

# 1. System requirements

AMEN was developed and tested on a PC workstation (Intel® Core™2 CPU 6700, 2GB of RAM) running under Windows XP, Linux operating systems and on a Mac OS X workstation. These specifications were sufficient for a 50x50'000 cell matrix. If much larger datasets are being used, computing power may need to be scaled up accordingly. A three-button mouse is required to use all options in the AMEN GUI (Graphical User Interface). A high-speed internet connection is required for efficient access to software and data repositories.

**Screenshot images.** Sometimes your analytical results may look different from those presented in the screenshot images in this manual. This could be because of changes made to publicly available annotation that has occurred since the production of this manual.

- **Project home page:** <http://sourceforge.net/projects/amen>
- **Operating system(s):** Platform independent
- **Programming language:** *Tcl/Tk, R, GraphViz*
- **R packages:** Biobase, splines, tools, affyio, affy, matchprobes, gcrma, lumi, annotate, mgcv, limma, cluster, survival, multtest, mclust, hopach, RankProducts, samr, gtools, cIValid, kohonen, dynamicTreeCut
- **Other requirements:** ActiveTcl version 8.5.2.0 (or Tcl/Tk aqua for Mac OS X), R version 2.7.1, GraphViz version 2.17 or higher
- **License:** GNU GPL

## 2. Where to download required programs

### Required programs:

Type	Release	URL
ActiveTcl or Tcl/Tk aqua	8.5.2.0	<a href="http://www.activestate.com/Products/activetcl/index.plex">http://www.activestate.com/Products/activetcl/index.plex</a> <a href="http://tcltkaqua.sourceforge.net">http://tcltkaqua.sourceforge.net</a> (for Mac OS X)
R	2.7.1	<a href="http://cran.r-project.org/bin/">http://cran.r-project.org/bin/</a>
	R and Bioconductor packages	Biobase, splines, tools, affyio, affy, matchprobes, gcrma, lumi, annotate, mgcv, limma, cluster, survival, multtest, mclust, hopach, RankProducts, samr, gtools, ciValid, kohonen, dynamicTreeCut
GraphViz	2.17	<a href="http://www.graphviz.org/Download..php">http://www.graphviz.org/Download..php</a> or <a href="http://www.pixelglow.com/graphviz">http://www.pixelglow.com/graphviz</a> (for Mac OS X)

### Required databases during the first AMEN execution:

The GO and InterPro databases contain functional annotation on proteins and their domains and must be downloaded prior to the first run of AMEN. One should update these databases periodically to keep them up-to-date.

Type	URL
InterPro database	<a href="ftp://ftp.ebi.ac.uk/pub/databases/interpro/names.dat">ftp://ftp.ebi.ac.uk/pub/databases/interpro/names.dat</a> <b>!!!this file is required during the first AMEN execution!!!</b>
Gene Ontology database	<a href="ftp://ftp.geneontology.org/go/ontology/gene_ontology.obo">ftp://ftp.geneontology.org/go/ontology/gene_ontology.obo</a> <b>!!!this file is required during the first AMEN execution!!!</b>

## 3. How to install AMEN

The AMEN interface calls up programs of the R software environment for statistical computing (<http://www.R-project.org/>) and the graphical tool GraphViz using the scripting language Tcl/Tk.

### 3.1 Automatic installation: AMEN installer for Windows

This version sequentially pre-installs Tcl/Tk, R, GraphViz and AMEN. At the end of the installation process, user should not uncheck the box “Launch AMEN” otherwise the three programs (Tcl/Tk, R, GraphViz) will be not installed.

### 3.2 Manual installation

#### 3.2.1 Tcl/Tk installation

We have developed AMEN using the Tcl/Tk development toolkit (version 8.5.2.0) from ActiveState Software. ActiveState distributes Tcl and major extensions freely for all major operating systems (Windows, Linux and Unix). ActiveTcl binaries for Windows and Linux are available for registered users from the ActiveState web site (<http://www.activestate.com/Products/ActiveTcl/>).

In case of **Mac OS X** operating system, one can use the Tcl/Tk aqua development toolkit. The following website, <http://tcltkaqua.sourceforge.net>, contains binaries, installer packages and explanations to install them on Mac OS X.

#### 3.2.2 R installation

Mathematical functions in AMEN rely upon R (version 2.7.1), an environment for statistical computing and graphics. Binaries and documentation for R can be obtained from “CRAN” (the Comprehensive R Archive Network) at <http://cran.r-project.org/mirrors.html>.

R scripts generated by AMEN require specific R libraries (CRAN & Bioconductor libraries: Biobase, splines, tools, affyio, affy, matchprobes, gcrma, lumi, annotate, mgcv, limma, cluster, survival, multtest, mclust, hopach, RankProducts, samr, gtools, cIValid, kohonen, dynamicTreeCut). If the AMEN installer fails to install them please refer to the R manual for a manual installation procedure. The main *package library* directory is *R\_HOME/library*. Packages may be distributed in source form or compiled binary form. Binary packages are platform specific and in general no special tools are needed to install them (see the documentation of your platform for more details).

After installation, please check that the “R” executable file location is correct (by default `C:\Program Files\R\R-2.7.1\bin\Rterm.exe` for Windows) because it is required for the first AMEN execution.

In case of **Mac OS X** operating system one can download and use the Mac OS X package installer in order to install R. User must check if the `usr/bin/R` file exists.

### 3.2.3 GraphViz installation

AMEN graphics utilize GraphViz (version 2.17), an open source graph visualization program suite. Sources, binaries and documentation for GraphViz can be obtained from [http://www.graphviz.org/Download\\_windows.php](http://www.graphviz.org/Download_windows.php) for Windows or [http://www.graphviz.org/Download\\_linux.php](http://www.graphviz.org/Download_linux.php) for Linux.

After installation, please check the executable file location (by default `c:\Program Files\ATT\GraphViz\bin\dot.exe` for Windows) because it is required for the first AMEN execution.

In case of **Mac OS X** operating system, one can download and install GraphViz in the following website, <http://www.pixelglow.com/graphviz>. User must drag-and-drop installation and it must check if the `/Applications/Graphviz.app/Contents/MacOS/dot` file exists.

## 4. How to run AMEN

→ **For Windows:** Install the ActiveTcl development toolkit program and associate its "wish.exe" program with the *tcl* extension. Double-click the AMEN\_1.1.2.tcl file. You may have to manually associate the *tcl* extension with the "wish" executable file (by default C:\Tcl\bin\wish.exe). In Windows this is done using the "browse" function of the "open with" window that appears when the system cannot identify a file type.

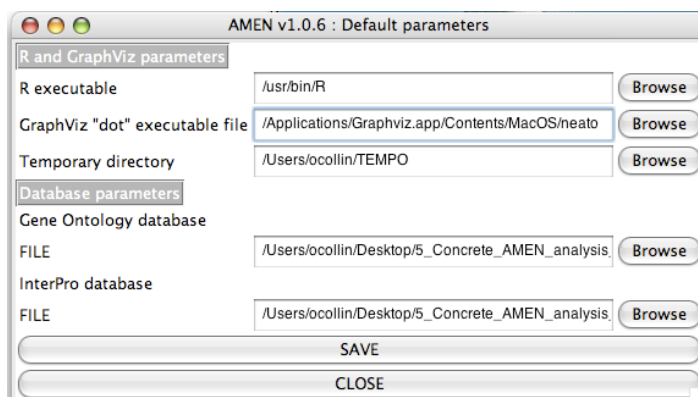
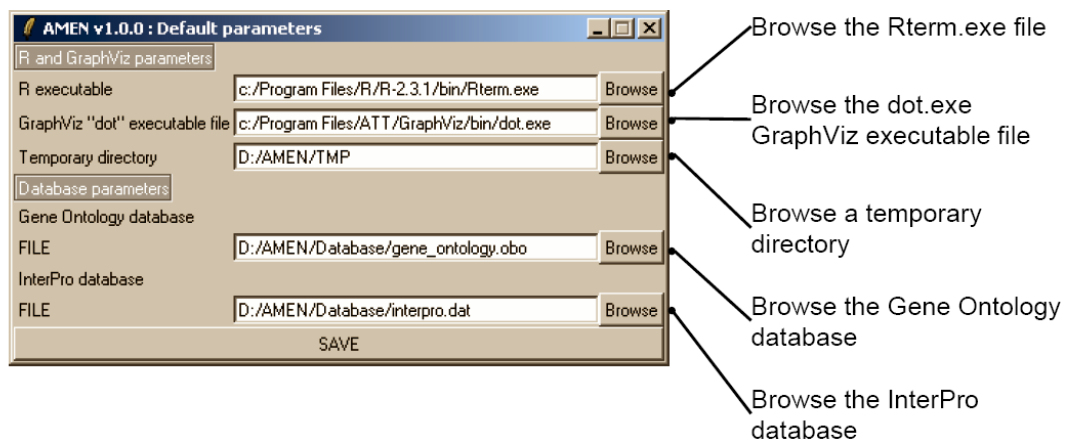
→ **For Linux and Mac OS X:** To run AMEN, enter the following command at the shell prompt in the directory in which the AMEN\_1.1.2.tcl file is located:

```
wish AMEN_1.1.2.tcl
```

**Important note for Windows users.** AMEN relies upon three programs which were developed on UNIX systems. Problem can occur if file- and folder names contain the **space character**. As a work-around use underscores for file and folder names.

### 4.1 Running AMEN for the first time

During the first launch of AMEN a number of directory and file paths must be assigned by the user:



→ The R binary file (by default C:\Program Files\R\R-2.7.1\bin\Rterm.exe for Windows, /usr/bin/R for Linux and Mac OS X)

→ The “dot” GraphViz executable file

(by default `c:\Program Files\ATT\GraphViz\bin\dot.exe` for Windows, `/Applications/Graphviz.app/Contents/MacOS/dot` for Mac OS X)

→ A directory for storing temporary AMEN files

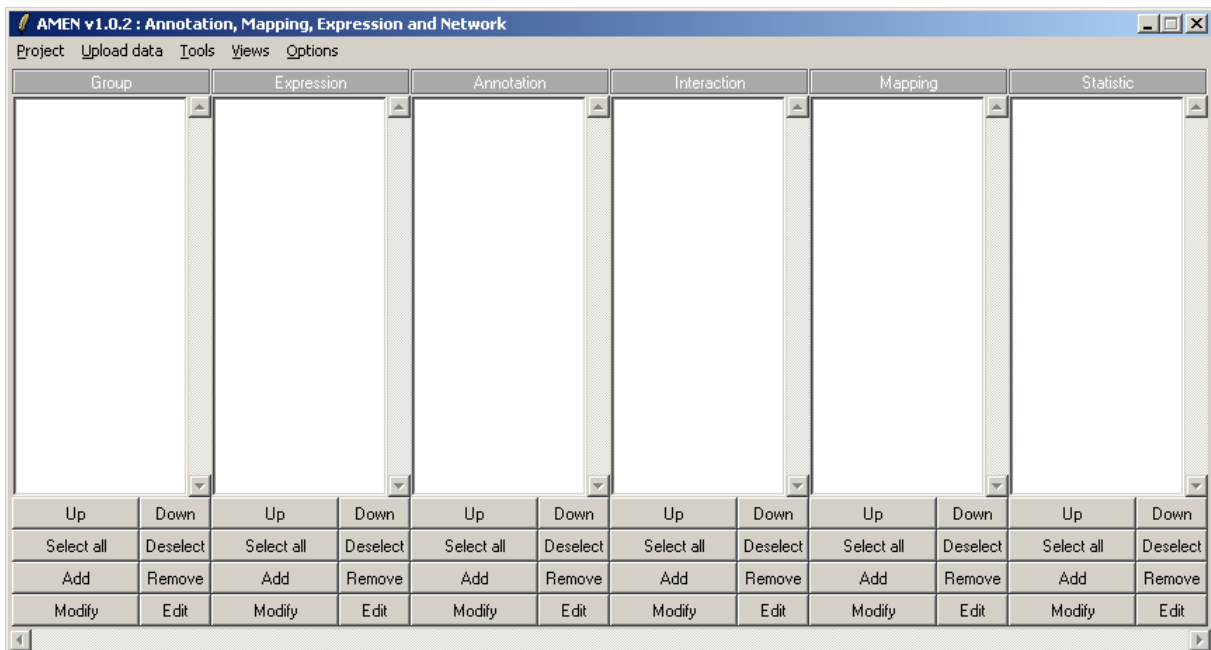
→ A Gene Ontology database file in the OBO format (usually named `gene_ontology.obo`) which can be downloaded via the following link [http://www.geneontology.org/ontology/gene\\_ontology.obo](http://www.geneontology.org/ontology/gene_ontology.obo)

→ An InterPro database file which can be downloaded using the following FTP links [ftp://ftp.ebi.ac.uk/pub/databases/interpro/short\\_names.dat](ftp://ftp.ebi.ac.uk/pub/databases/interpro/short_names.dat) or <ftp://ftp.ebi.ac.uk/pub/databases/interpro/names.dat>

Once this process is completed, click the “save” button and an `AMEN.tcl.param` file containing all these parameters will be created in the same directory.

## 4.2 Running AMEN

When AMEN is launched the default parameters are configured and the main AMEN Graphical User Interface is displayed.



AMEN v1.0.6 : Annotation, Mapping, Expression and Network

Group	Expression	Annotation	Interaction	Mapping	Statistic
groupe		Mouse430_2.n	IntAct_MINT_BioGRID		

Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Select all	Deselect	Select all	Deselect	Select all	Deselect	Select all	Deselect	Select all	Deselect	Select all	Deselect
Add	Remove	Add	Remove	Add	Remove	Add	Remove	Add	Remove	Add	Remove
Modify	Edit	Modify	Edit	Modify	Edit	Modify	Edit	Modify	Edit	Modify	Edit