NGS workshop software installation instruction

0. Preparation

Prior to attending the workshop, you need to install a number of software tools and download the data to your laptop. All the tools required for this tutorial are provided in the data package except Java and Perl. You may need to switch to root account to install certain software packages.

0.a. Software environment. Install the latest version of Java. See appendix for details.
PC users, install Cygwin after Java. See appendix for detailed instructions. Open the command line terminal after installation. A home folder will be created.

0.b. Data. All data and required software packages are in the ngs_workshop.zip file. Unzip the file after download.
PC users, copy the ngs_workshop folder under Cygwin installation folder. For example, if your Cygwin is installed at D:\cygwin, you can copy the ngs_workshop folder to D:\cygwin\home\username. Once you open the Cygwin terminal for the first time, a home folder will be created.

0.c. Terminal. Open a terminal, change your current path to ngs_workshop. The entire tutorial assumes your current path is ngs_workshop folder. Otherwise, you will need to change the paths of input, output, reference files in order to run the codes correctly. You can use `pwd` to find out your current path. All tasks in this tutorial are to be done in terminal by default.
PC users, you can use "Shift+Ins" to paste texts into the command line window.

0.d. Extract FastQC.
FastQC v.0.11.5 is provided under /tools.
Mac/Linux users, extract the package as follow:

```
$ cd tools (do not type in the "$")
$ unzip fastqc_v0.11.5.zip
$ chmod 755 FastQC/fastqc
$ cd ..
```

PC users, extract the package in Windows not in Cygwin terminal. Locate the file fastqc_v0.11.5.zip in file explorer, right click the zip file, click extract here. It should create a FastQC folder.

0.e. Install BWA.
BWA v.0.7.15 is provided under /tools. The human reference genome hg19 is provided under /ref.
Extract the BWA package and compile as follow:

```
$ cd tools
$ tar xvjf bwa-0.7.15.tar.bz2
$ cd bwa-0.7.15
$ make
$ cd ../..
```

0.f. Install Samtools.
Samtools v.1.5 is provided under /tools. Extract the package and compile as follow:

```
$ cd tools
$ tar xvjf samtools-1.5.tar.bz2
$ cd samtools-1.5
$ ./configure
$ make
```

```
$ make install
$ cd ../..
```

0.g. Extract IGV.

IGV v.2.3.97 is provided under /tools.

Mac/Linux users, extract the package as follow:

```
$ cd tools
$ unzip IGV_2.3.97.zip
$ cd ..
$ chmod 755 tools/IGV_2.3.97/igv.sh
```

PC users, extract the package in Windows not in Cygwin terminal. Locate the file IGV_2.3.97.zip in file explorer, right click the zip file, click extract here. It should create a folder IGV_2.3.97.

0.h. Install Vcf2maf and ensemble tools.

Vcf2maf and ensembl tools release 88 are provided under /tools. The relevant reference files are provided under /ref/homo_sapiens/88_GRCh37.

0.h.1. Extract the vcf2maf and ensembl tools.

```
$ cd tools
$ unzip vcf2maf.zip
$ unzip ensembl-tools-release-88.zip
```

0.h.2. Install tabix:

```
$ cd samtools-1.5/htslib-1.5
$ make
$ make install
```

0.h.3. Install VEP:

```
$ cd ../../ensemble-tools-release-
88/scripts/variant_effect_predictor/
$ perl INSTALL.pl --NO_HTSLIB
```

(Do not install cache files, fasta files, plugins, only install bioperl when prompted.)

```
$ cd ../../../..
```

If encounter the error "./Bio: No such file or directory", create a folder called "Bio" under "variant_effect_predictor" folder before running INSTALL.pl.

You may need to update your Perl and install certain packages if you have problem installing VEP. To install Perl packages, do the following:

```
$ cpan install Package::Name
```

Appendix

Java installation:

Windows/Linux: Go to java.com, click on Free Java Download, select “See all Java downloads”, select the appropriate offline installer. If you are using a 64bit operating system, select the 64bit version.

OSX: On newer versions of OSX you need to install the Java Development Kit. The normal Java runtime environment IS NOT enough. Go to java.com, click "Free Java Download", select "See all java downloads", on the next screen select "Looking for the JDK?" from the left hand menu and select the link to "JDK downloads" in the first paragraph. You can then click the "Download" button underneath JDK in the page you are taken to.

If you're not sure whether you have java installed, you can test from command line.

Windows: Select Start > Run, and type 'cmd' (no quotes) in the box which appears, press OK

MacOSX: Run Applications > Utilities > Terminal

Linux: From your applications menu look for an application called 'Terminal' or 'Konsole'.

Either of these will give you a usable shell. Run the following command:

```
$ java –version
```

You should see something like:

```
java version "1.8.0_60"
Java(TM) SE Runtime Environment (build 1.8.0_60-b27)
Java HotSpot(TM) 64-Bit Server VM (build 25.60-b23, mixed mode)
```

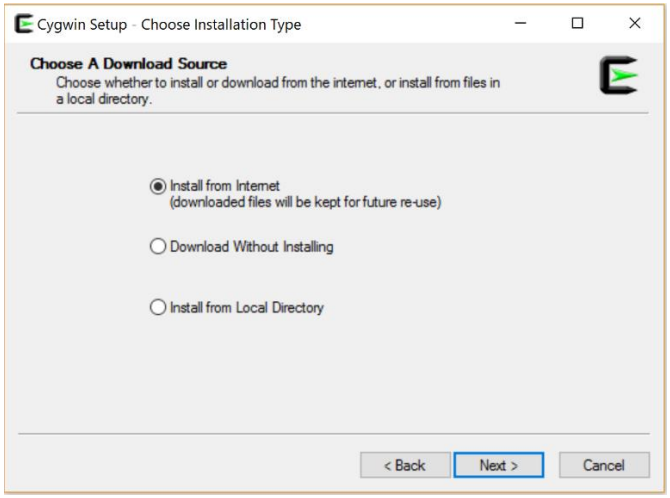
If the version listed on the first line is less than 1.6, you need to update your Java.

Cygwin installation for Windows:

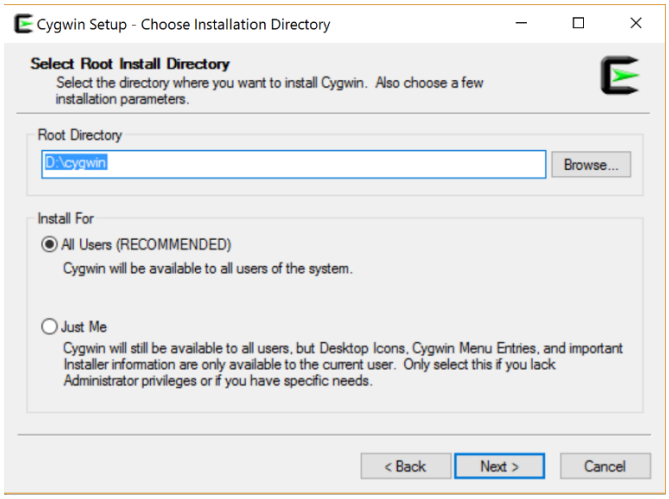
The 32 bit and 64 bit of installers are provided in the /ngs_workshop/tools/Cygwin folder. Most likely, you will need the 64 bit installer. If your laptop has at least 4GB of RAM, your Windows should be 64 bit and you should install the 64 bit version. Here is the step by step guide:

1. Double click the setup-x86_64.exe.

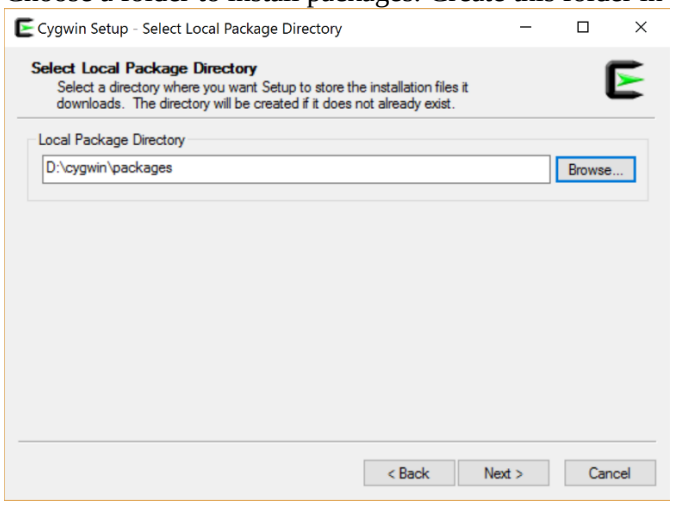
2. Select Install from Internet. Click next.



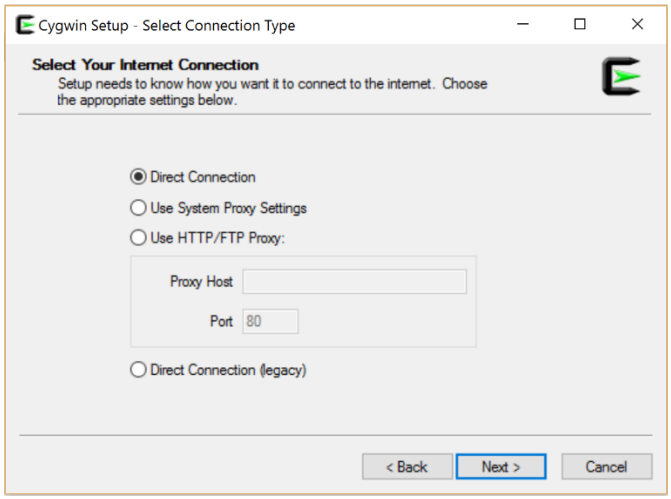
3. Choose a target folder. Create this folder in Windows first.



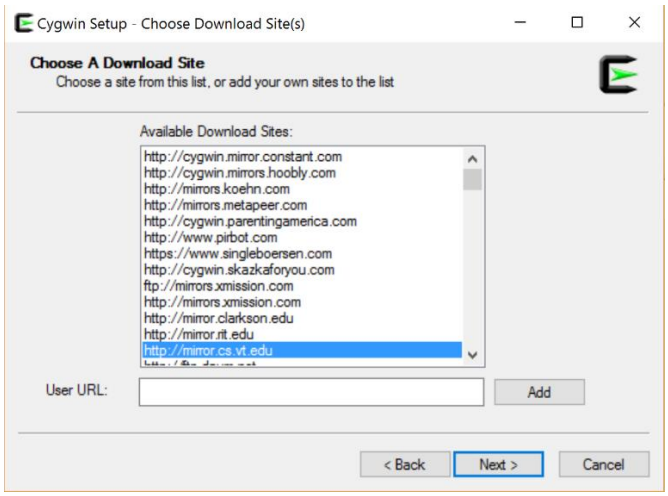
4. Choose a folder to install packages. Create this folder in Windows first.



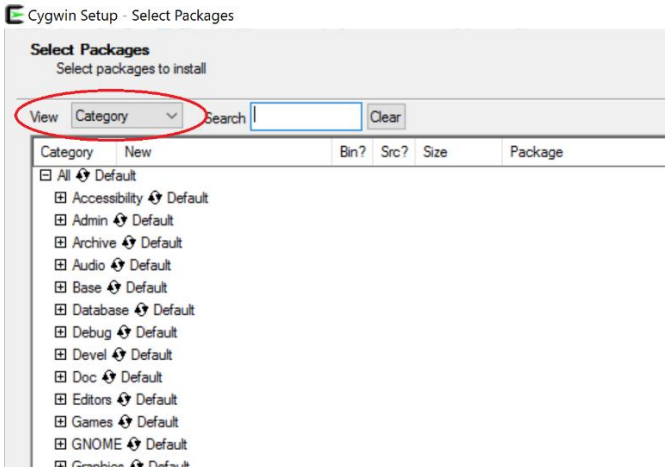
5. Select “direct connection”.



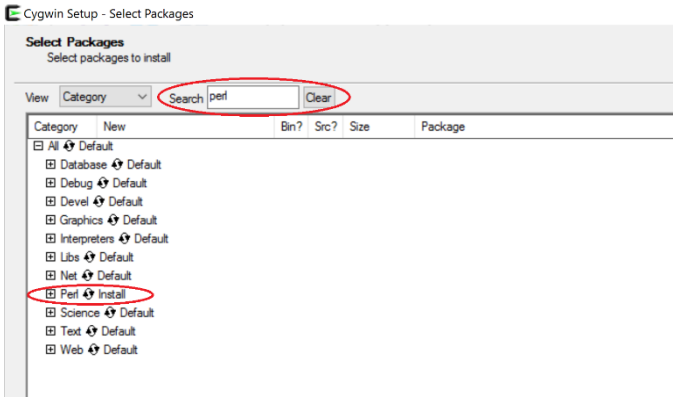
6. Choose any of the listed download sites.



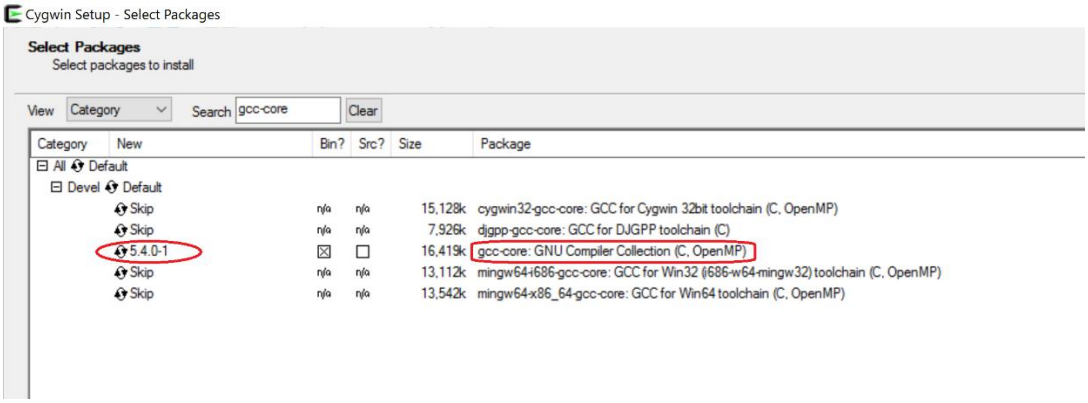
7. On “Select Packages” screen, choose category in the View dropdown list.



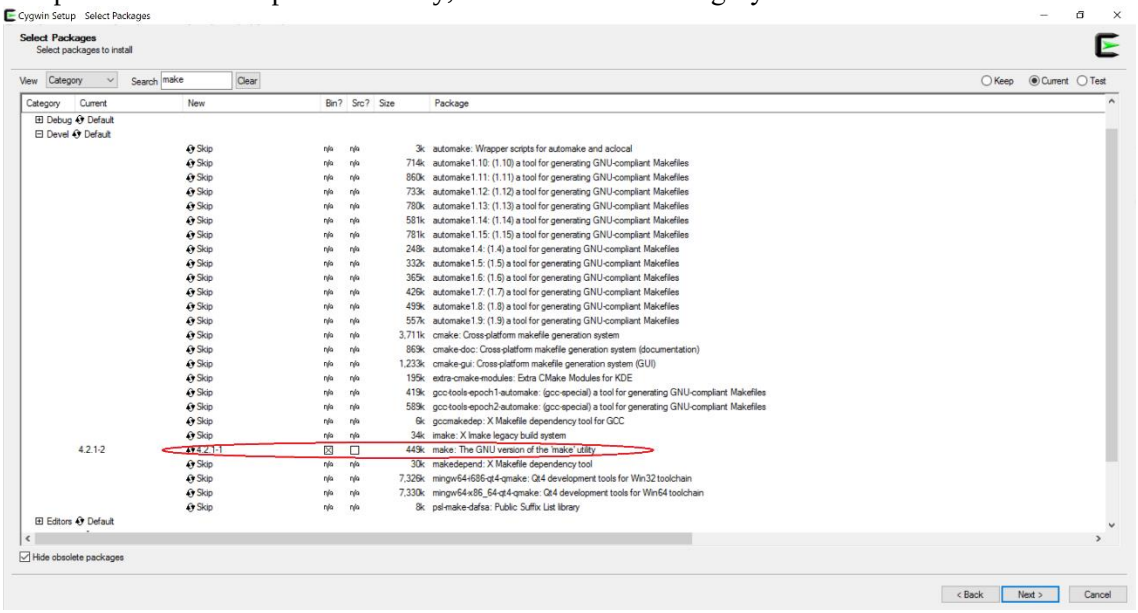
8. Search for “perl” in the search box. Click “Default” next to “Perl” and change it to “Install”. Do not hit “enter” on this screen.



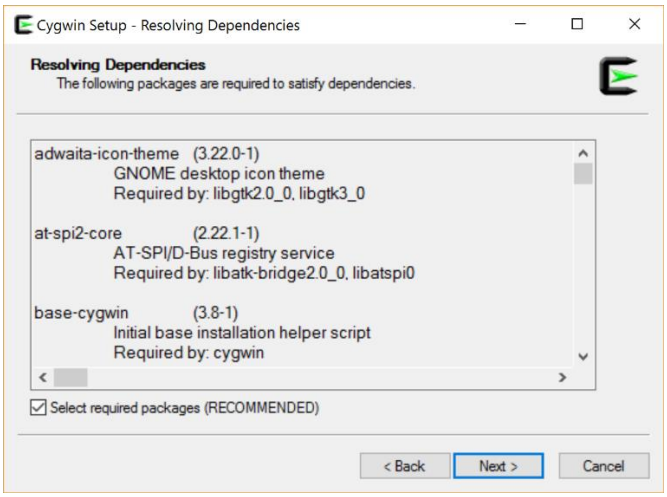
9. Search for “gcc-core”. Click the “+” sign on the left of “Devel” to expand the category. Find “gcc-core: GNU Compiler Collection”. Click “skip” and a version number will show in place of “skip”.



10. Repeat step 8 to install the following packages:
    make: The GNU version of the 'make' utility, under "Devel" category (see screenshot below);
    libncurses-devel: Terminal display library, under "Libs" category;
    libbz2-devel: BZip file de/compressor, under "Archive" category;
    liblzma-devel: LZMA de/compressor library (development), under "Libs" category;
    unzip: Info-ZIP decompression utility, under "Archive" category.



11. Install the dependencies by selecting "Select required packages" option.



12. If there are any packages not installed, you can repeat from step 1 to reinstall.

13. Enable Java in Cygwin. There should be a shortcut for Cygwin on desktop or start menu. Double click to open the command line window and type in the following command:

```
$ export PATH=$PATH:"/cygdrive/C/Program Files (x86)/Java/jre1.8.0_141/bin/"
```