

## multiPrime2: Designing degenerate primers for maximum coverage with minimal degeneracy.

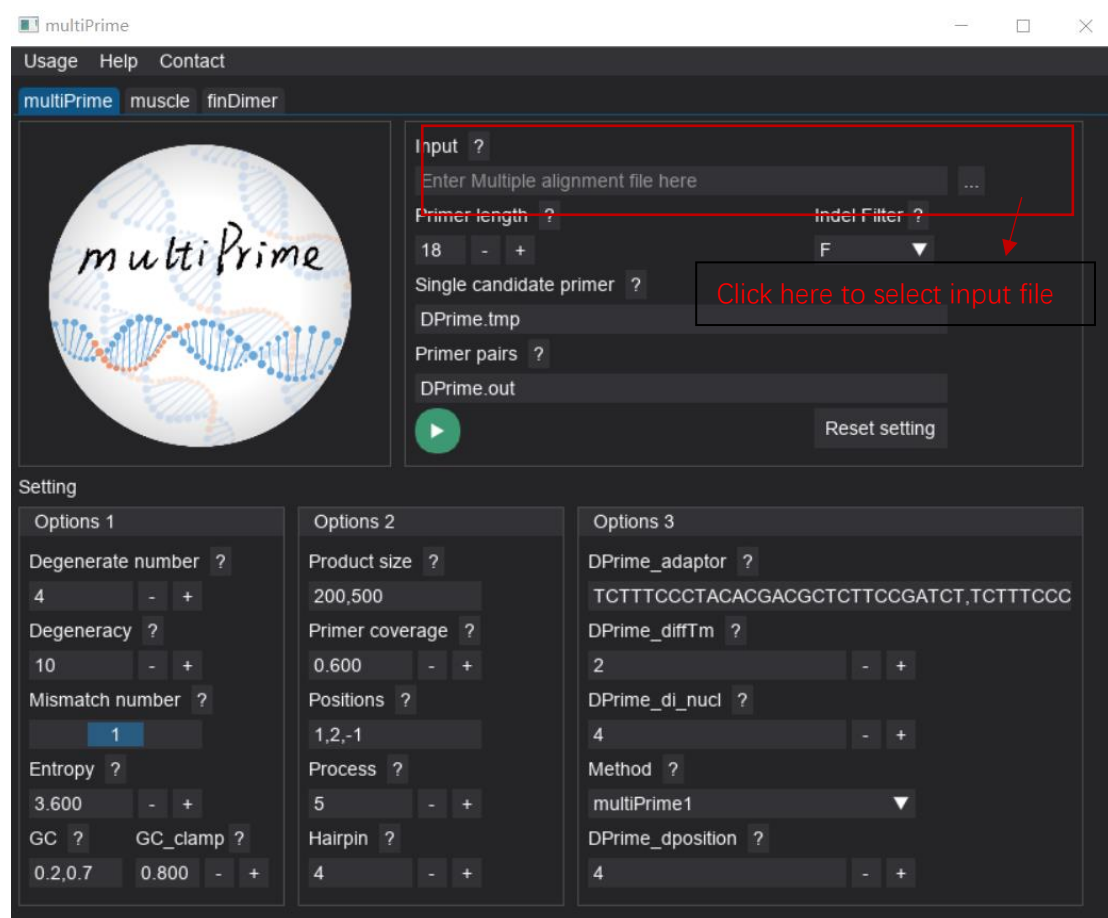
Welcome to multiPrime2! MultiPrime2 is a user-friendly and one-step tool for designing degenerate primer pairs. It integrates degenerate primer design theory with mismatch handling, resulting in improved accuracy and specificity in detecting broad spectrum sequences. It outperformed conventional programs in terms of run time, primer number, and primer coverage. In the Method, the 'multiPrime1' approach represents a local optimum solution, resembling the core primer design module of multiPrime, available at <https://github.com/joybio/multiPrime>. In contrast, 'multiPrime2', available at <https://github.com/joybio/multiPrime2-GUI>, stands as a global optimum solution, capable of achieving maximum primer coverage with minimal degeneracy.

There are two versions of multiPrime2: the web-based version, accessible at <https://multiprime.cn/>, and the GUI-based version, multiPrime2 GUI.exe. The server and GUI version of multiPrime2 can be freely utilized without the need for login credentials.

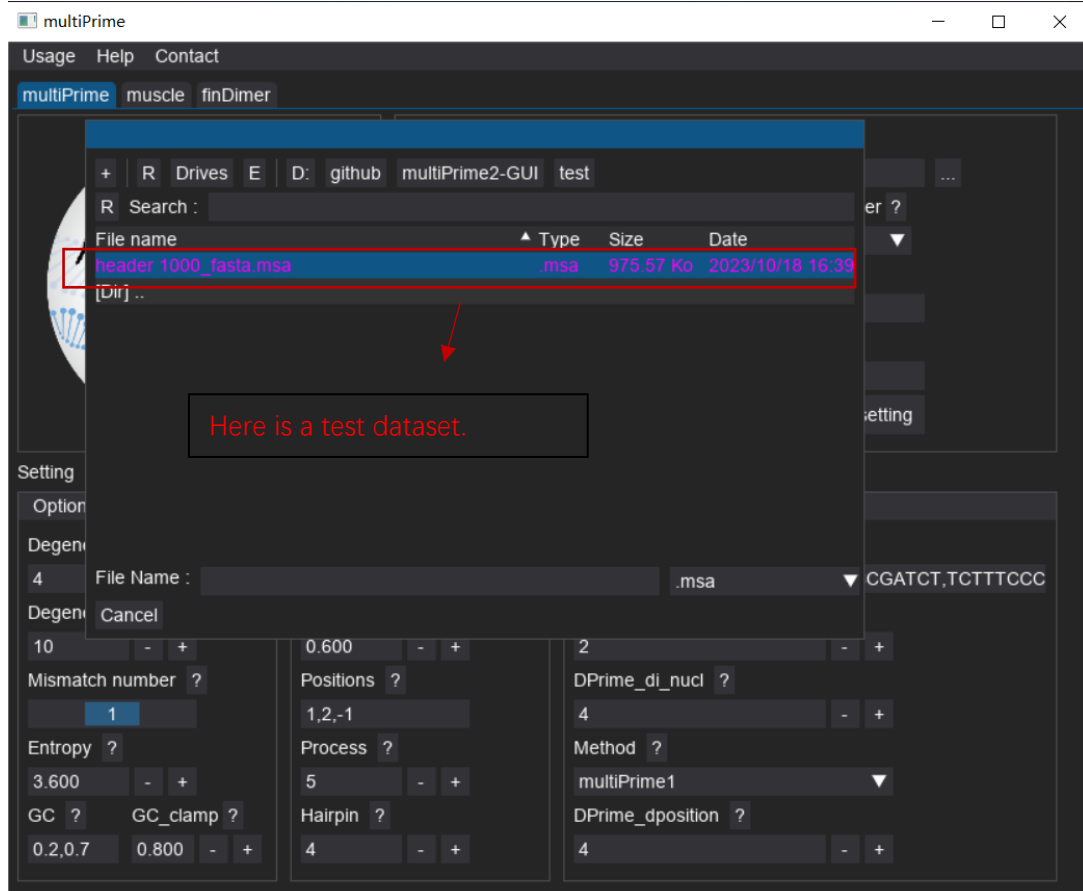
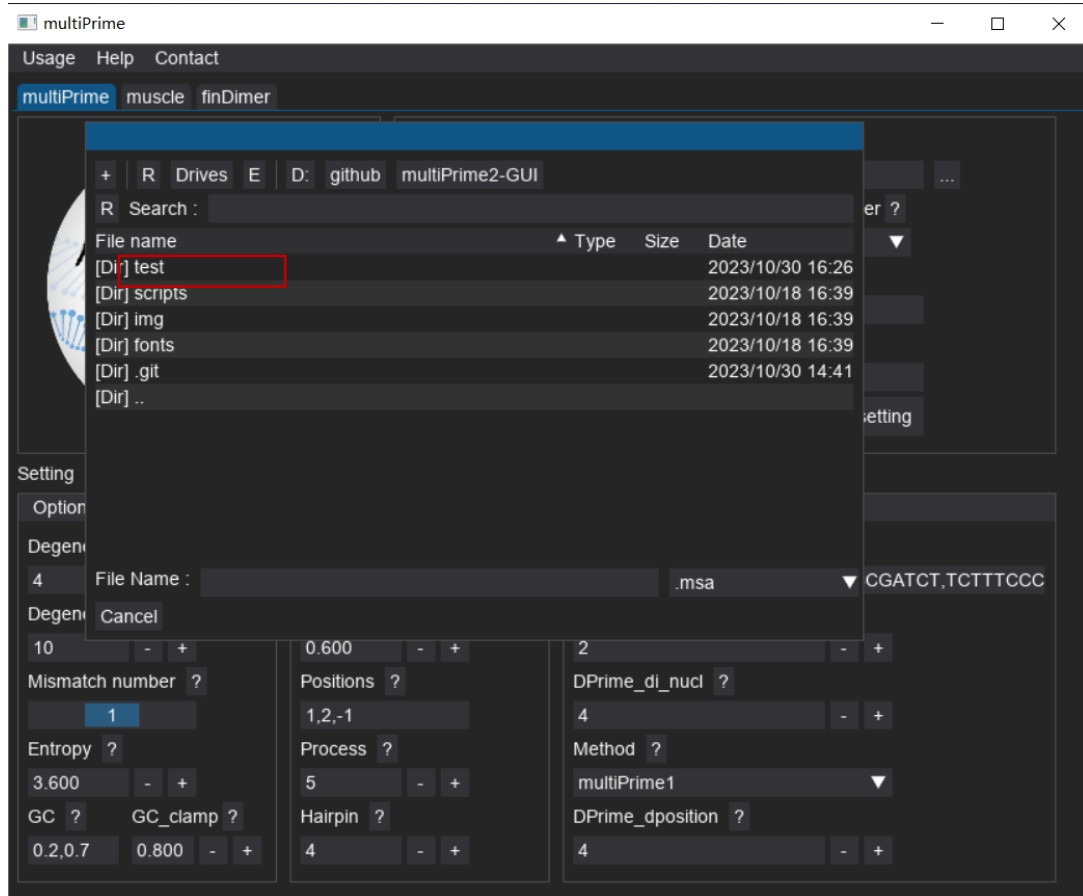
### How to Use GUI-based multiPrime

To utilize multiPrime2-GUI.exe effectively, follow these steps:

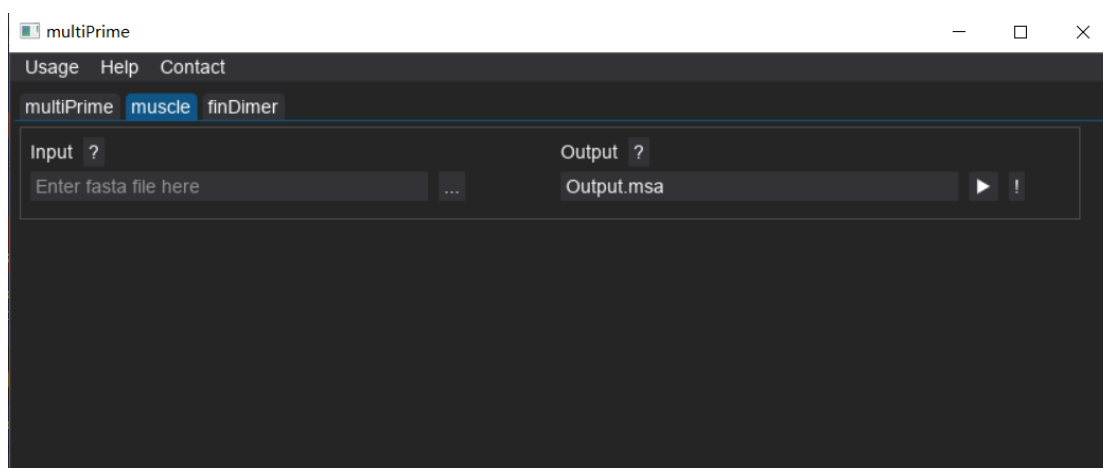
1. **Input Data:** Prepare your input data in a msa format (multiple alignment file). If you're unsure about the meaning of a parameter, simply click the question mark symbol (?), and it will provide you with an explanation.



Example:

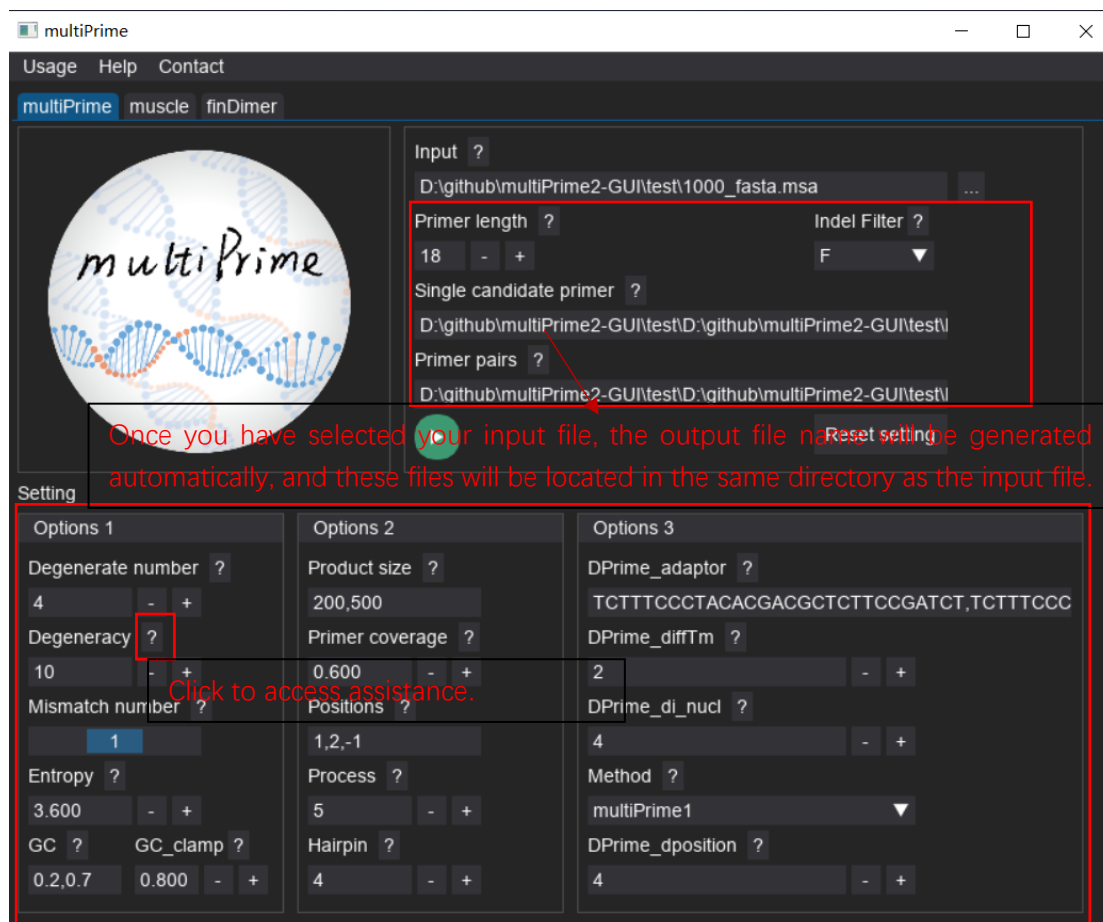


If you don't have multiple alignment file, you can run muscle in the muscle window.

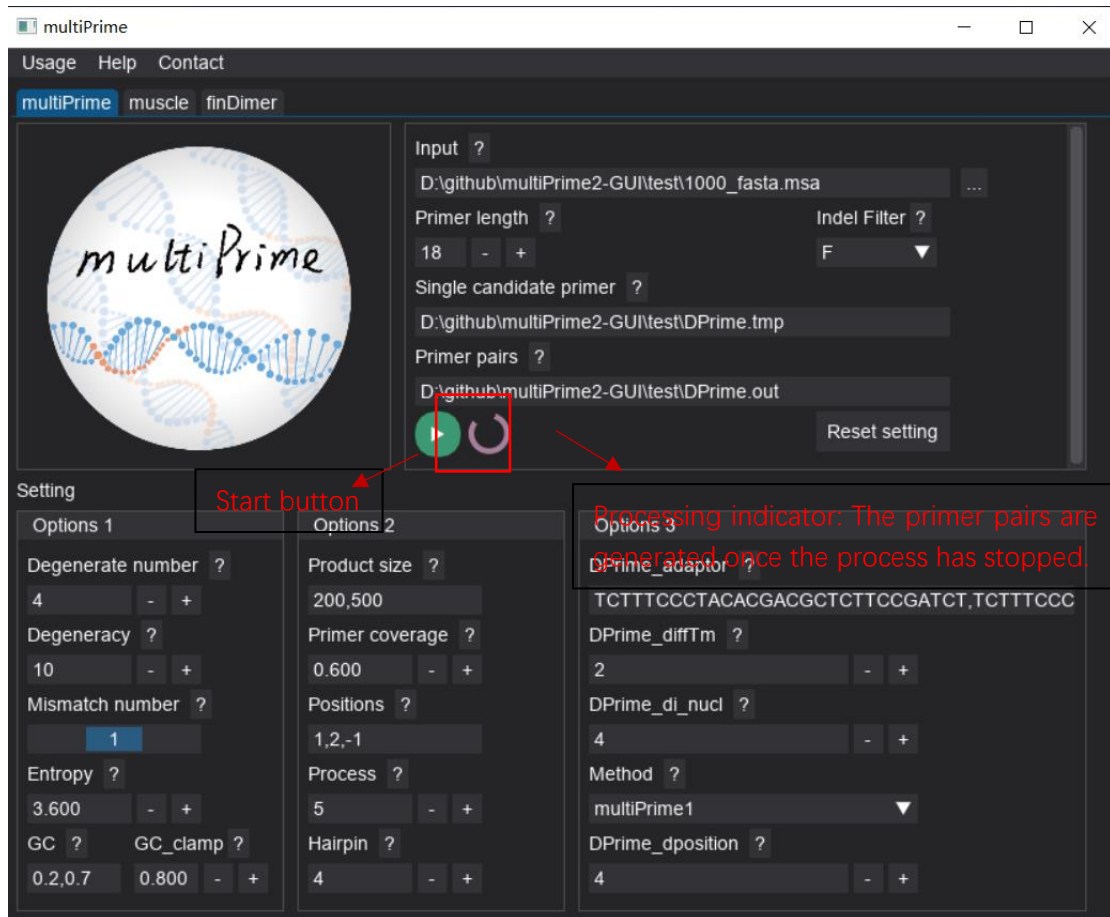


## 2. General Settings: Customize Options to Suit Your Preferences

In the use multiPrime2-GUI application, we understand the importance of flexibility in tailoring the tool to your specific needs. As such, all the available options can be easily configured according to your preferences. You have the freedom to set them as you see fit. Empower yourself with complete control over the tool's behavior, allowing for seamless integration into your research and analysis workflows.



## 3. Primer Pair Generation: Run multiPrime2-GUI.exe on your input data to initiate the primer pair design module. The tool will use its mismatch-tolerant approach to generate highly specific and effective degenerate primer pairs.

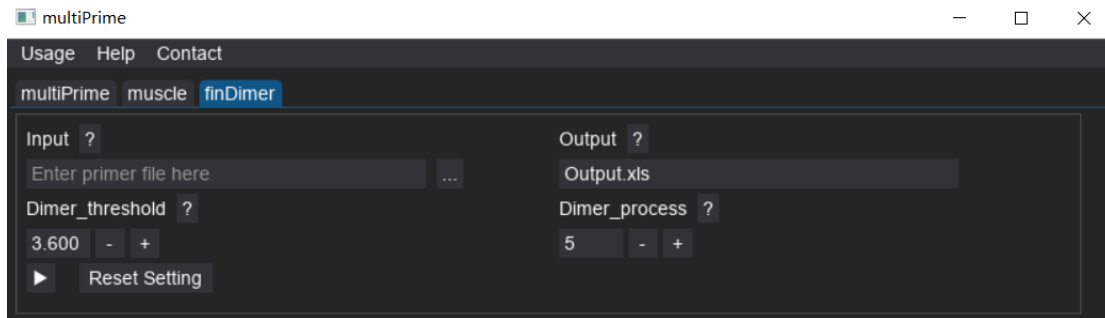


4. **Analyze Results:** Once the primer design step is complete, carefully review the results, including the generated primer pairs, PCR product lengths, melting temperatures, and other relevant factors.
5. **Result Interpretation:** The multiPrime2-GUI.exe generates four result files, including a temporary file (\*.tmp), two JSON files (\*.json), and one Excel file (\*.xls). The temporary file records the best candidate primers for each position, which can be viewed using Excel. The JSON files record the sequences ID that each primer at each position can capture and the sequences they cannot capture. The Excel file, on the other hand, contains information about the candidate primer pairs.

Example (.xls file):

Primer_F_seq	Primer_R_seq	Product length.Tm:coverage_percentage	Target nui	Primer_start_end	Uncovered ID
GACCTCAAYHACACATTG	YTGACGYCTCATYTCTTT	295.49.7:0.948	948	61:355	{>AB008235.1'
GACCTCAAYHACACATTG	RYTGACGTCTCATYTCTT	296.49.5:0.939	939	61:356	{>MF940629.1'
CGACCTCAAYHACACATT	YTGACGYCTCATYTCTTT	296.50.49:0.937	937	60:355	{>AB196492.1'
YCCWRGAATGGCGTATTT	YTGACGYCTCATYTCTTT	230.51.02:0.93	930	126:355	{>AB008235.1'
CGACCTCAAYHACACATT	RYTGACGTCTCATYTCTT	297.50.3:0.928	928	60:356	{>MF940629.1'
YCCWRGAATGGCGTATTT	RYTGACGTCTCATYTCTT	231.50.82:0.922	922	126:356	{>MF940629.1'
GATYCWGAATGGCGTAT	YTGACGYCTCATYTCTTT	232.50.36:0.683	683	124:355	{>AB326963.1'
GATYCWGAATGGCGTAT	RYTGACGTCTCATYTCTT	233.50.16:0.675	675	124:356	{>AB326963.1'
GACCTCAAYHACACATTG	ACAAYTCTRTCCAKCTGT	276.49.77:0.671	671	61:336	{>MN066873.1'
YCCWRGAATGGCGTATTT	ACAAYTCTRTCCAKCTGT	211.51.08:0.666	666	126:336	{>MN066873.1'
CGACCTCAAYHACACATT	ACAAYTCTRTCCAKCTGT	277.50.56:0.659	659	60:336	{>MN066873.1'
WAAGYTRCCGACCTCAA	ACAAYTCTRTCCAKCTGT	286.51.47:0.657	657	51:336	{>MN066873.1'
GATYCWGAATGGCGTAT	YARCTCAACCTCTYACAT	446.49.86:0.603	603	124:569	{>AB326963.1'

6. **Dimer examination:** We have also integrated a dimer examination tool into the multiPrime2-GUI.exe. This tool accepts FASTA files and provides the capability to assess the potential for dimer formation within the FASTA sequences.



## How to Use Web-based multiPrime2

To utilize Web-based multiPrime2 effectively, follow these steps:

**A**

1. Input

2. Parameters

3. Run

**B**

4. Primer pairs

4. Coverage of primer pairs

Other information is the same as that provided by the GUI-based multiPrime2.

## System Requirements

- Operating System: Windows
- CPU: NA
- RAM: NA
- Disk Space: NA

## Dependencies

Please ensure you have the following dependencies installed on your system before using multiPrime2-GUI.exe:

- NA

## Installation and Execution

- NA

## Support and Contact

If you encounter any issues, have questions, or need further assistance, please don't hesitate to

contact me at [[yang\\_junbo\\_hi@126.com](mailto:yang_junbo_hi@126.com)] or [[1806389316@pku.edu.cn](mailto:1806389316@pku.edu.cn)]. We value your feedback and are committed to enhancing the multiPrime for a better user experience.

### **Acknowledgments**

We would like to express our gratitude to all contributors and developers who have made the multiPrime2-GUI pipeline possible. Your dedication and support have been invaluable in creating this powerful tool for the scientific community.

### **License**

The MIT License (MIT)

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Thank you for choosing multiPrime2-GUI! We hope this tool proves to be an invaluable asset in your target sequence detection endeavors using tNGS. Happy sequencing!