%hash = ('red fruit', 'apple',
  'yellow fruit', 'banana', 'nine
  squared', 81);
```

Reminder: Hash Syntax

- Accessing individual Hash element

```
$hash{'red fruit'};
```

- Equivalently

```
$key = 'red fruit';
$hash{$key};
```

Reminder: Hash Syntax

- Assigning individual Hash element

```
$hash{'purple veggie'} =
  'eggplant';
```

- Can initialize a array using a Hash

```
@array = %hash;
```

Reminder: The Big Arrow

```
%hash = (
      'red fruit'      => 'apple',
      'yellow fruit'   => 'banana',
      'red berry'      => 'cranberry',
      'white tuber'    => 'daikon',
      'purple veggie'  => 'eggplant',
      'nine squared'   => 81,
      );
```

Reminder: Hash Functions

- keys(%hash): returns an Array of the Keys

```
@hash_keys = keys (%hash);
@hash_keys = keys %hash;
```

- values(%hash): returns an Array of the Values

```
@hash_values = values (%hash);
@hash_values = values %hash;
```

Reminder: Hash Functions

- each(%hash): returns the next Key Value pair as a 2 element array

```
@pair = each (%hash);
print "$pair[0] = $pair[1]\n";


while (@pair = each (%hash))
{
    print "$pair[0] = $pair[1]\n";
}
```

Session 5 Exercises Review

1. Modify the code from session 3 exercise 3 to generate a 9kb long random DNA sequence. Save this sequence to a variable.

2. Create a hash where the keys are unique sequences of 3 base pairs and the values are the counts of how often the key appeared in the randomly generated sequence. Print out/save to a file these keys and values

Bonus: Print out/save keys and values sorted by values in decreasing order

/home/jarekp/perl_05/exercise1.pl

Person with the most number (at least 3) of distinct methods of populating a hash from a string wins 50 FREE CBSU computing hours!

/home/jarekp/perl_05/exercise2.pl
/home/jarekp/perl_05/exercise3.pl
/home/jarekp/perl_05/exercise4.pl
/home/jarekp/perl_05/exercise5.pl

Simple Line Input

Each program has three default input/output objects associated with it

- *input stream* – usually keyboard input: STDIN
- *output stream* – usually screen: STDOUT
- *error stream* – usually screen: STDERR

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

$svar = <STDIN>;        #get one line of std input

print STDOUT "1. [$svar]\n";

chomp($svar);

print STDERR "2. [$svar]\n";

print "3. [$svar]\n";
```

Opening files as streams

You can open file and create a stream with *open* function

```
open HANDLE, "/path/filename";
```

name of the stream, also called *filehandle* or *iohandle* or *handle*

file name: relative or absolute

the above opens the file for reading (default)

Opening files as streams

You can open file for writing:

```
open HANDLE, ">/path/filename";
```

The file will be created if it doesn't exist. If it does exist it will be overwritten.

Opening file for append requires ‘>>’

```
open HANDLE, ">>/path/filename";
```

Function **open** returns value indicating success or failure

```
$res = open HANDLE, ">/path/filename";
if($res)
{
        print "open successful\n";
}
```

or

```
if(!open HANDLE, ">/path/filename")
{
        print "open failed\n";
}
```

If there is an error special variable **$!** is set to an error text message generated by the system (like “access denied”)

```
if(!open HANDLE, ">/path/filename")
{
        print "open failed\nError is: $!";
}
```

There is a short version of **if**, especially useful in one-line statements:

```
open HANDLE, ">filename" or die "Open failed\nError is: $!";
```

if substitute: execute whatever is past '**or**' if previous statement returned **false**

print the message into STDERR and terminate the program

There is a short version of **if**, especially useful in one-line statements:

```
open HANDLE, ">filename" or print "Open failed\nError: $!";
```

if substitute: execute whatever is past '**or**' if previous statement returned **false**

print the message into STDOUT

Once opened, the file can be read the same way as <STDIN>

```
$svar=<in>;
```

the file should be closed with close when not needed – it will flush the buffers

```
close(out);
```

script1.pl (1)

```
Script to read file1 and copy the content to file2

File names read from arguments of the script

Every other new line replaced with a space
```

script1.pl (1)

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

#we want 2 file names as parameters
if($#ARGV != 1)
{
        print STDERR "USAGE: script1.pl file_name1 file_name2\n";
        exit;
}

#now try to open files
open in, $ARGV[0] or die "ERROR1: $!\n";
open out, ">" . $ARGV[1] or die "ERROR2: $!\n";
```

script1.pl (2)

```
#lets read file 1 and write file 2 in a loop
#lets replace line endings with space on every other line
#when writing to file 2
$n=1;
while($txt=<in>)
{
        chomp $txt; #remove ending \n character
        print "line $n length is " . length($txt) . "\n";
        print out "$txt";
        if($n % 2 == 0)
        {
                print out "\n";
        }
        else
        {
                print out " ";
        }
        $n++;
}
#close files
close(in);
close(out);
```

What happens when we forget to open the file?

If reading, we will always get an empty string.

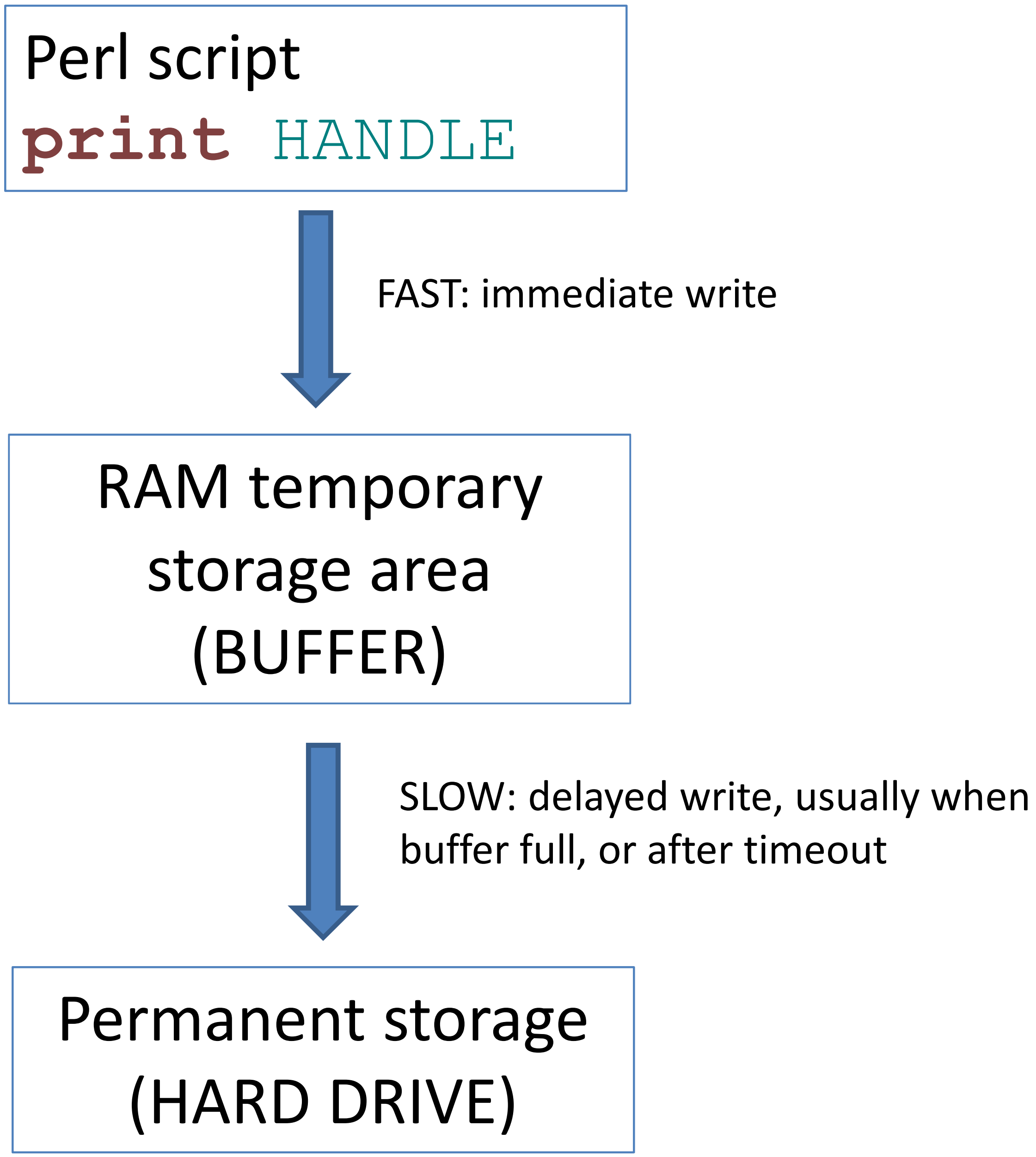
If writing, the data is ignored (goes to /dev/null).

What happens when we forget to close the file?

The file will be closed automatically when program exits, or when the handle is reused (opened again).

However, if the program crashes, the data being written to a file may be lost.

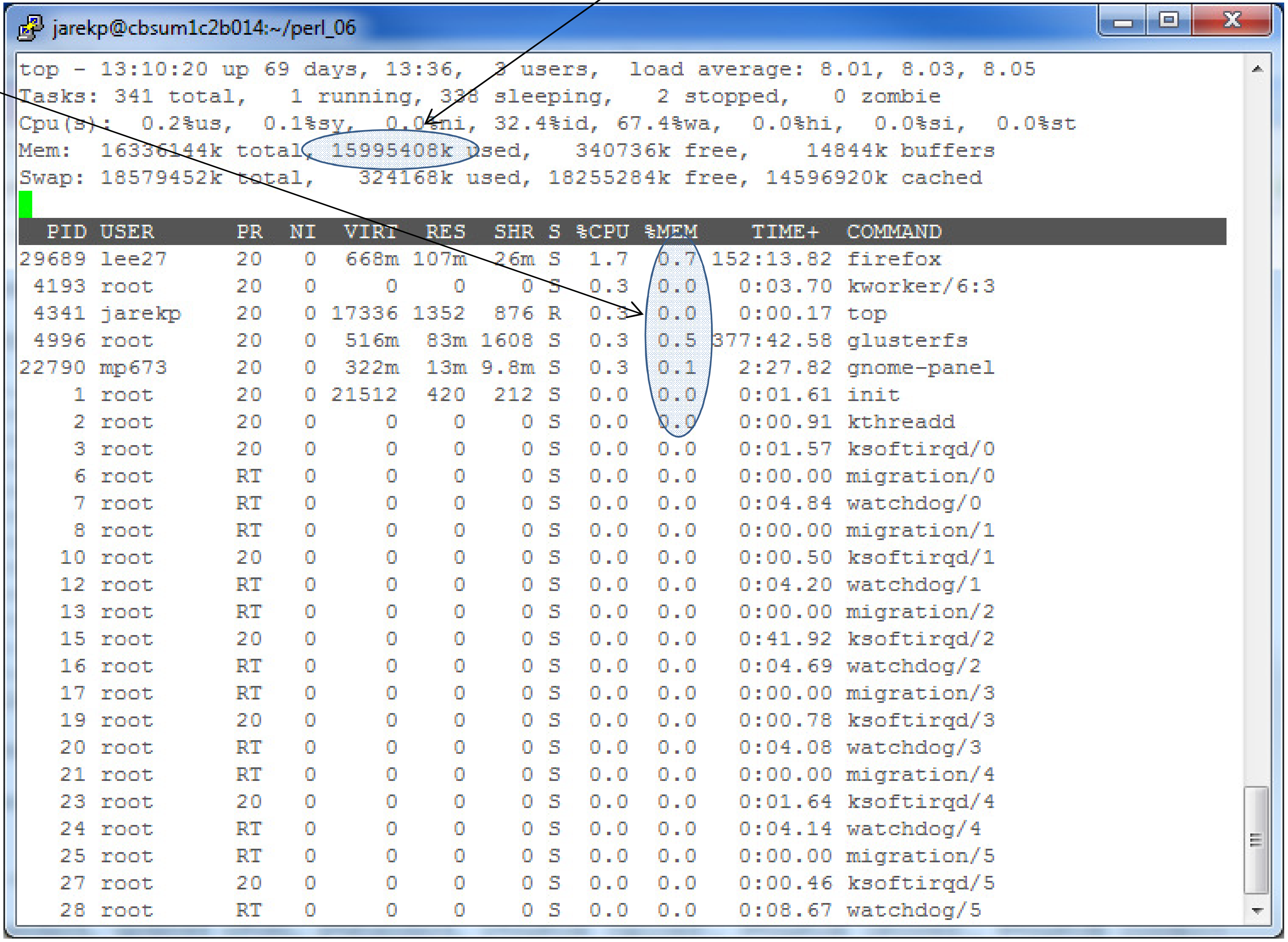
The data is written to a **buffer** first, then transferred to the disk later. This procedure speeds up read/write a lot, but if interrupted data may be lost.


Perl script
RAM temporary storage area (BUFFER)
Permanent storage (HARD DRIVE)
FAST: immediate write
SLOW: delayed write, usually when buffer full, or after timeout

```
print HANDLE
```

Very little memory used by processes

.. yet the memory is almost full – here are the buffers



```
top - 13:10:20 up 69 days, 13:36,  3 users,  load average: 8.01, 8.03, 8.05
Tasks: 341 total,   1 running, 338 sleeping,   2 stopped,   0 zombie
Cpu(s):  0.2%us,  0.1%sy,  0.0%ni, 32.4%id, 67.4%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:  16336144k total, 15995408k used,   340736k free,    14844k buffers
Swap: 18579452k total,   324168k used, 18255284k free, 14596920k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
29689 lee27     20   0  668m 107m  26m S  1.7  0.7 152:13.82 firefox
 4193 root      20   0     0    0    0 S  0.3  0.0   0:03.70 kworker/6:3
 4341 jarekp    20   0 17336 1352  876 R  0.3  0.0   0:00.17 top
 4996 root      20   0  516m  83m 1608 S  0.3  0.5 377:42.58 glusterfs
22790 mp673     20   0  322m  13m 9.8m S  0.3  0.1   2:27.82 gnome-panel
    1 root      20   0 21512  420  212 S  0.0  0.0   0:01.61 init
    2 root      20   0     0    0    0 S  0.0  0.0   0:00.91 kthreadd
    3 root      20   0     0    0    0 S  0.0  0.0   0:01.57 ksoftirqd/0
    6 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/0
    7 root      RT   0     0    0    0 S  0.0  0.0   0:04.84 watchdog/0
    8 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/1
   10 root      20   0     0    0    0 S  0.0  0.0   0:00.50 ksoftirqd/1
   12 root      RT   0     0    0    0 S  0.0  0.0   0:04.20 watchdog/1
   13 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/2
   15 root      20   0     0    0    0 S  0.0  0.0   0:41.92 ksoftirqd/2
   16 root      RT   0     0    0    0 S  0.0  0.0   0:04.69 watchdog/2
   17 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/3
   19 root      20   0     0    0    0 S  0.0  0.0   0:00.78 ksoftirqd/3
   20 root      RT   0     0    0    0 S  0.0  0.0   0:04.08 watchdog/3
   21 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/4
   23 root      20   0     0    0    0 S  0.0  0.0   0:01.64 ksoftirqd/4
   24 root      RT   0     0    0    0 S  0.0  0.0   0:04.14 watchdog/4
   25 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/5
   27 root      20   0     0    0    0 S  0.0  0.0   0:00.46 ksoftirqd/5
   28 root      RT   0     0    0    0 S  0.0  0.0   0:08.67 watchdog/5
```

Buffering is a feature of both system and Perl interpreter, and it can be controlled by setting special variable $|

```
$| = 1; #don't buffer current stream
$| = 0; #do buffer current stream
```

A stream is made current by using select() function

```
$prev = select(out);
```

previously selected steam

stream now selected for changing

A stream handle can be kept in a variable instead of bareword

```
open $handle, "filename";
```

script2.pl (1)

```
Script printing random numbers to a file

File name is the argument

User decides to buffer the output or not
```

script2.pl (1)

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

if($#ARGV < 0)
{
        print STDERR "USAGE: script2.pl file_name\n";
        exit;
}

print "Do you want to flush? (y/n) ";
while($fl=<STDIN>)
{
        chomp $fl;
        if($fl ne "y" && $fl ne "n")
        {
                print "error: invalid input $fl\n";
                print "Do you want to flush? (y/n) ";
        }
        else
        {
                last;
        }
}
```

script2.pl (2)

```
open out, ">" . $ARGV[0] or die "ERROR: $!\n";
if($fl eq "y")
{
     $prev = select(out);  #choose which stream buffer we will modify
     $| = 1;               #switch buffering off in selected stream
     select($prev);        #switch back to previously selected stream
}


$n=0;
while($n<1000_000_000)
{
     $n++;
     if($n % 1000 == 0)
        {printf out "%010d %17.16f\n", $n, sqrt(rand(100));}
}
close(out);
```

Binary files

By default, any stream opened is treated as ASCII (text) stream.

Reading a file in ASCII (text) mode means some binary characters may be lost (converted) and in general the written binary file becomes corrupted.

See what happens when script1.pl is used for ~jarekp/perl_06/picture.jpg (you can view picture with eog).

File handle must be marked as binary in order to stop character conversion.

Binary files

```
open HANDLE1, "/path/filename1";
binmode(HANDLE1);
$count = read(HANDLE1, $data , $size);
```

how many bytes have been read

binary data from file is stored in a variable

how many bytes to read

```
open HANDLE2, ">/path/filename2";
binmode(HANDLE2);
print HANDLE2 $data;
```

script3.pl

```
Script copying binary file

Source file is argument 1, destination file argument 2

Print number of bytes copied
```

script3.pl

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

#we want 2 file names as parameters
if($#ARGV != 1)
{
        print STDERR "USAGE: script3.pl file_name1 file_name2\n";
        exit;
}

open in, $ARGV[0] or die "ERROR1: $!\n";
binmode(in);
open out, ">" . $ARGV[1] or die "ERROR2: $!\n";
binmode(out);

#lets read file 1 and write file 2 in a loop
$n=0;
while($cnt=read(in, $data, 1024))
{
        $n += $cnt;
        print "$n bytes total read so far, $cnt this iteration\n";
        print out $data or die "Error writing file\n$!";
}
print "$n bytes copied\n";
#close files
close(in);
close(out);
```

Opening pipelines

Perl can open output stream of a program and read it as a file.

output from program1 goes
to our file handle

```
open HANDLE, "program1 |";
```

The file handle will reach the end when program1 ends.

```
open HANDLE, "program1 | program2 |";
```

output from program1 goes
as input to program2

output from program2 goes
to our file handle

Executing a program inside Perl

Perl can execute any program from inside a script:

```
system("program1 arg1 arg2");
```

STDOUT from program1 will go to script's STDOUT
STDERR will go to script's STDERR

```
system("program1 arg1 arg2 1> out");
```

STDOUT from program1 will go to file out
STDERR will go to script's STDERR

```
system("program1 arg1 arg2 1> out 2> err");
```

STDOUT from program1 will go to file out
STDERR will go to file err

Perl script will WAIT until program1 finishes

Executing a program inside Perl

```
system("program1 arg1 arg2 1> out 2> err &");
```

now Perl WILL NOT WAIT for program1 to finish, will continue immediately and program1 will run in parallel.

Executing a program inside Perl

```
$n = system("program1 arg1 arg2");
```

system() returns an integer representing completion code of the program

usually 0 for success and something else for error.

Executing a program inside Perl

```
system("program1 arg1 arg2");
```

can be also written using back quotes, in this case the return is the OUTPUT of the command

```
$str = `program1 arg1 arg2`
```

script4.pl

```
Script to find the number of sequences and

number of amino acids in swissprot BLAST database
```

script4.pl

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

open in, "fastacmd -d /shared_data/genome_db/BLAST_NCBI/swissprot
-p T -D 1 |" or die "ERROR: $!\n";

$n=0;
$aa=0;
$|=1;
while($txt=<in>)
{
        if(substr($txt, 0, 1) eq ">")
        {
                $n++;
                if($n % 1000 == 0){print ".";}
                if($n % 80_000 == 0){print "\n";}
        }
        else
        {
                $aa += length($txt) - 1;
        }
}
if($n % 80_000 != 0){print "\n";}
close(in);
print "swissprot contains $n sequences and $aa aa\n";
```

Logical operators for files and directories

-e "name"	file or directory exists
-f "name"	*name* is a file
-d "name"	*name* is a directory
-s "name"	*name* is non-zero size
-r "name"	*name* is readable
-w "name"	*name* is writable
-x "name"	*name* is executable
-z "name"	*name* exists and has zero size

… and more

Functions operating on files and directories

mkdir(“name”)	create directory *name*
rmdir(“name”)	delete directory *name*
chdir(“name”)	change current SCRIPT directory to *name*
unlink(“name”)	delete file *name*
rename(“name”)	rename file or directory *name*

… and more

Functions operating on files and directories

Many Perl files and directories functions do the same as system functions:

chdir("name");	⬄	system("cd name");	NOT THE SAME

changes current directory of Perl script

changes current directory of system command shell, Perl script current directory is NOT affected

mkdir("name");	⬄	system("mkdir name")	OK, but system dependent
unlink("name");	⬄	system("rm name")	OK, but system dependent

The most important difference between the functions and system calls is that the system calls will only work on one system (e.g. Linux if using "rm name", on Windows it should be "del name"), while the functions will work on ANY system script4a.pl

```
#!/usr/local/bin/perl
$pwd = `pwd`;
print "1. Our curent directory is: $pwd";
mkdir("tmpdir");
if(!-e "tmpdir")
{
        print "ERROR!\n";
        exit;
}
else
{
        print "mkdir worked!\n";
}
chdir("tmpdir");

print "2. Our curent directory is: ";
system("pwd");

print "----\n";
system("cd /tmp; pwd ");
print "----\n";

print "3. Our curent directory is: ";
system("pwd");

chdir("/tmp");

print "4. Our curent directory is: ";
system("pwd");
```

Opening and reading a directory

Perl can open a directory and retrieve all its entries:

```
opendir DIRHANDLE, "/path/dirname";
```

Similar as to file **open**, **opendir** returns success or failure code

```
@ent = readdir(DIRHANDLE);
```

returns an array containing all entries in a directory – i.e. names of all files and directories it contains (including '.' and '..').

```
closedir DIRHANDLE;
```

script5.pl

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

opendir DIR, "/home/jarekp";

foreach $entry (readdir DIR)
{
        $fullentry = "/home/jarekp/$entry";
        if(-d $fullentry)
        {
                print "directory  $entry\n";
        }
        elsif(-x $fullentry)
        {
                print "executable $entry\n";
        }
        elsif(-f $fullentry)
        {
                print "file       $entry\n";
        }
        else
        {
                print "other entry $entry\n";
        }
}
```

Exercises

1. Directory /home/jarekp/perl_06/files contains a set of fastq files with short reads. Write a script that lists all the files in this directory.

2. Modify the script from exercise 1 to open each file, read it, and produce a hash containing the distribution of sequence lengths in ALL files. Print the distribution out in descending order to a file. Plot it in Excel (no binning).

   Hint 1: Fastq file contains 4 lines for each sequence: header (starting with @), sequence itself, '+' line, and quality score line. Check script4.pl – there we had two lines per sequence.

   Hint 2: Create a hash where sequence lengths are the keys and values are frequencies of the lengths.

3. Modify the script from exercise 2 to produce <u>fasta</u> file containing ALL the sequences from ALL *fastq* files.

   Hint: Open fasta file at the beginning, then write each header (replacing first @ with >) followed by sequence.