4MRNA User Manual

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Contents

[GitHub repository]

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1. Introduction

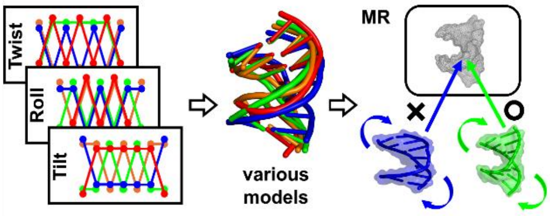
1-1. What is 4MRNA?

4MRNA is an abbreviation for “**M**assive **M**ulti-type **M**odel **M**olecular **R**eplacement for **N**ucleic **A**cids”. This is a novel method designed to enhance molecular replacement (MR) for phasing in X-ray crystallography of nucleic acids.

Applying MR, which is widely used in structure determination (phase determination), to nucleic acids presents unique challenges that are not encountered with proteins. To overcome these issues, we developed an innovative strategy termed 4MRNA.

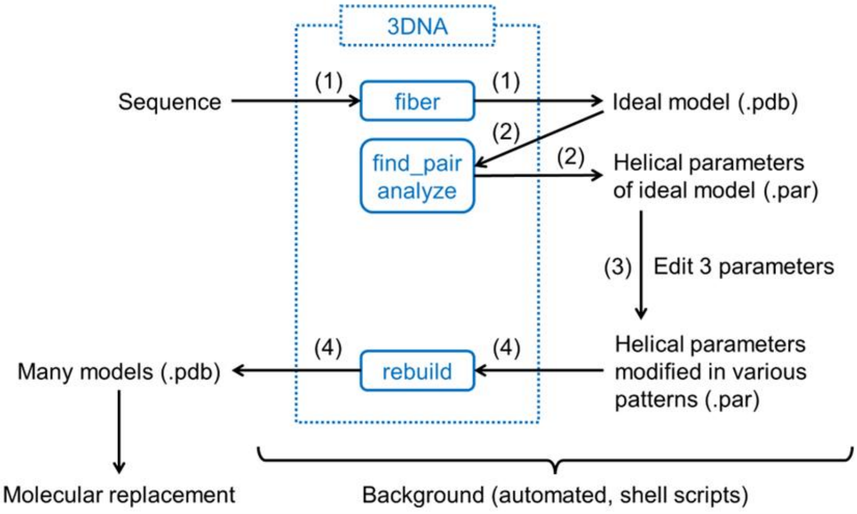
One major difficulty is that nucleic acids can adopt different 3D structures even with the same sequence. As a result, models existing in the database may not be suitable as search models, and sequence-based structure prediction methods such as AlphaFold are also limited in applicability [1,2]. Moreover, it is empirically known that MR of nucleic acids can fail even when the search model and the target structure differ only slightly. [3] To address these issues, the new strategy 4MRNA includes creating a large number of diverse search models (= massive multi-type models) and applying them to MR.

We found that by varying three out of the twelve parameters that control the three-dimensional structures of nucleic acids according to different patterns, the resulting set of models included ones that closely matched the correct structure, thereby increasing the success rate of MR.



(Strategy of 4MRNA)

Building on this strategy, we employed **3DNA** [4], which is a software that generates nucleic acid structural models based on parameters, to create a wide variety of models. The processes of parameter adjustment and model creation have been automated using **Shell** scripts. Subsequently, MR is carried out for each of the many created models. Since this operation must be repeated many times, we also automated this process using **Shell** scripts.



(Tactics of 4MRNA)

1-2. Two modes to execute 4MRNA

There are two modes to execute 4MRNA: default mode and customize mode.

For both mode, you need to install the `4MRNA` command on your computer in advance.

In the **default mode**, you can simply type the command `4MRNA` in Ubuntu/Terminal and run it. Thus, it is well suited for all users.

In the **customize mode**, you can obtain some programming codes by runnning the command `4MRNA`. Basically, you just run them in the prescribed order, but if needed, you can edit and customize the code. For example, you can adjust parameters by yourself to get more diverse models, or add models prepared by other methods to perform molecular replacement. Such customization represents a highly advanced option and is generally intended for experts in structural biology or X-ray crystallography.

Style	**Procedure**	**Pre-setup**	**Usability**	**Customization**	**Target users**
Default	Run the command `4MRNA`	Required	Very good	Not possible	For all users
Customize	Download codes → Run them in order	Required	Fair	Possible	For experts

For details on how to make the preparations, please refer to the Preparations section.

⇒ Preparations

For details on the usage of each mode, please refer to the corresponding sections.

⇒ Default mode

⇒ Customize mode

2. Preparations

2-1. How to download and install 4MRNA

Please run the following commands in order.

```
cd $HOME
git clone https://github.com/S-Ando-Biophysics/4MRNA-Install.git
cd 4MRNA-Install
chmod +x install.sh
./install.sh
echo 'export PATH="$HOME/4MRNA-Install/bin:$PATH"' >> ~/.bashrc
source ~/.bashrc
```

2-2. Additional preparation

Please install and set up the following softwares in advance.

Name	**Download link**	**Remarks**
Ubuntu	https://apps.microsoft.com/search?query=Ubuntu	Required only for Windows to run Shell scripts. Not required for macOS or Linux, since they use Terminal.
3DNA	http://forum.x3dna.org	It can be downloaded after registering on the site and receiving approval.
Phenix	https://phenix-online.org/download	The command-line version is required.
Phaser	https://www.ccp4.ac.uk/download	The program Phaser is included in CCP4, so please install CCP4.

2-2-1. Ubuntu

Step 1. Enable “**Windows Subsystem for Linux (WSL)**” and “**Virtual Machine Platform**”. Go to *Control Panel > Programs > Programs and Features > Turn Windows features on or off*, and check the boxes for “*Windows Subsystem for Linux*” and “*Virtual Machine Platform*”.

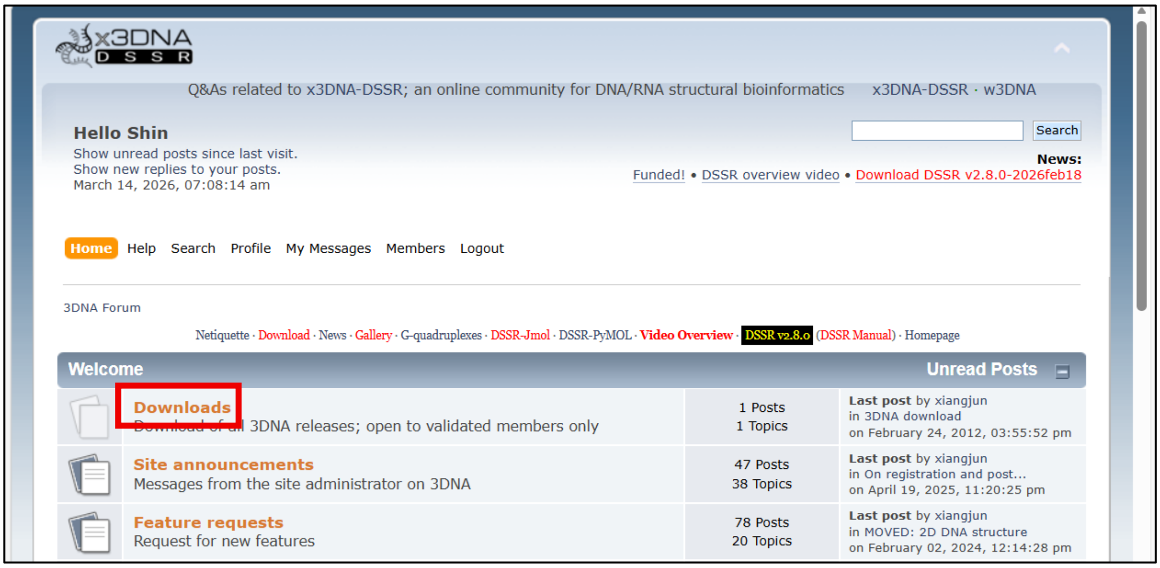
Step 2. Download Ubuntu from here. Basically, download the latest version labeled “Ubuntu XX.XX.X LTS”.

Step 3. After the download is complete, launch Ubuntu and run the following commands.

```
sudo apt update
sudo apt upgrade
sudo apt install -y bzip2 curl git wget
sudo apt install libgomp1 libstdc++6 libgcc-s1 libc6
sudo ln -s /usr/bin/python3 /usr/bin/python
```

2-2-2. 3DNA

Step 1. Visit the official website and register on the site. Once you receive the approval email a few days later, you will be able to download 3DNA.



Q&As related to x3DNA-DSSR; an online community for DNA/RNA structural bioinformatics x3DNA-DSSR · w3DNA

Hello Shin
Show unread posts since last visit.
Show new replies to your posts.
March 14, 2026, 07:08:14 am

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3DNA Forum

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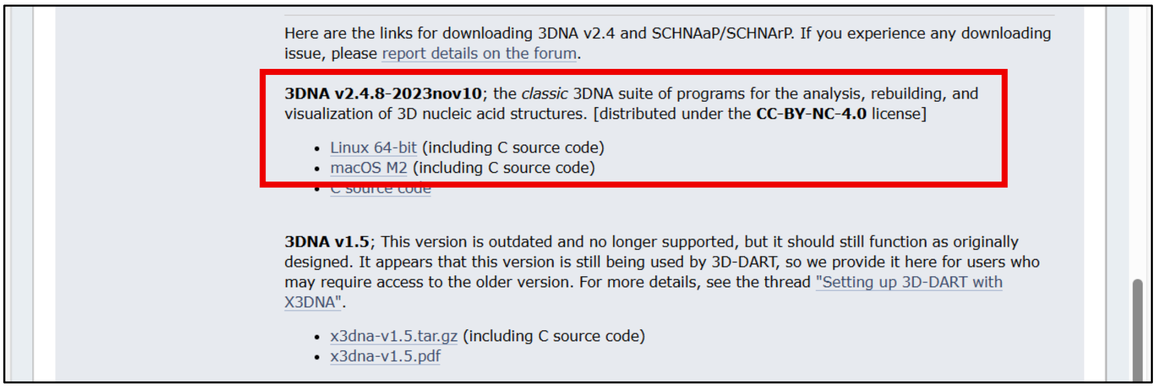
Welcome		Unread Posts
Downloads Download of all 3DNA releases; open to validated members only	1 Posts 1 Topics	**Last post** by xiangjun in 3DNA download on February 24, 2012, 03:55:52 pm
Site announcements Messages from the site administrator on 3DNA	47 Posts 38 Topics	**Last post** by xiangjun in On registration and post... on April 19, 2025, 11:20:25 pm
Feature requests Request for new features	78 Posts 20 Topics	**Last post** by xiangjun in MOVED: 2D DNA structure on February 02, 2024, 12:14:28 pm

Step 2. Download the installer for the Linux or macOS version. Choose the Linux version even if you're using Windows.



Here are the links for downloading 3DNA v2.4 and SCHNAaP/SCHNArP. If you experience any downloading issue, please report details on the forum.

3DNA v2.4.8-2023nov10; the *classic* 3DNA suite of programs for the analysis, rebuilding, and visualization of 3D nucleic acid structures. [distributed under the **CC-BY-NC-4.0** license]

- Linux 64-bit (including C source code)
- macOS M2 (including C source code)
- C source code

3DNA v1.5; This version is outdated and no longer supported, but it should still function as originally designed. It appears that this version is still being used by 3D-DART, so we provide it here for users who may require access to the older version. For more details, see the thread "Setting up 3D-DART with X3DNA".

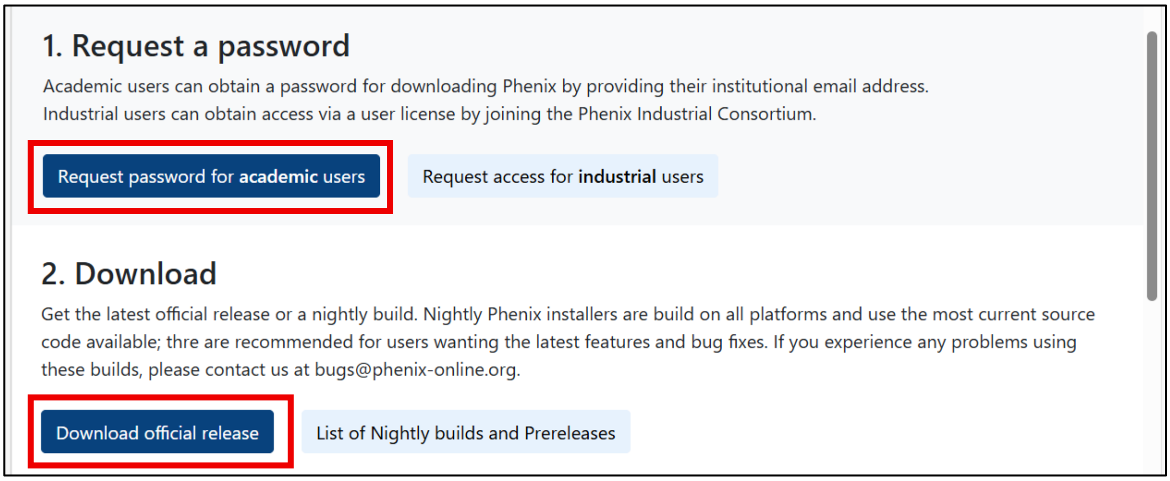
- x3dna-v1.5.tar.gz (including C source code)
- x3dna-v1.5.pdf

Step 3. Once the download is complete, run the following commands in order to complete the installation. The following commands are for Ubuntu on Windows, but similar commands should be used for macOS and Linux. (These commands assume that the file “x3dna-v2.4-linux-64bit.tar.gz” has been downloaded to “C:\Users\name\Downloads”. You will need to adjust the version number and directory path as appropriate.)

```
sudo su
cd /usr/local
mv /mnt/c/Users/name/Downloads/x3dna-v2.4-linux-64bit.tar.gz .
tar -pzxvf x3dna-v2.4-linux-64bit.tar.gz
cd x3dna-v2.4/bin
./x3dna_setup
exit
echo 'export X3DNA=/usr/local/x3dna-v2.4' >> ~/.bashrc
echo 'export PATH="$X3DNA/bin:$PATH"' >> ~/.bashrc
source ~/.bashrc
```

2-2-3. Phenix

Step 1. Visit the official website and request a password. Once you receive the email a few minutes later, you will be able to download Phenix.



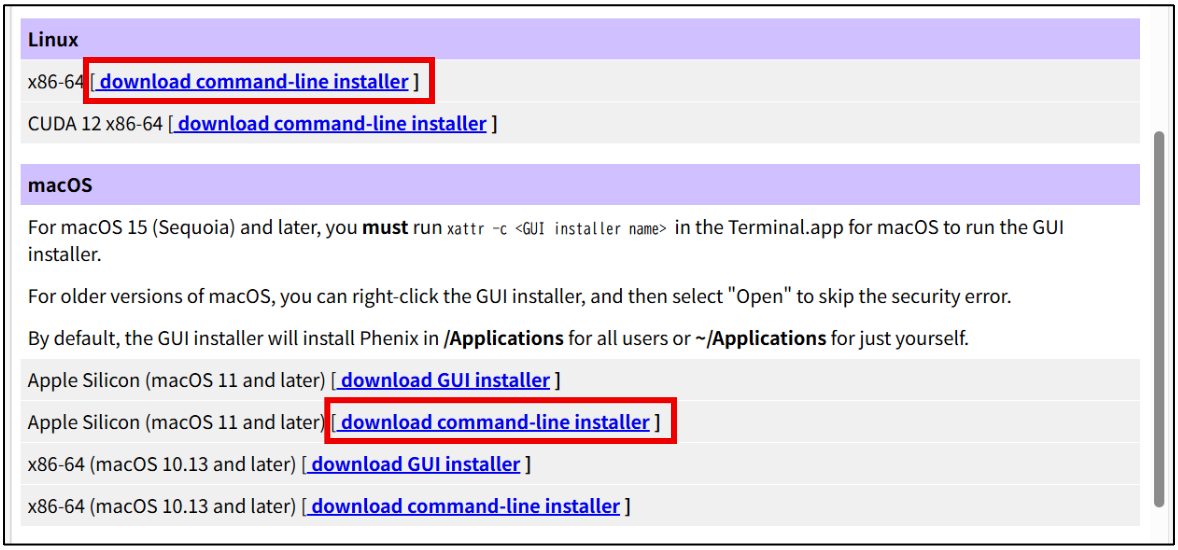
1. Request a password

Academic users can obtain a password for downloading Phenix by providing their institutional email address.
Industrial users can obtain access via a user license by joining the Phenix Industrial Consortium.

2. Download

Get the latest official release or a nightly build. Nightly Phenix installers are build on all platforms and use the most current source code available; thre are recommended for users wanting the latest features and bug fixes. If you experience any problems using these builds, please contact us at bugs@phenix-online.org.

Step 2. Download the command-line installer for the Linux or macOS version. Choose the Linux version even if you're using Windows.



Linux

x86-64 [download command-line installer]

CUDA 12 x86-64 [download command-line installer]

macOS

For macOS 15 (Sequoia) and later, you **must** run `xattr -c <GUI installer name>` in the Terminal.app for macOS to run the GUI installer.

For older versions of macOS, you can right-click the GUI installer, and then select "Open" to skip the security error.

By default, the GUI installer will install Phenix in **/Applications** for all users or **~/Applications** for just yourself.

Apple Silicon (macOS 11 and later) [download GUI installer]

Apple Silicon (macOS 11 and later) [download command-line installer]

x86-64 (macOS 10.13 and later) [download GUI installer]

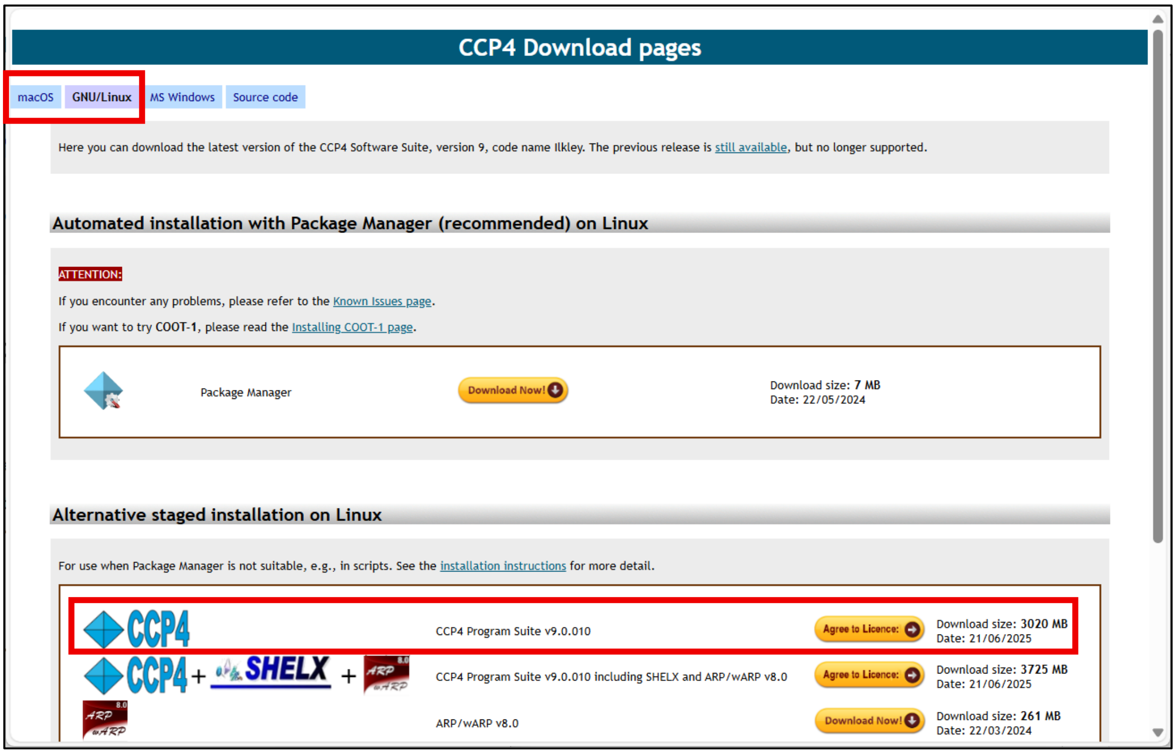
x86-64 (macOS 10.13 and later) [download command-line installer]

Step 3. Once the download is complete, run the following commands in order to complete the installation. The following commands are for Ubuntu on Windows, but similar commands should be used for macOS and Linux. (These commands assume that the file "Phenix-2.0-5936-Linux-x86_64.sh" has been downloaded to "C:\Users\name\Downloads". You will need to adjust the version number and directory path as appropriate.)

```
sudo su
cd /usr/local
mv /mnt/c/Users/name/Downloads/Phenix-2.0-5936-Linux-x86_64.sh .
bash Phenix-2.0-5936-Linux-x86_64.sh -b -p /usr/local/phenix-2.0-5936
exit
echo "source /usr/local/phenix-2.0-5936/phenix_env.sh" >> ~/.bashrc
source ~/.bashrc
```

2-2-4. Phaser

Step 1. Phaser is included in a software suite termed CCP4. Visit the official website and download the Linux or macOS version. Choose the Linux version even if you're using Windows.



CCP4 Download pages

Here you can download the latest version of the CCP4 Software Suite, version 9, code name Ilkley. The previous release is still available, but no longer supported.

Automated installation with Package Manager (recommended) on Linux

ATTENTION:

If you encounter any problems, please refer to the Known Issues page.

If you want to try COOT-1, please read the Installing COOT-1 page.

Package Manager	Download Now!	Download size: 7 MB Date: 22/05/2024

Alternative staged installation on Linux

For use when Package Manager is not suitable, e.g., in scripts. See the installation instructions for more detail.

CCP4 Program Suite v9.0.010	Agree to Licence:	Download size: 3020 MB Date: 21/06/2025
CCP4 Program Suite v9.0.010 including SHELX and ARP/wARP v8.0	Agree to Licence:	Download size: 3725 MB Date: 21/06/2025
ARP/wARP v8.0	Download Now!	Download size: 261 MB Date: 22/03/2024

Step 2. Once the download is complete, run the following commands in order to complete the installation. The following commands are for Ubuntu on Windows, but similar commands should be used for macOS and Linux. (These commands assume that the file “ccp4-9.0.010-linux64.tar.gz” has been downloaded to “C:\Users\name\Downloads”. You will need to adjust the version number and directory path as appropriate.)

```
sudo su
cd /usr/local
mv /mnt/c/Users/name/Downloads/ccp4-9.0.010-linux64.tar.gz .
gunzip ccp4-9.0.010-linux64.tar.gz
tar -xvf ccp4-9.0.010-linux64.tar
cd ccp4-9
./BINARY.setup
exit
echo "source /usr/local/ccp4-9/bin/ccp4.setup-sh" >> ~/.bashrc
source ~/.bashrc
```

3. How to use the default mode

In this mode, you need to install the `4MRNA` command on your computer in advance. Installation is, of course, required only once at the initial use. This Section 3 describes the usage of the `4MRNA` command after installation has been completed.

3-1. Summary of the procedure

Step 1. Create a new directory on your computer for executing 4MRNA. After creating it, place your reflection file (.mtz) for molecular replacement into that directory.

Step 2. Open Ubuntu/Terminal, change to the directory created in Step 1, and run the command `4MRNA`.

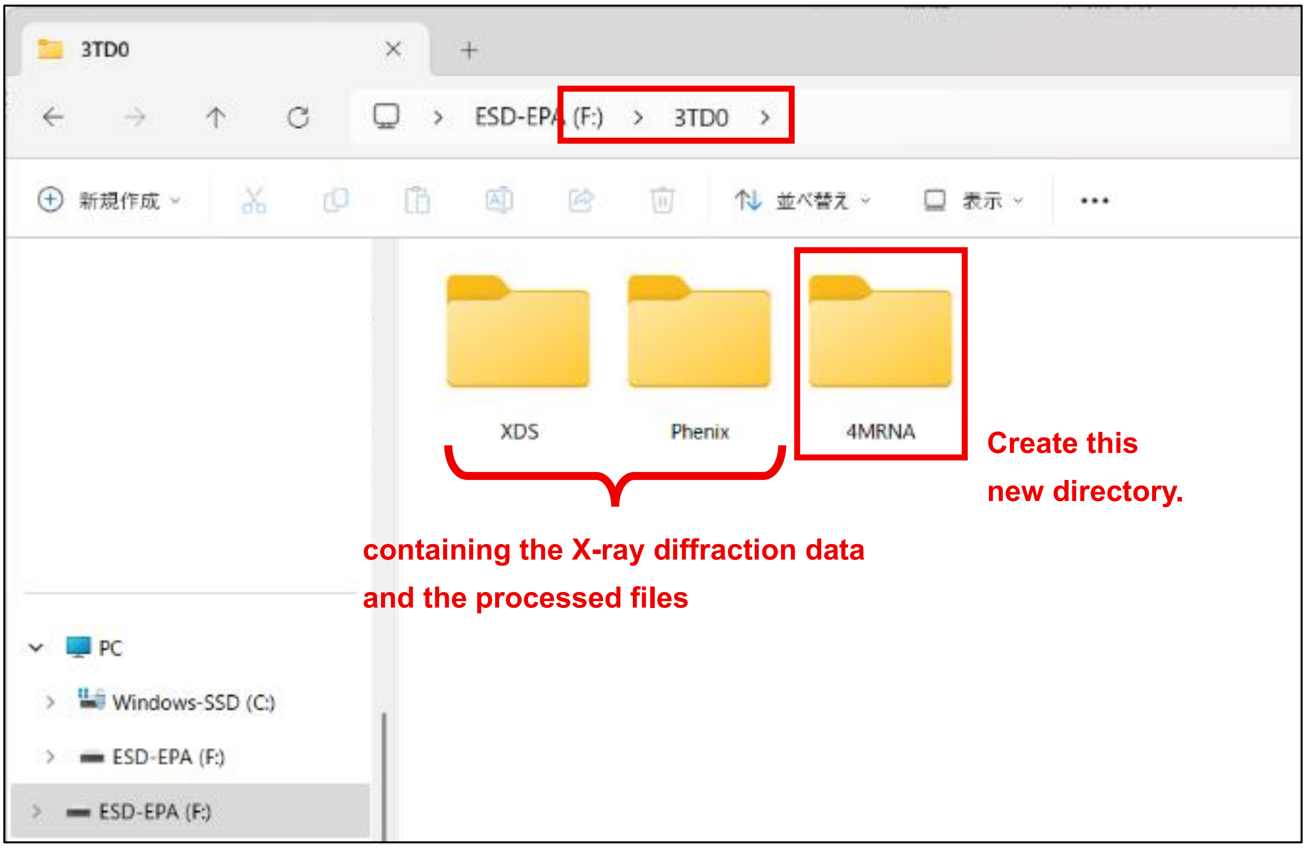
Step 3. When prompted to choose the execution mode, select "**default**".

Step 4. When 4MRNA finishes, a directory named “**4MRNA-Results**” will be created, containing up to seven candidate solutions for molecular replacement.

3-2. Step-by-step instructions with screenshots

■ Create a new directory on your computer for executing 4MRNA.

[e.g.] Suppose your sample is named 3TD0, and the X-ray diffraction data is stored in a directory 3TD0 on a USB drive (F:). In that case, create a new directory named “4MRNA” inside “F:\3TD0”.

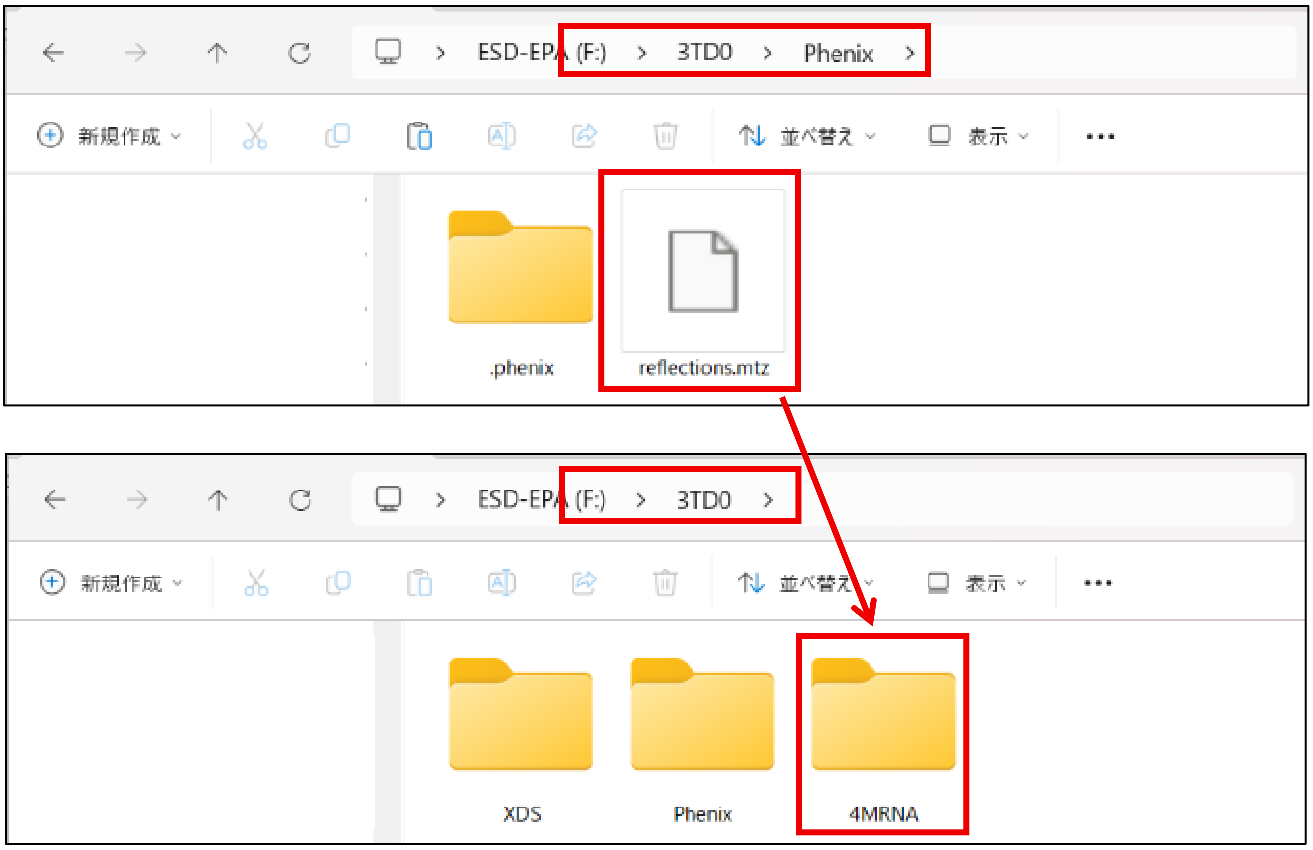


Create this new directory.

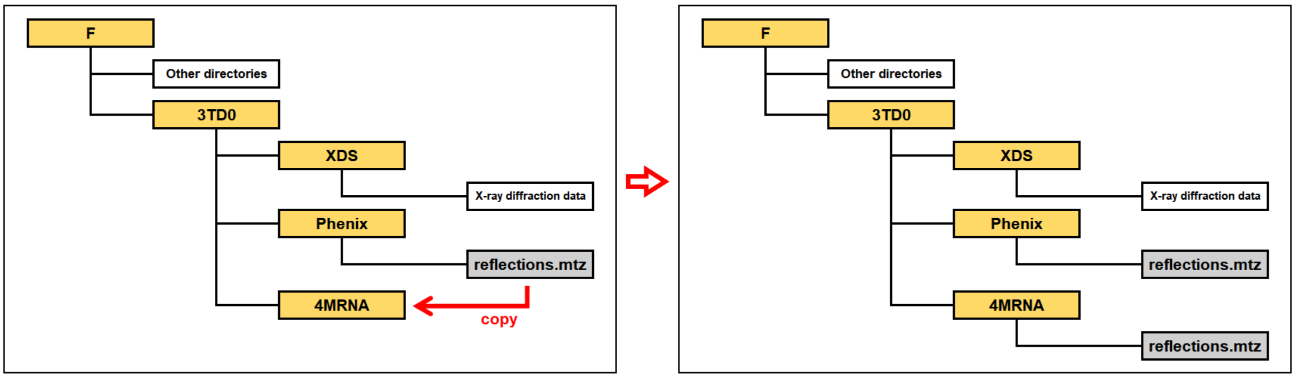
containing the X-ray diffraction data and the processed files

- Place your reflection file (.mtz) for molecular replacement into the created directory.

[e.g.] When using the software Phenix, the reflection file is typically named “reflections.mtz”. If this file is in “F:\3TD0\Phenix”, copy it, and paste to “F:\3TD0\4MRNA”.

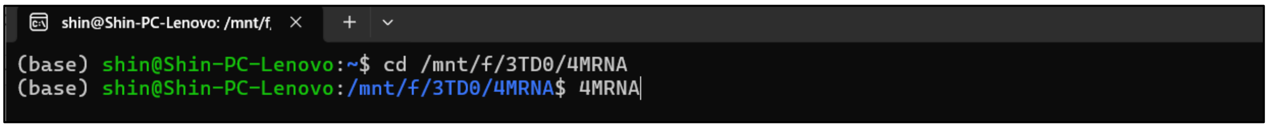


- The directory structure after these operations is as illustrated in the example below.



- Open Ubuntu or Terminal, change to the created directory, and run the command `4MRNA` as follows.

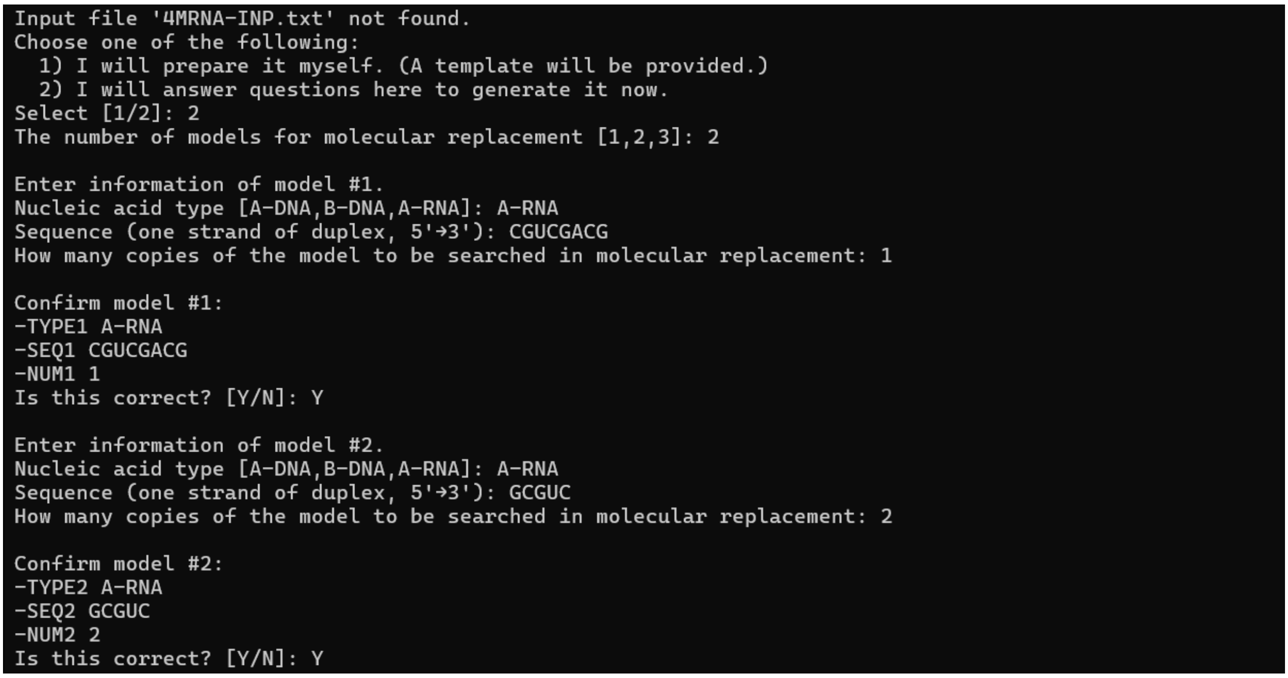
[e.g.] If the created directory is “F:\3TD0\4MRNA”, enter the command in Ubuntu as shown in the figure below. (This example is for Windows.)



```
(base) shin@Shin-PC-Lenovo:~$ cd /mnt/f/3TD0/4MRNA
(base) shin@Shin-PC-Lenovo:/mnt/f/3TD0/4MRNA$ 4MRNA
```

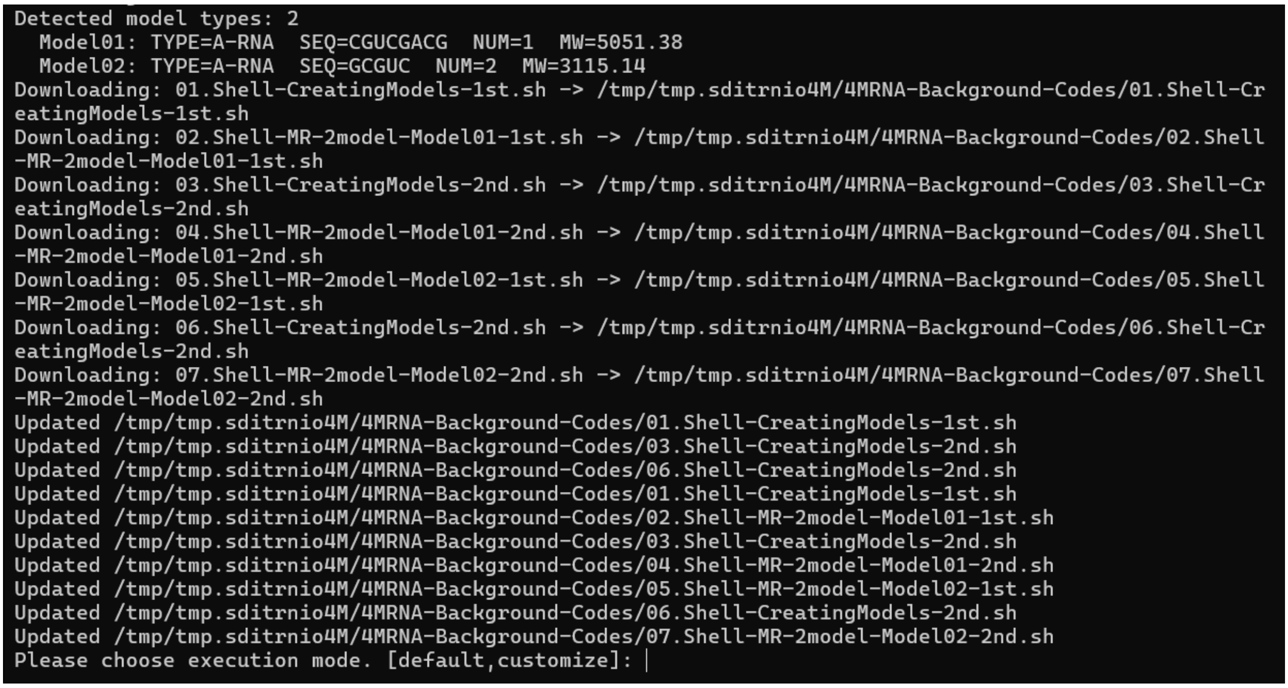
- Actually, for the command **4MRNA** to work, an input file called "**4MRNA-INP.txt**" is required. The user is prompted to choose from two options: (1) preparing the file yourself (a template will be provided), or (2) answering questions on the screen so that the program automatically generates it.
- Option **(2)** is strongly recommended. For option (1), refer to the supplementary notes in Section 3-3.
- If you choose option (2), several questions will be asked. Please answer them. Once all questions have been answered, the 4MRNA workflow will start automatically.

[e.g.] The figure below shows an example of molecular replacement using two different RNAs as models. In this example, during molecular replacement, one copy of the first model and two copies of the second model are searched for in the asymmetric unit of the crystal.



```
Input file '4MRNA-INP.txt' not found.
Choose one of the following:
  1) I will prepare it myself. (A template will be provided.)
  2) I will answer questions here to generate it now.
Select [1/2]: 2
The number of models for molecular replacement [1,2,3]: 2

Enter information of model #1.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): CGUCGACG
How many copies of the model to be searched in molecular replacement: 1

Confirm model #1:
-TYPE1 A-RNA
-SEQ1 CGUCGACG
-NUM1 1
Is this correct? [Y/N]: Y

Enter information of model #2.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): GCGUC
How many copies of the model to be searched in molecular replacement: 2

Confirm model #2:
-TYPE2 A-RNA
-SEQ2 GCGUC
-NUM2 2
Is this correct? [Y/N]: Y
```

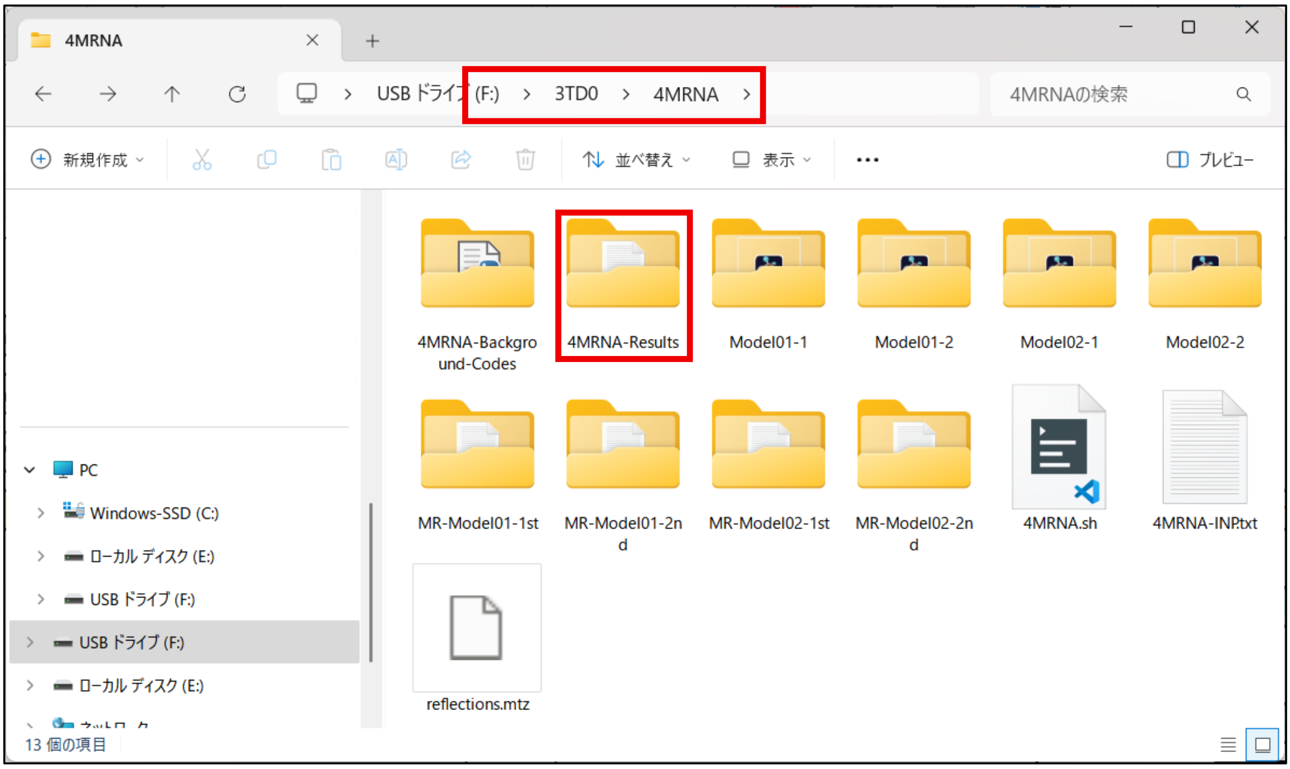
- When prompted with "Please choose execution mode.", please enter "**default**".



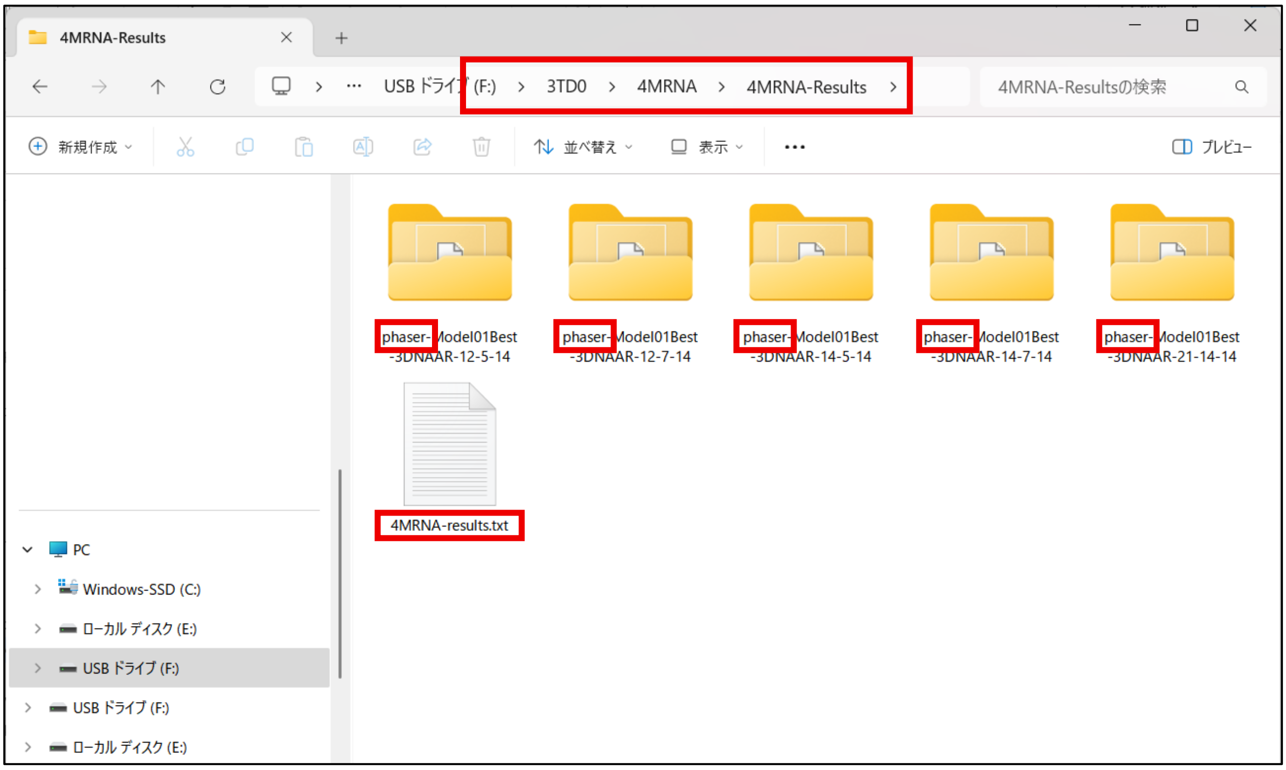
```
Detected model types: 2
  Model01: TYPE=A-RNA  SEQ=CGUCGACG  NUM=1  MW=5051.38
  Model02: TYPE=A-RNA  SEQ=GCGUC  NUM=2  MW=3115.14
Downloading: 01.Shell-CreatingModels-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-Cr
eatingModels-1st.sh
Downloading: 02.Shell-MR-2model-Model01-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/02.Shell
-MR-2model-Model01-1st.sh
Downloading: 03.Shell-CreatingModels-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-Cr
eatingModels-2nd.sh
Downloading: 04.Shell-MR-2model-Model01-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/04.Shell
-MR-2model-Model01-2nd.sh
Downloading: 05.Shell-MR-2model-Model02-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/05.Shell
-MR-2model-Model02-1st.sh
Downloading: 06.Shell-CreatingModels-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-Cr
eatingModels-2nd.sh
Downloading: 07.Shell-MR-2model-Model02-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/07.Shell
-MR-2model-Model02-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-CreatingModels-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-CreatingModels-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/02.Shell-MR-2model-Model01-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/04.Shell-MR-2model-Model01-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/05.Shell-MR-2model-Model02-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/07.Shell-MR-2model-Model02-2nd.sh
Please choose execution mode. [default,customize]: 
```

- When the entire workflow is finished, the message “All finished” will be displayed, and a directory named “**4MRNA-Results**” will be created inside the 4MRNA execution directory (i.e. the directory you created at the beginning).

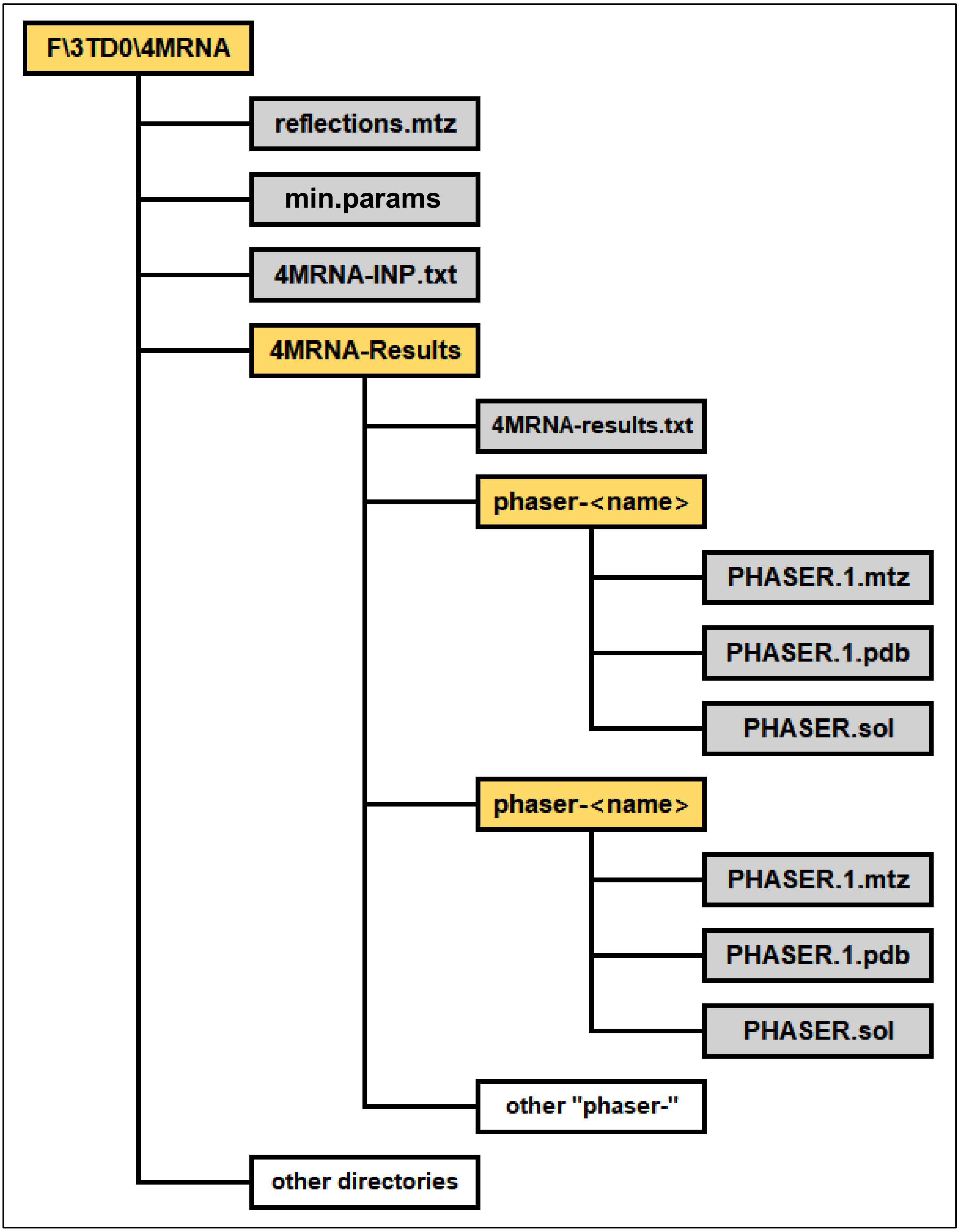
```
=== All finished ===
```



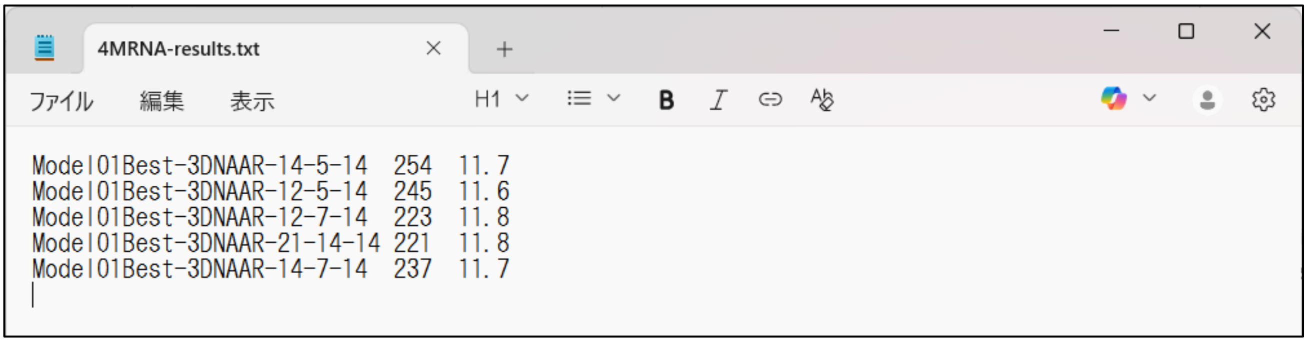
- Inside the “4MRNA-Results” directory, there is a file named “**4MRNA-results.txt**” and up to seven subdirectories. Each subdirectory name begins with “**phaser-**” and contains the results of molecular replacement (MR).



- Inside each "phaser-" subdirectory, there is a structural file named "**PHASER.1.pdb**." This file is a solution of molecular replacement, that is, a candidate for the correct structure.
- The following figure is an example of the final directory structure.



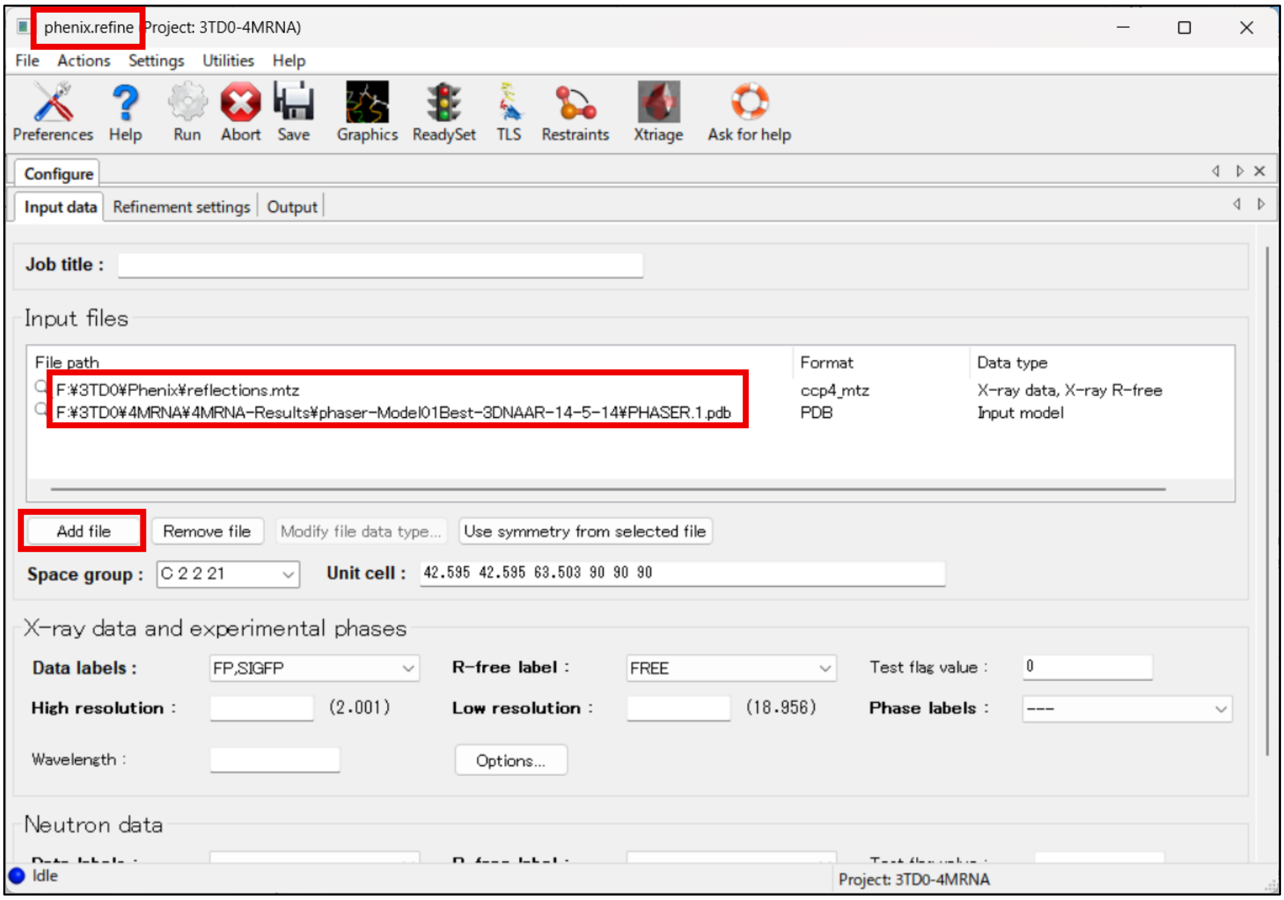
- Each run of molecular replacement produces a solution, but it must be validated against the experimental data (electron density) to confirm its correctness. This validation is guided by two statistical values, **LLG** and **TFZ**. The program is designed to automatically select up to seven solutions with the most favorable statistics. These statistics are compiled in “**4MRNA-results.txt**.” Each line in this file lists the ID of molecular replacement (i.e. the name of directory) , LLG, and TFZ in that order.



```
Model01Best-3DNAAR-14-5-14  254  11.7
Model01Best-3DNAAR-12-5-14  245  11.6
Model01Best-3DNAAR-12-7-14  223  11.8
Model01Best-3DNAAR-21-14-14 221  11.8
Model01Best-3DNAAR-14-7-14  237  11.7
```

- With reference to the statistics provided in “**4MRNA-results.txt**,” open the structural file (“**PHASER.1.pdb**”) together with the electron density map (“**PHASER.1.mtz**”) in software like Coot. By overlaying them, decide which of the up to seven candidate solutions is the best.
- After the optimal structure has been determined, it will be used as the initial structure for refinement.

[e.g.] For example, if the structure with the molecular replacement ID “Model01Best-3DNAAR-14-5-14” (i.e., “PHASER.1.pdb” within the directory “phaser-Model01Best-3DNAAR-14-5-14”) is decided as the initial structure, you should add this file. In addition, add the reflection file (“reflections.mtz”).



Job title :

Input files

File path	Format	Data type
F:¥3TD0¥Phenix¥reflections.mtz	ccp4_mtz	X-ray data, X-ray R-free
F:¥3TD0¥4MRNA¥4MRNA-Results¥phaser-Model01Best-3DNAAR-14-5-14¥PHASER.1.pdb	PDB	Input model

Space group : C 2 2 21

Unit cell : 42.595 42.595 63.503 90 90 90

X-ray data and experimental phases

Data labels : FP,SIGFP

R-free label : FREE

Test flag value : 0

High resolution : (2.001)

Low resolution : (18.956)

Phase labels : ---

Wavelength :

Neutron data

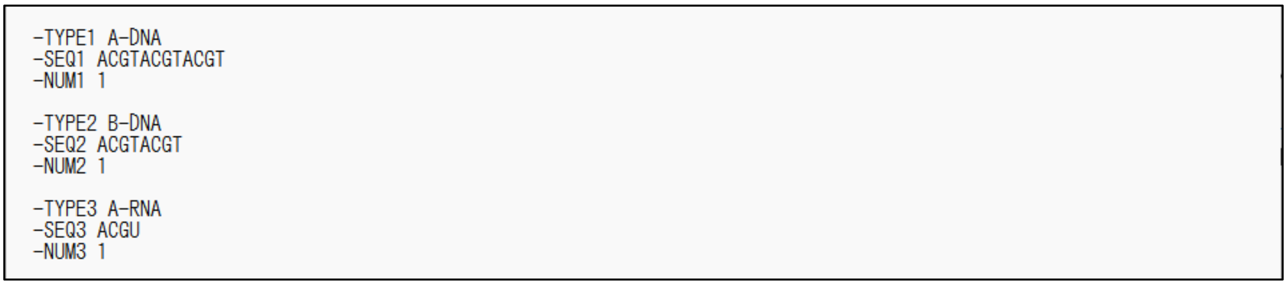
3-3. Supplementary notes

- Actually, for the `4MRNA` command to work, an input file called “**4MRNA-INP.txt**” is required.
- There are two ways to prepare the input file “4MRNA-INP.txt”:
  1. Prepare the input file yourself before running the `4MRNA` command.
     ⇒ Please refer to the template below.
  2. **[Strongly recommended]** Do not prepare the input file in advance. Instead, answer the questions prompted by the `4MRNA` command, and the program will automatically create the input file.
     ⇒ Please refer to Section 3-2.
- The template of the input file “4MRNA-INP.txt” looks like the screenshot below. The following three pieces of information should be repeated for each model used in molecular replacement.

  -TYPE: This is the nucleic acid type. Choose from A-form DNA, B-form DNA, or A-form RNA.

  -SEQ: This is the sequence. Only the sequence of one strand of the duplex is required. The complementary strand is processed automatically by the program.

  -NUM: This means how many copies of the model to be searched in molecular replacement.



```
-TYPE1 A-DNA
-SEQ1 ACGTACGTACGT
-NUM1 1

-TYPE2 B-DNA
-SEQ2 ACGTACGT
-NUM2 1

-TYPE3 A-RNA
-SEQ3 ACGU
-NUM3 1
```

4. How to use the customize mode

In this mode, after executing the `4MRNA` command, the 4MRNA workflow does not start automatically as it does in the default mode. Instead, the programming code that runs in the background is downloaded. You can then edit and customize the code.

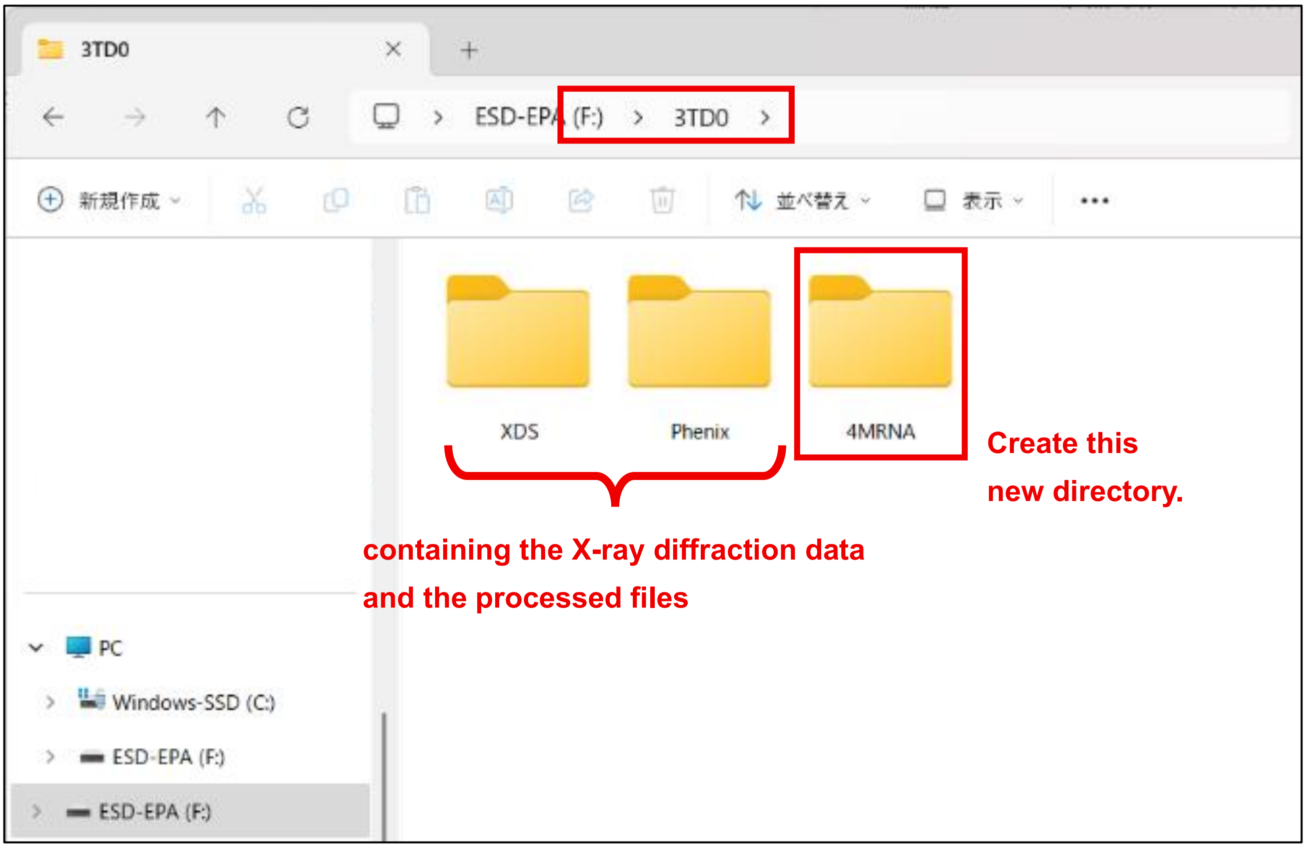
4-1. Summary of the procedure

Step 1. Create a new directory on your computer for executing 4MRNA. After creating it, place your reflection file (.mtz) for molecular replacement into that directory.
Step 2. Open Ubuntu/Terminal, change to the directory created in Step 1, and run the command `4MRNA`.
Step 3. When prompted to choose the execution mode, select "**customize**".
Step 4. You can edit each downloaded code.
Step 5. Please run the edited scripts with the `bash` command on Ubuntu/Terminal in the order indicated by the number at the beginning of each file.
Step 6. When 4MRNA finishes, a directory named "**4MRNA-Results**" will be created, containing up to seven candidate solutions for molecular replacement.

4-2. Step-by-step instructions with screenshots

■ Create a new directory on your computer for executing 4MRNA.

[e.g.] Suppose your sample is named 3TD0, and the X-ray diffraction data is stored in a directory 3TD0 on a USB drive (F:). In that case, create a new directory named "4MRNA" inside "F:\3TD0".

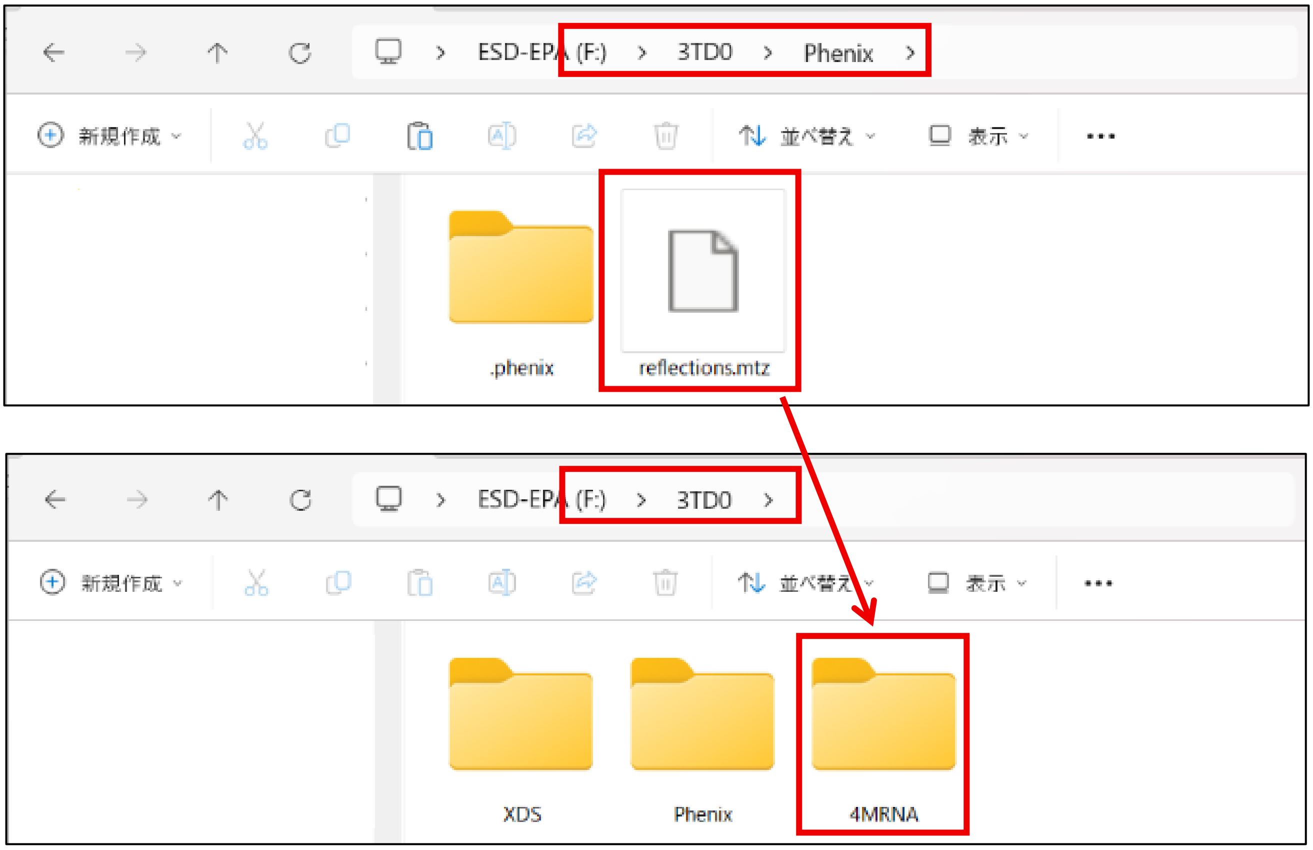


Create this new directory.

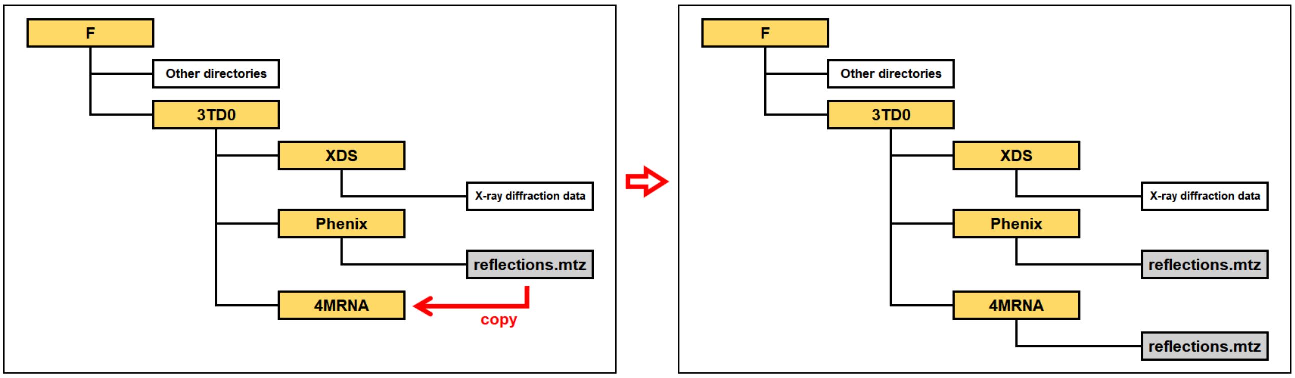
containing the X-ray diffraction data and the processed files

- Place your reflection file (.mtz) for molecular replacement into the created directory.

[e.g.] When using the software Phenix, the reflection file is typically named “reflections.mtz”. If this file is in “F:\3TD0\Phenix”, copy it, and paste to “F:\3TD0\4MRNA”.

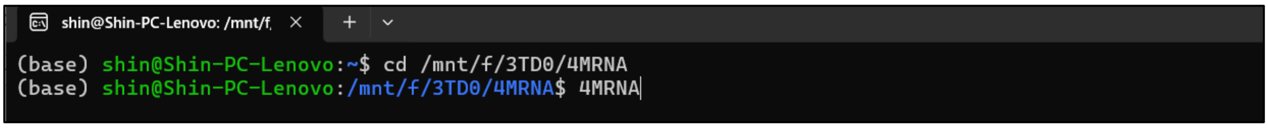


- The directory structure after these operations is as illustrated in the example below.



- Open Ubuntu or Terminal, change to the created directory, and run the command `4MRNA` as follows.

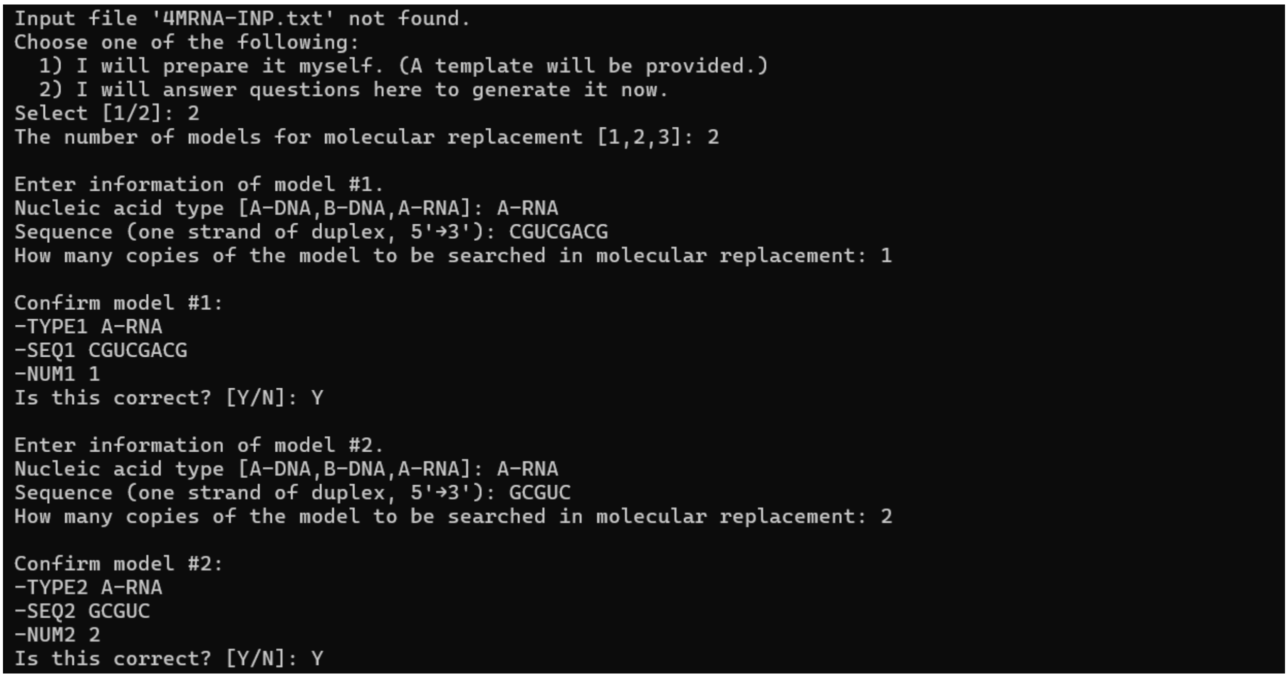
[e.g.] If the created directory is “F:\3TD0\4MRNA”, enter the command in Ubuntu as shown in the figure below. (This example is for Windows.)



```
(base) shin@Shin-PC-Lenovo:~$ cd /mnt/f/3TD0/4MRNA
(base) shin@Shin-PC-Lenovo:/mnt/f/3TD0/4MRNA$ 4MRNA
```

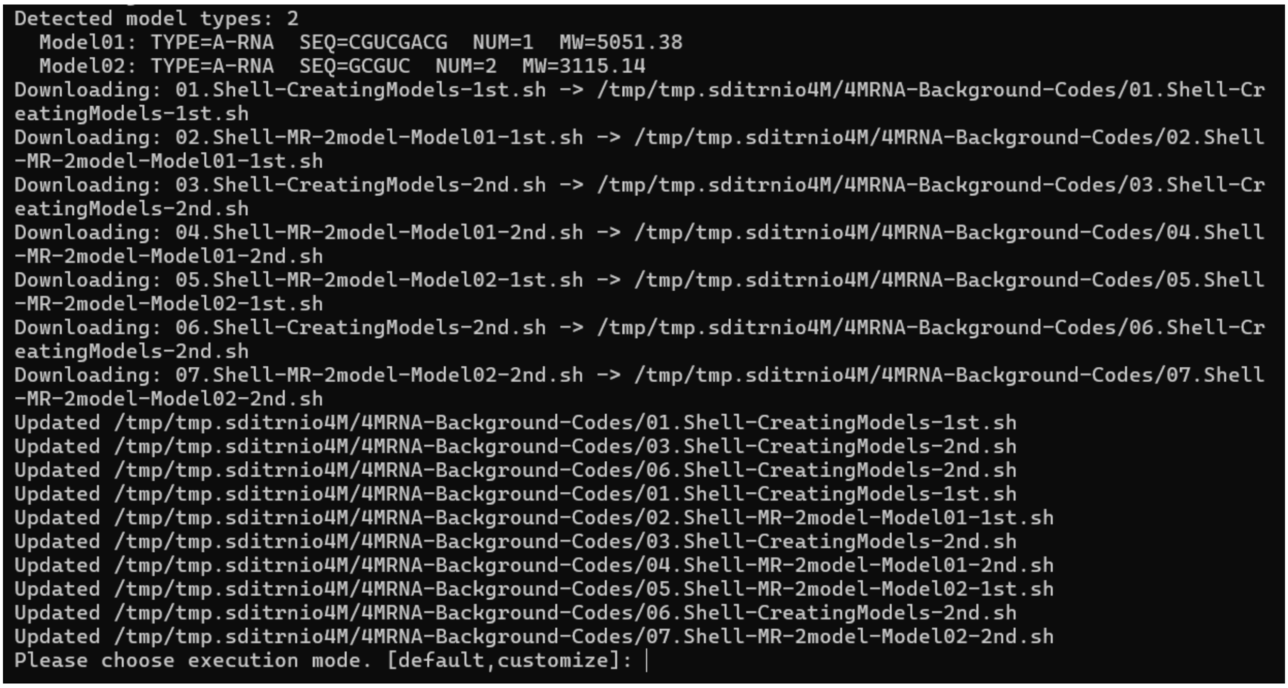
- Actually, for the command **4MRNA** to work, an input file called “**4MRNA-INP.txt**” is required. The user is prompted to choose from two options: (1) preparing the file yourself (a template will be provided), or (2) answering questions on the screen so that the program automatically generates it.
- Option **(2)** is strongly recommended. For option (1), refer to the supplementary notes in Section 3-3.
- If you choose option (2), several questions will be asked. Please answer them. Once all questions have been answered, the 4MRNA workflow will start automatically.

[e.g.] The figure below shows an example of molecular replacement using two different RNAs as models. In this example, during molecular replacement, one copy of the first model and two copies of the second model are searched for in the asymmetric unit of the crystal.



```
Input file '4MRNA-INP.txt' not found.
Choose one of the following:
  1) I will prepare it myself. (A template will be provided.)
  2) I will answer questions here to generate it now.
Select [1/2]: 2
The number of models for molecular replacement [1,2,3]: 2

Enter information of model #1.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): CGUCGACG
How many copies of the model to be searched in molecular replacement: 1

Confirm model #1:
-TYPE1 A-RNA
-SEQ1 CGUCGACG
-NUM1 1
Is this correct? [Y/N]: Y

Enter information of model #2.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): GCGUC
How many copies of the model to be searched in molecular replacement: 2

Confirm model #2:
-TYPE2 A-RNA
-SEQ2 GCGUC
-NUM2 2
Is this correct? [Y/N]: Y
```

- When prompted with “Please choose execution mode.”, please enter “**customize**”.



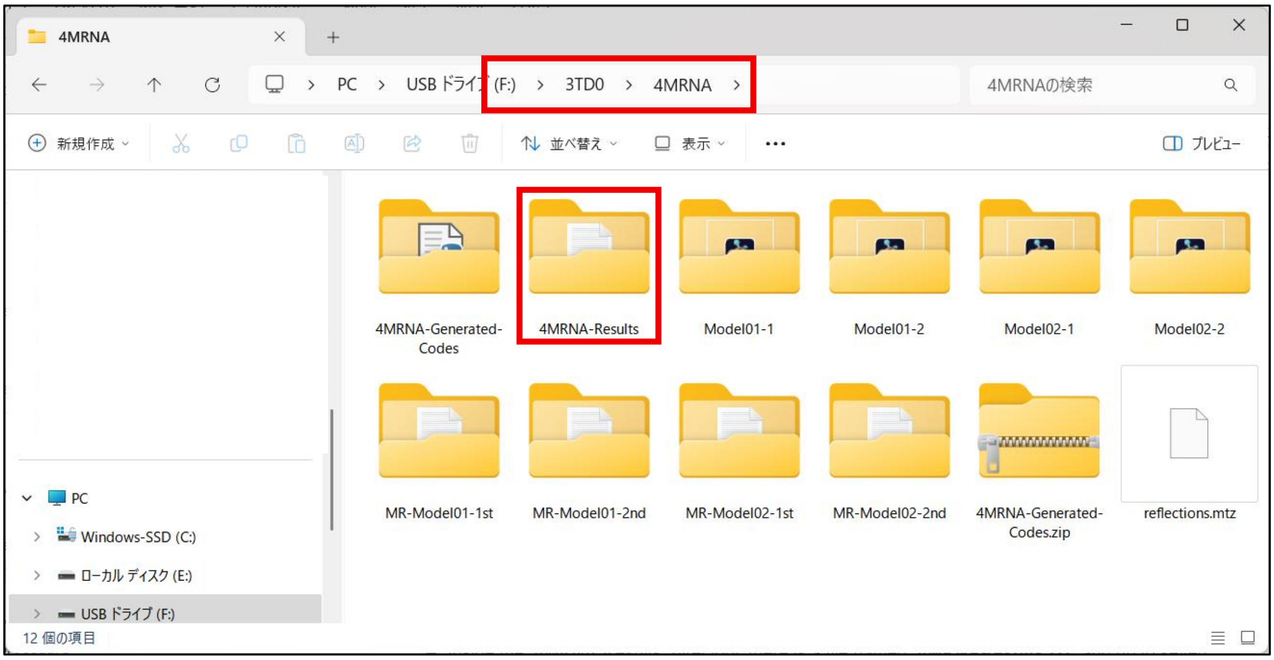
```
Detected model types: 2
  Model01: TYPE=A-RNA  SEQ=CGUCGACG  NUM=1  MW=5051.38
  Model02: TYPE=A-RNA  SEQ=GCGUC  NUM=2  MW=3115.14
Downloading: 01.Shell-CreatingModels-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-Cr
eatingModels-1st.sh
Downloading: 02.Shell-MR-2model-Model01-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/02.Shell
-MR-2model-Model01-1st.sh
Downloading: 03.Shell-CreatingModels-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-Cr
eatingModels-2nd.sh
Downloading: 04.Shell-MR-2model-Model01-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/04.Shell
-MR-2model-Model01-2nd.sh
Downloading: 05.Shell-MR-2model-Model02-1st.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/05.Shell
-MR-2model-Model02-1st.sh
Downloading: 06.Shell-CreatingModels-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-Cr
eatingModels-2nd.sh
Downloading: 07.Shell-MR-2model-Model02-2nd.sh -> /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/07.Shell
-MR-2model-Model02-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-CreatingModels-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/01.Shell-CreatingModels-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/02.Shell-MR-2model-Model01-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/03.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/04.Shell-MR-2model-Model01-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/05.Shell-MR-2model-Model02-1st.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/06.Shell-CreatingModels-2nd.sh
Updated /tmp/tmp.sditrnio4M/4MRNA-Background-Codes/07.Shell-MR-2model-Model02-2nd.sh
Please choose execution mode. [default,customize]: |
```

- The codes running in the background of 4MRNA are downloaded as a folder named “**4MRNA-Background-Codes**”.
- Basically, you only need to run the codes in the order indicated by the numbers at the beginning of each file, and the entire 4MRNA workflow will be completed. Please open Ubuntu or Terminal, change to the directory “4MRNA-Background-Codes”, and execute the code as follows.

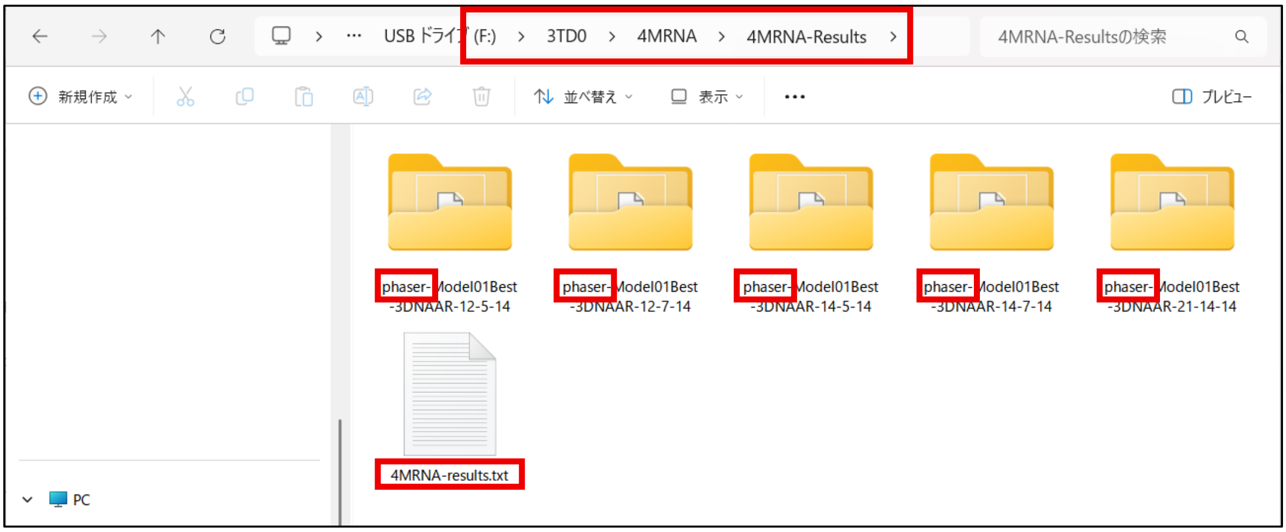
```
bash <file name>.sh
```

- However, it is also possible to review and modify the contents of each file if necessary.

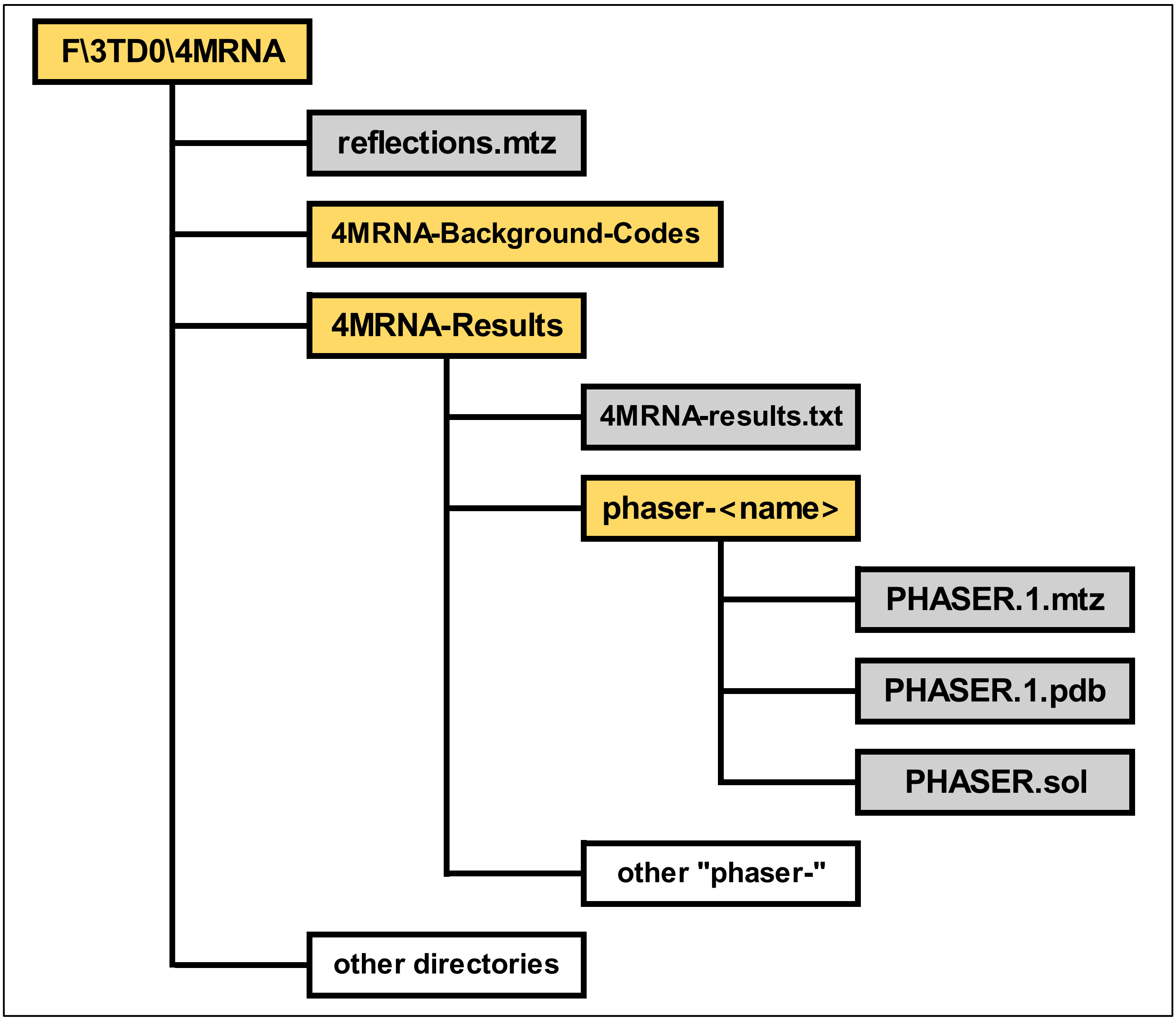
- After all the codes have finished running, a directory named “**4MRNA-Results**” will be created.



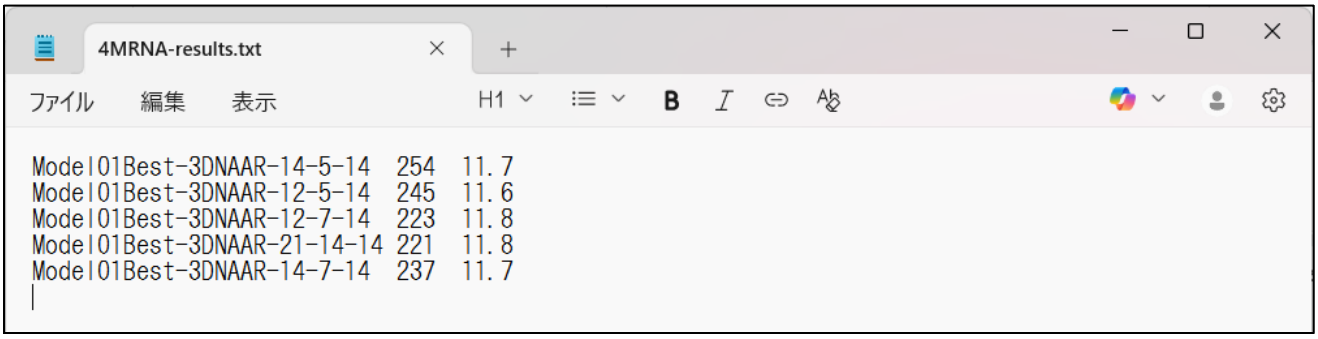
- Inside the “4MRNA-Results” directory, there is a file named “**4MRNA-results.txt**” and up to seven subdirectories. Each subdirectory name begins with “**phaser-**” and contains the results of molecular replacement (MR).



- Inside each “phaser-” subdirectory, there is a structural file named “**PHASER.1.pdb**.” This file is a solution of molecular replacement, that is, a candidate for the correct structure.
- The following figure is an example of the final directory structure.



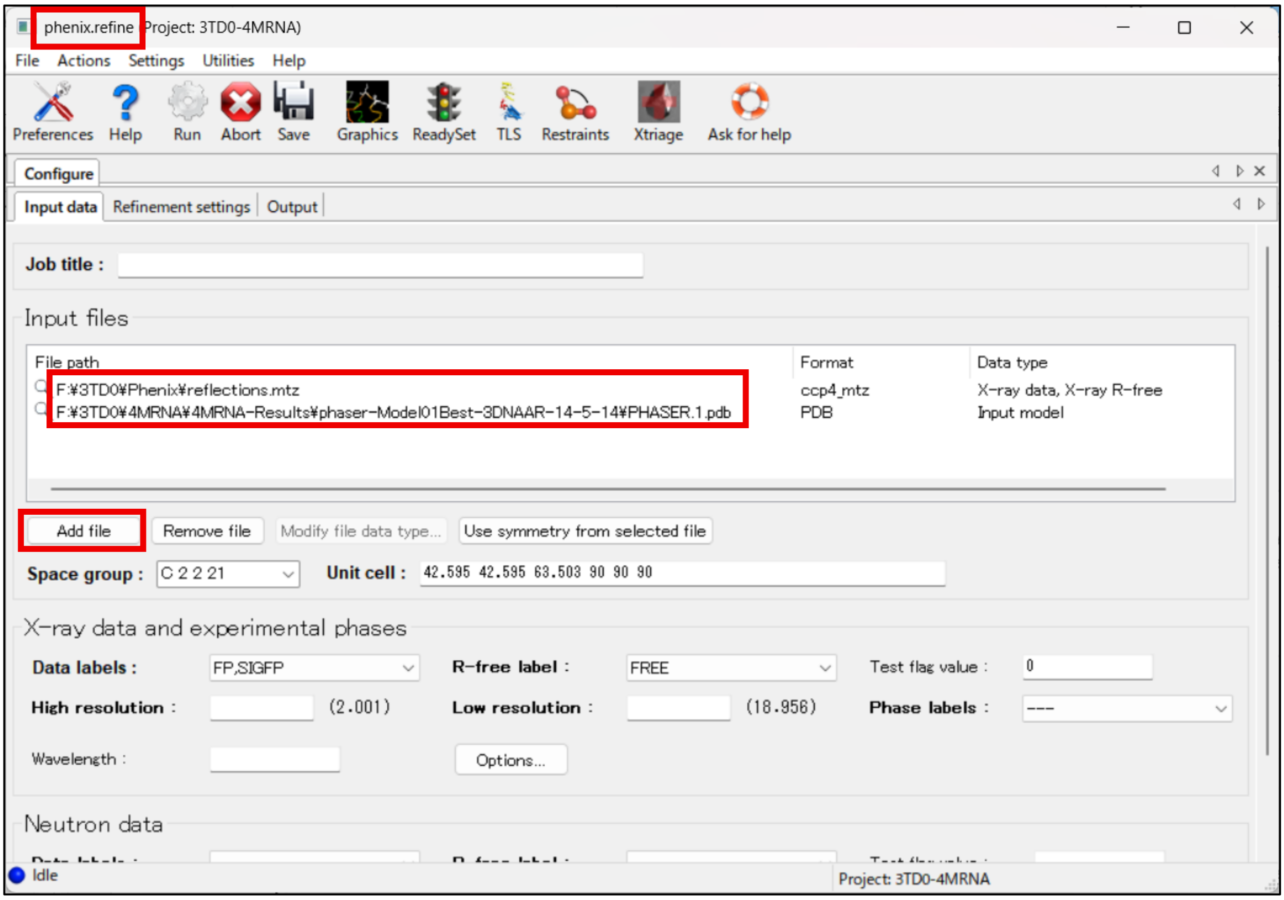
- Each run of molecular replacement produces a solution, but it must be validated against the experimental data (electron density) to confirm its correctness. This validation is guided by two statistical values, **LLG** and **TFZ**. The program is designed to automatically select up to seven solutions with the most favorable statistics. These statistics are compiled in “**4MRNA-results.txt**.” Each line in this file lists the ID of molecular replacement (i.e. the name of directory) , LLG, and TFZ in that order.



```
Model01Best-3DNAAR-14-5-14  254  11.7
Model01Best-3DNAAR-12-5-14  245  11.6
Model01Best-3DNAAR-12-7-14  223  11.8
Model01Best-3DNAAR-21-14-14 221  11.8
Model01Best-3DNAAR-14-7-14  237  11.7
```

- With reference to the statistics provided in “**4MRNA-results.txt**,” open the structural file (“**PHASER.1.pdb**”) together with the electron density map (“**PHASER.1.mtz**”) in software like Coot. By overlaying them, decide which of the up to seven candidate solutions is the best.
- After the optimal structure has been determined, it will be used as the initial structure for refinement.

[e.g.] For example, if the structure with the molecular replacement ID “Model01Best-3DNAAR-14-5-14” (i.e., “PHASER.1.pdb” within the directory “phaser-Model01Best-3DNAAR-14-5-14”) is decided as the initial structure, you should add this file. In addition, add the reflection file (“reflections.mtz”).



File path	Format	Data type
F:¥3TD0¥Phenix¥reflections.mtz	ccp4_mtz	X-ray data, X-ray R-free
F:¥3TD0¥4MRNA¥4MRNA-Results¥phaser-Model01Best-3DNAAR-14-5-14¥PHASER.1.pdb	PDB	Input model

Space group : C 2 2 21
Unit cell : 42.595 42.595 63.503 90 90 90

Data labels : FP,SIGFP
R-free label : FREE
Test flag value : 0
High resolution : (2.001)
Low resolution : (18.956)
Phase labels : ---
Wavelength :

5. Notes

5-1. Supported environment

4MRNA can run in any environment that supports shell scripts and the external software packages 3DNA and Phaser. It has been tested on the following operating systems.

- Windows (via Windows Subsystem for Linux, Ubuntu)
- macOS
- Linux (Rocky Linux)

5-2. License information

- **4MRNA itself is distributed under the MIT License.** This means that it can be used completely freely, provided that the copyright notice is included.
- However, please pay attention to the licenses of the dependencies. 4MRNA relies on several external resources, including 3DNA, Phenix, and CCP4/Phaser, but these components are not included in the distribution of 4MRNA. Users are responsible for installing them separately and complying with their respective licenses.
- A summary of these dependencies is provided in “DEPENDENCIES.txt” on GitHub.

5-3. Citation

If you use 4MRNA in your research, please cite the following reference. In addition, please also cite the external software packages used by 4MRNA.

- **4MRNA:** The manuscript describing 4MRNA is currently in preparation. Until it becomes available, please cite the following GitHub repository: https://github.com/S-Ando-Biophysics/4MRNA.
- **3DNA:** Lu, X. J., & Olson, W. K. (2008). 3DNA: a versatile, integrated software system for the analysis, rebuilding and visualization of three-dimensional nucleic-acid structures. *Nature protocols*, *3*(7), 1213–1227. https://doi.org/10.1038/nprot.2008.104
- **Phenix:** Liebschner, D., Afonine, P. V., Baker, M. L., Bunkóczi, G., Chen, V. B., Croll, T. I., Hintze, B., Hung, L. W., Jain, S., McCoy, A. J., Moriarty, N. W., Oeffner, R. D., Poon, B. K., Prisant, M. G., Read, R. J., Richardson, J. S., Richardson, D. C., Sammito, M. D., Sobolev, O. V., Stockwell, D. H., … Adams, P. D. (2019). Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology*, *75*(Pt 10), 861–877. https://doi.org/10.1107/S2059798319011471
- **Phaser:** McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., & Read, R. J. (2007). Phaser crystallographic software. *Journal of applied crystallography*, *40*(Pt 4), 658–674. https://doi.org/10.1107/S0021889807021206

6. References

[1] Bernard, C., Postic, G., Ghannay, S., & Tahi, F. (2025). Has AlphaFold3 achieved success for RNA?. *Acta crystallographica. Section D, Structural biology*, *81*(Pt 2), 49–62.

[2] Kwon D. (2025). RNA function follows form - why is it so hard to predict?. *Nature*, *639*(8056), 1106–1108.

[3] Kondo, J., Urzhumtseva, L., & Urzhumtsev, A. (2008). Patterson-guided ab initio analysis of structures with helical symmetry. *Acta crystallographica. Section D, Biological crystallography*, *64*(Pt 10), 1078–1091.

[4] Lu, X. J., & Olson, W. K. (2008). 3DNA: a versatile, integrated software system for the analysis, rebuilding and visualization of three-dimensional nucleic-acid structures. *Nature protocols*, *3*(7), 1213–1227.