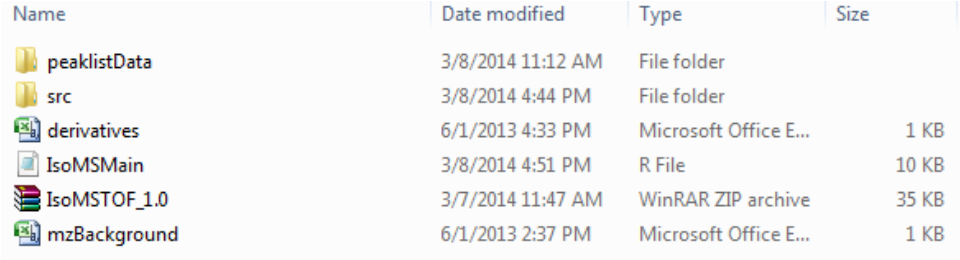
IsoMS User Manual for TOF- or QTOF-MS (version 1.0; March 9, 2014)

- IsoMS is a program written in R for processing data generated by differential chemical isotopic labeling LC-MS. It consists of two scripts, IsoMS and IsoMS-align. IsoMS is for peak picking, peak pairing and peak ratio calculation from an LC-MS dataset. IsoMS-align is for alignment of peak pairs and their peak ratios from multiple datasets. These two scripts are freely available for non-commercial use from www.mycompoundid.org/IsoMS.
- The overall workflow of IsoMS for processing multiple LC-MS datasets produced from running multiple samples in a typical metabolomics study includes three steps: 1) converting the raw LC-MS data into a centroid peak list file and storing all the converted files in a folder, 2) using IsoMS to process all the files automatically, and 3) using IsoMS-align to align the peak pairs and their ratios from all the datasets to produce the final CSV file that can be exported for data and statistics analysis.
- The instruction for data conversion in Step 1 can be downloaded from the MyCompoundID website.
- The instructions for installing and using the IsoMS and IsoMS-align scripts are given below.

1. Installation of IsoMS

1) Two versions of IsoMS, IsoMSFT and IsoMSTOF, have been developed for processing the FT-ICR-MS data and TOF- or QTOF-MS data, respectively. For processing the TOF- or QTOF-MS data, download IsoMSTOF (zipped file).
2) R graphic user interface (RGui) should be installed in a computer with Windows XP or Windows 7. RGui can be downloaded from the following URL: http://www.r-project.org/. Since IsoMS has been packaged with RGui 3.0, it is recommended to install the same version to run IsoMS.
3) If a different version of RGui is installed, R-tools is required for packaging the IsoMS source files which can be found in the src folder within the IsoMS unzipped folder (Figure 1). R-tools can be downloaded from the URL: http://cran.rstudio.com/.



Name	Date modified	Type	Size
peaklistData	3/8/2014 11:12 AM	File folder	
src	3/8/2014 4:44 PM	File folder	
derivatives	6/1/2013 4:33 PM	Microsoft Office E...	1 KB
IsoMSMain	3/8/2014 4:51 PM	R File	10 KB
IsoMSTOF_1.0	3/7/2014 11:47 AM	WinRAR ZIP archive	35 KB
mzBackground	6/1/2013 2:37 PM	Microsoft Office E...	1 KB

Figure 1. Unzipped IsoMS folder (for TOF-MS version).

4) During the installation of R-tools, the environmental variables should be checked (see Figure 2). Otherwise a user needs to edit the R environmental variables after installation by right clicking Computer → Properties → Advance system properties → Advance → Environmental variables.

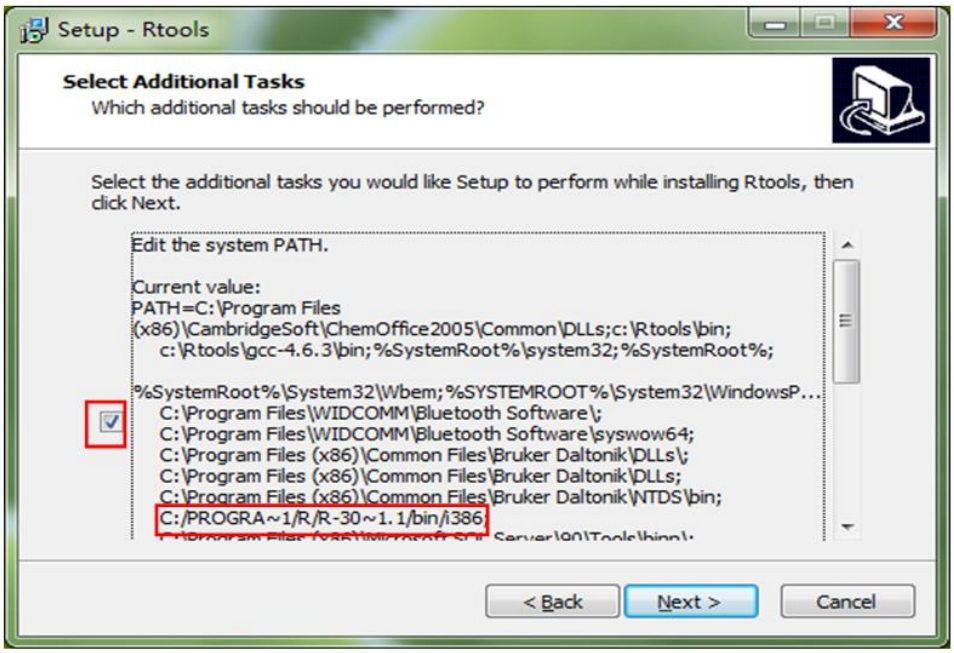


Figure 2. Select/check the environmental variables during the installation of R-tools.

5) Windows 7 users must change "user account control" to the lowest level in order to package the IsoMS scripts by opening Control panel → User account → User account settings.
6) After downloading IsoMSTOF.zip, unzip it. In the unzipped file folder (Figure 1), there is another zipped file, IsoMSTOF_1.0; DO NOT unzip it. Install it in RGui by clicking Packages → Install packages(s) from local zip files (Figure 3), and then select the zipped IsoMS package named as "IsoMSTOF_1.0".

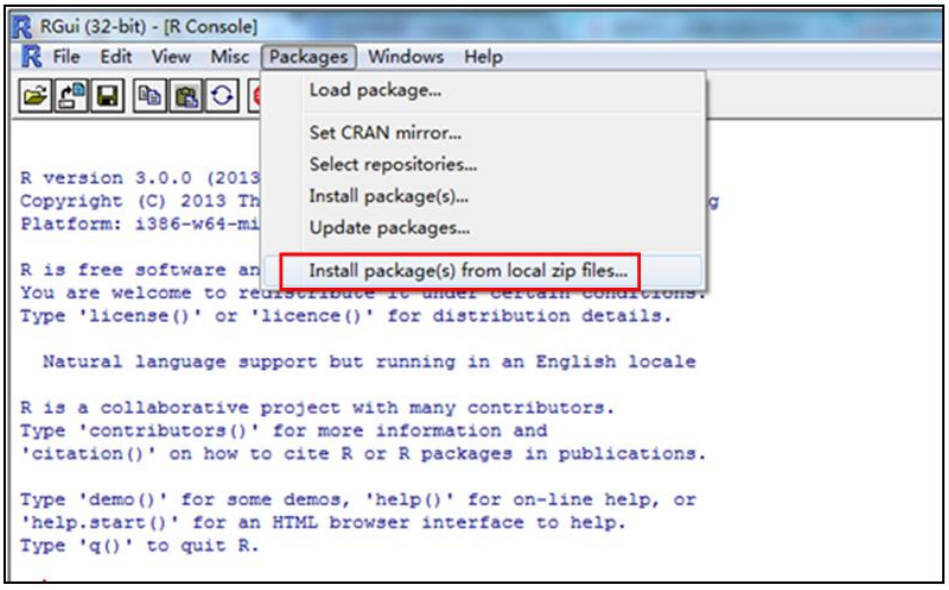


Figure 3. Install the IsoMS package in RGui.

7) Assign the unzipped folder of IsoMS (Figure 1) as the working folder of RGui by clicking: File → Change dir..... (Figure 4).

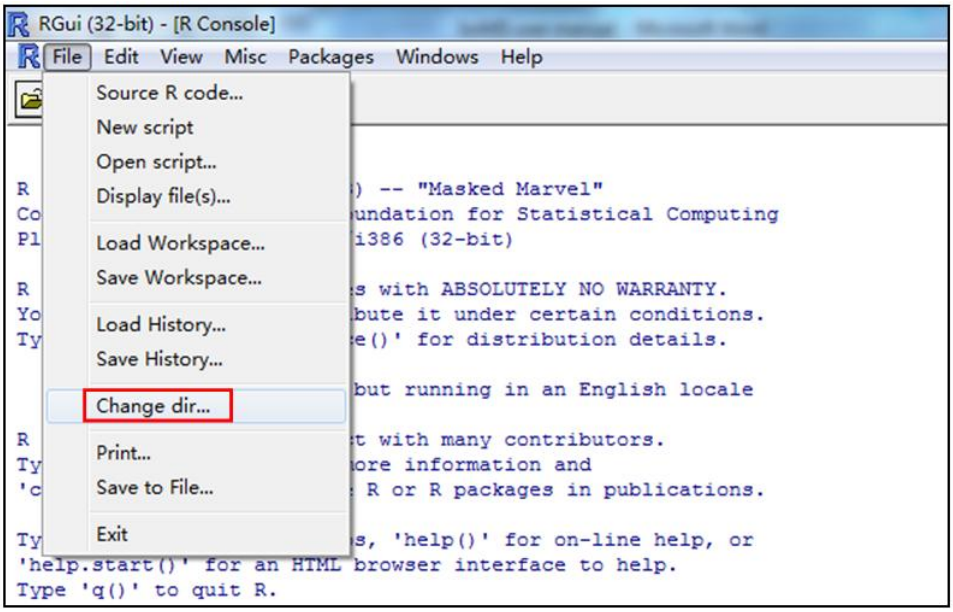


Figure 4. Change the working folder of RGui.

2. Running IsoMS

1) Run IsoMS by clicking: File → Source R code... to open the working folder (Figure 5), and then click "IsoMSMain".

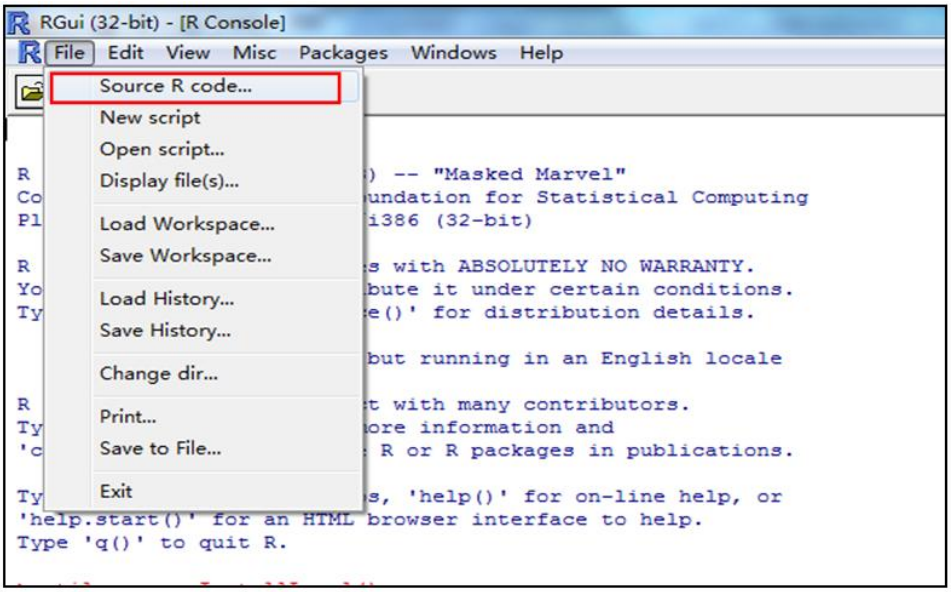
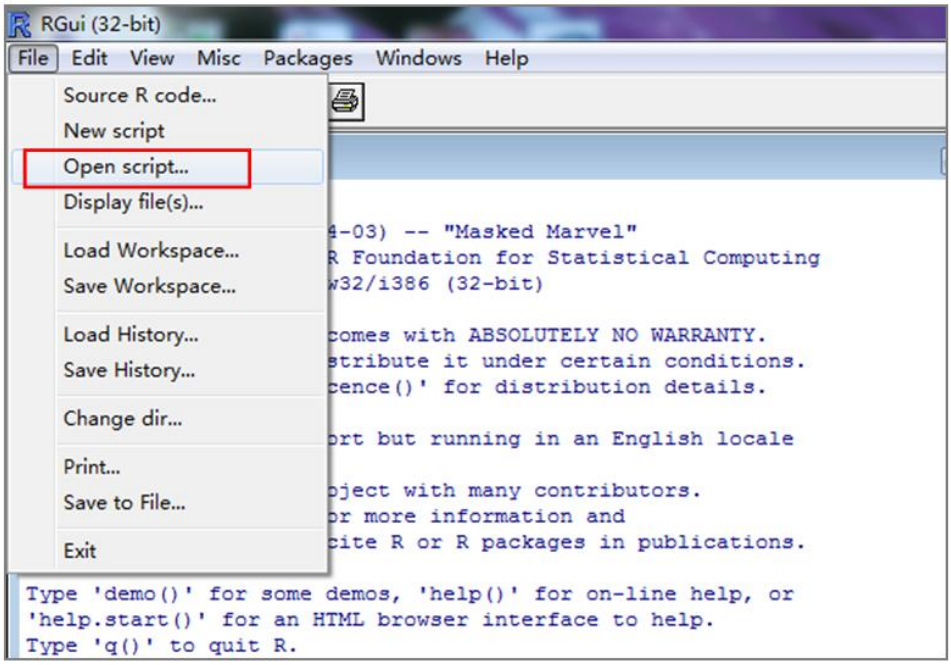


Figure 5. Run IsoMSMain in RGui.

2) A user has the option to change the IsoMS parameters based on the LC-MS data acquired and the desired output. To open the parameter page in RGui, click File → Open scripts → IsoMSMain (Figure 6A). A partial list of the parameter page is shown in Figure 6B.

(A)



(B)

```
library(IsoMSTOF)

#------------------------------Start of parameter table--------------------------------------#

peaklistDataFolder<-"./peaklistData/"          # Assign a folder for raw data
convertedDataFolder<-"./convertedPeaklist/"    # Assign a folder for converted peak lists
dataSaveFolder<-"./outputPairlist/"            # Assign a folder for detected pair lists
companyNO<-1                                   # Instrument manufacturer NO: Agilent=1, Bruker=2,Waters=3.

dataSaveFolderDebug<-paste(dataSaveFolder,"debug/",sep="")

if (companyNO==1) {
   AgilentDataConvert(peaklistDataFolder,convertedDataFolder)
   dataFolder<-"./convertedPeaklist/"
}  else if (companyNO==3) {
   WatersDataConvert(peaklistData,convertedDataFolder)
   dataFolder<-"./convertedPeaklist/"
}  else {
   dataFolder<-"./peaklistData/"
}

isFT<-FALSE                        # This is TOF version, users needn't
rtWinMin<-100                      # Starting time of chromatography that will be processed.
rtWinMax<-1800                     # End time of chromatography that will be processed.
mzWinMin<-265                      # Minimal m/z that will be processed.
mzWinMax<-1000                     # Maximal m/z that will be processed.
intThreshold<-1500                 # Threshold that cuts off low abundant features.
isFTHomo<-FALSE
isTOFTailing<-FALSE
```

Figure 6. (A) Open IsoMSMain script. (B) Partial list of the parameter page of IsoMSMain.

3) Change the parameters. Table 1 lists the parameters and their functions. These parameters should be adjusted according to the LC-MS data obtained. For most applications, only the parameters shown with a comment "#..." need to be adjusted.

Table 1. IsoMS parameters that need to be changed according to the LC-MS data obtained.

Parameter	Function
peaklistDataFolder	Location of source data files (i.e., the peak list files converted in Step 1). Default folder is "peaklistData" in the working folder (see Figure 1). Note: if a user opts to use a folder containing all the peak list files outside this working folder, replace "peaklistData" with the directory of the folder containing the peak list files.
convertedDataFolder	Location of converted data files. A default folder called "convertedPeaklist" is automatically created in the working folder. Note: this folder contains converted data files from Agilent or Waters MS which can be read more easily than the original peak list data files. The converted data files from Bruker MS can be read directly. In most instances, there is no need to look at these data.
dataSaveFolder	Location of resultant data files. A default folder called "outputPairlist" is automatically created in the working folder.
isFT	FALSE means that IsoMS is for TOF. There is no need to change this value.
rtWinMin	Chromatogram cutoff window: Spectra before this retention time are not processed. Note: use this parameter to exclude any mass spectra showing only background peaks at the beginning of the chromatogram where no metabolite peak pairs are expected.

rtWinMax	Chromatogram cutoff window: Spectra after this retention time are not processed. Note: use this parameter to exclude any mass spectra collected after metabolite elution where no metabolite peak pairs are expected.
mzWinMin	Mass spectrum cutoff window: Spectral peaks with lower m/z than the cutoff value are not processed. Note: this window can be useful to exclude mass spectral region where no peak pairs are expected (e.g., below the m/z value of a labeling reagent).
mzWinMax	Mass spectrum cutoff window: Spectral peaks with higher m/z than the cutoff value are not processed.
intThreshold	Intensity threshold. Spectral peaks with lower intensity are not processed.
isFTHomo	Bruker DataAnalysis4.0 gives homogeneous peaks during exporting FT-ICR-MS peak list, but the latest version has fixed this problem. When value is TRUE, IsoMS can filter out the homogeneous peaks from the peak list. Default value is FALSE. There is no need to change this value.
isTOFTailing	Old TOF- or QTOF-MS might give some centroid peaks after a high abundant peak (e.g., within 1 Da) due to its asymmetry peak shape. The newer instruments do not have this issue. Default value is FALSE. There is no need to change this value.
intSaturated	Upper limit of intensity or ion count to define the peak saturation. Note: the value of this limit is dependent on the dynamic range of an instrument. It can be determined by inspecting the mass spectra obtained under the experimental conditions; the upper limit is the max intensity or ion count where the peak saturation occurs.
isMZBackground	No change is needed. Always select =TRUE to use a user-defined mzBackground csv file to filter out the background peaks. This file should be placed in the working folder (see Figure 1). Note 1: The mzBackground.csv file contains a list of masses of m/z_light peaks of known background peak pairs to be excluded in the final results. An example of the mzBackground csv file is given in the working folder. Users should change the mass values in this file according to the background peak pairs found in their own work. Note 2: Any other peaks a user wants to remove can be added to this mzBackground file. For example, if a blank sample is run and the blank dataset has been processed by IsoMS, the m/z_light masses of the peak pairs found in the blank can be added to the mass column in the mzBackground file. Only the m/z_light masses need to be added. The program automatically removes the corresponding m/z_heavy peaks. Note 3: If there is no background peak to be removed, the mzBackground csv file should still contain "m/z" in row 1, but leave all the other cells blank.

mzTolGroup	Mass tolerance to group the same peak pairs from the neighboring spectra.	Note: the mass tolerance value used is depending on the mass measurement accuracy of the instrument used. There is no need to adjust the individual mass tolerance values (i.e., use the default values already given), unless a user wants to fine-tune these values to see if any additional improvement on the specificity and sensitivity of IsoMS can be made.
mzTolIso	Mass tolerance to search ^{13}C isotopologues within a pair.	
mz_tol_dt	Mass tolerance to filter derivative pairs.	
mzTolChargeVec	Mass tolerance to calculate the charge number of an ion.	
mzTolFixLev3	Mass tolerance to pair the peaks for level 3 pairs.	
mzTolDimer	Mass tolerance to filter dimer or other multimer pairs.	
mzTolBackground	Mass tolerance to filter background noises.	
mz_tol_ctfilter	Mass tolerance to filter multiply charged ions.	
minIntensity	Lowest intensity of exported peak pairs.	
minRatio	The peak intensity ratio range of exported peak pairs.	
MaxRatio		
minSN	Only for FTMS data: the lowest S/N of exported peak pairs. There is no need to change this parameter for TOFMS data.	

3. Installing and running IsoMS-align

1) Download the IsoMS-align script from the MyCompoundID website.
2) Assign the folder of IsoMS-align as the working folder of RGui by cliking: File → Change dir…(Figure 7).

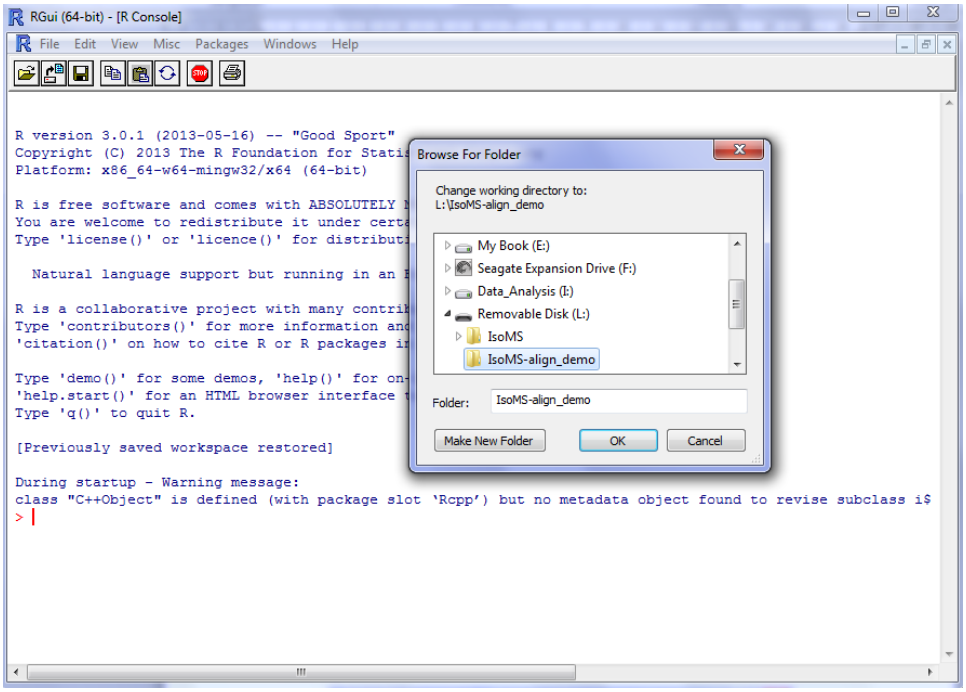


Figure 7. Select the alignment script.

3) Open the IsoMS-align script (Figure 8) and change the parameters therein (see Table 2 for the explanation of these parameters).

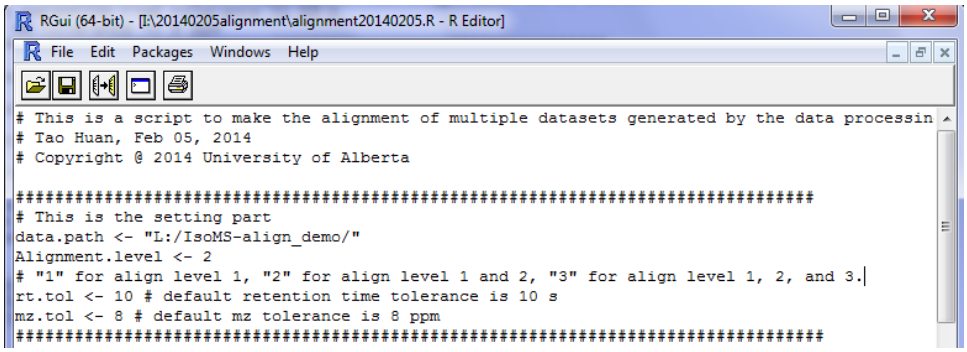


Figure 8. Change the parameters in IsoMS-align.

Table 2. IsoMS-align parameters that need to be changed.

Parameter	Function
data.path	Set the data path to the folder that contains all the peak pair csv files.
Alignment.level	Select the levels of peak pairs to perform the alignment.
rt.tol	Set the retention time tolerance during the alignment process.
mz.tol	Set the mz tolerance during the alignment process.

4) Save the parameter changes to the script. Type in the command code in RGui as shown in red in Figure 9 and press enter to run the script.

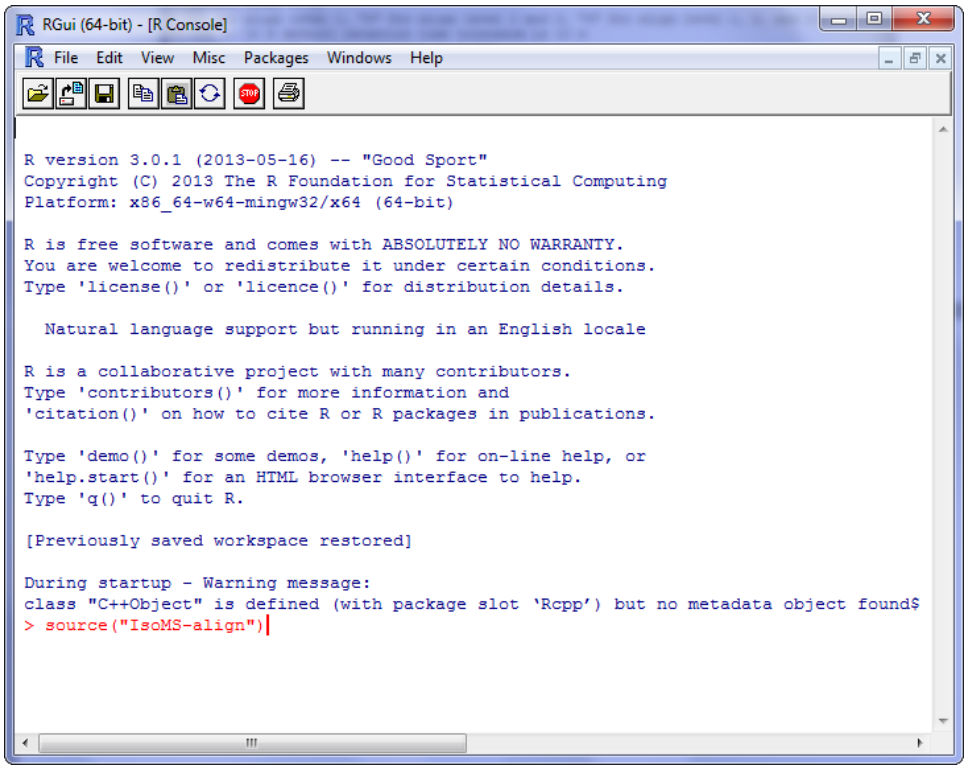


Figure 9. Type in the command code in RGui and then run the script.

5) After running the script, a new csv file named "Alignment_result.csv" will be created. This csv file contains the alignment result.