



# Novofinder Manual

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The image shows the Novofinder software interface, which is a web-based application for gene analysis. The interface is divided into several sections: "Project Build" at the top, "Gene List Input" in the middle, and "Properties Filter" at the bottom. The "Project Build" section includes fields for "Project Name" and "Analyze Results Directory", with a "Browse" button next to the latter. The "Gene List Input" section has three tabs: "FromUser", "FromFile", and "FromFilter". Below these tabs are input fields for "Sample", "Compare Group", "Annotation", "GO ID", "GO KeyWords", and "Other KeyWords", along with a "Filter" button. The "Properties Filter" section has five tabs: "Gene Annotation", "Gene Expression", "Sequence", "Differentially Expressed", and "Enrichment Analysis". The "Gene Annotation" tab is selected, showing a list of checkboxes for various gene properties: "Gene Info", "Swissprot Info", "GO Biological Pathway", "BP Description", "GO Molecular Function", "MF Description", "GO Cellular Component", and "CC Description". Each checkbox has a corresponding "Select All" and "Select Invert" button. At the bottom of the interface, there is a "One Key Start" button.

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## 1 Novofinder Description

Novofinder is a powerful software of RNA-seq designed by RNA team in Novogene, which can be used in browsing and integrating your complicated RNA-seq results. Novofinder can present gene sequence, marker, annotation, gene expression result, differential expression analysis result and functional enrichment result, basing on a user defined gene list and filter. We hope Novofinder can efficiently and effectively deliver customer services.

## 2 Novofinder Instruction

### 2.1 Start

Open Novofinder → Typing your project name: Project Name (The name of result folder) → Loading the results address by “Browse” button.

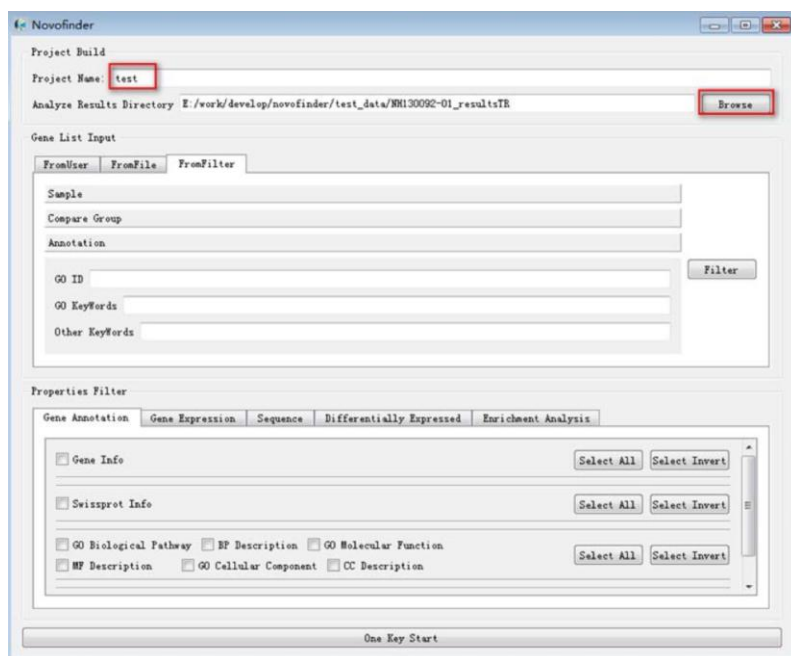


Figure 2.1

After opening Novofinder, Figure 2.1

1. Put your Project Name in corresponding box, For example, “test”, inquiry results will be saved to folder named as “test”;
2. In Analysis Results Directory box, you can load the address of results through “Browse”. For example: E:/work/develop/Novofinder/test\_data/NH130092-01\_results\_TR

Thus, the result will be saved under directory:

C:/Users/Administrator/Desktop/NH130776\_Sansejin\_noRef\_DGE\_results/NH130776\_Sansejin\_DGE\_results/test/

#### Tips:

Please load the address after entering the project name.

## 2.2 Gene List Input

To make the Input more flexible, Novofinder provide three different methods of gene list input.

### 2.2.1 Manually paste your target gene list

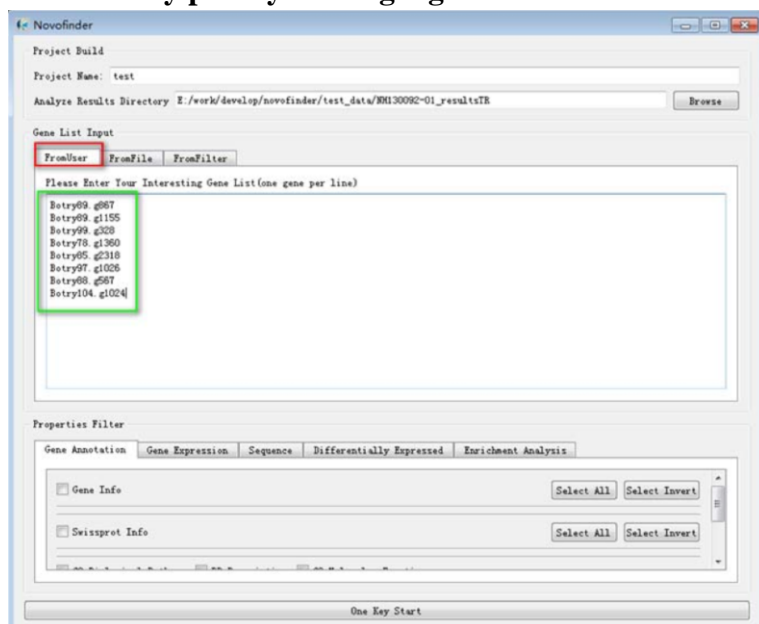
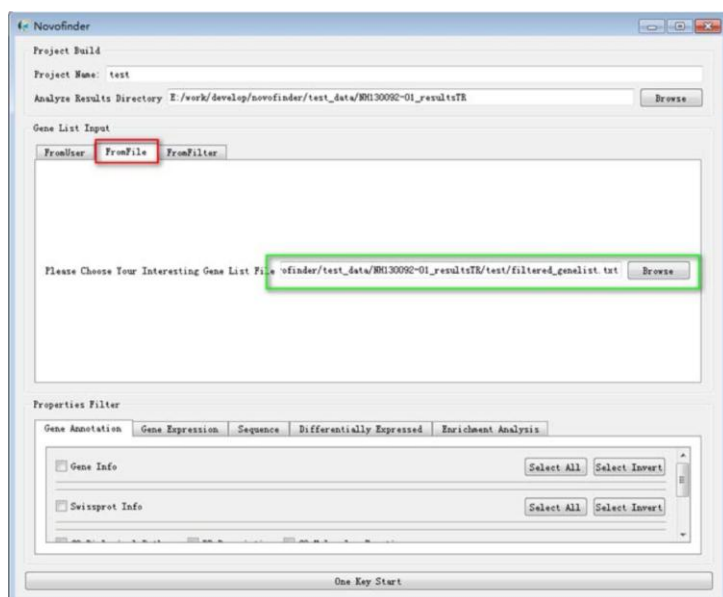


Figure 2.2.1

1. Under Gene List Input selection box, choose “From User”, Figure 2.2.1;

2. In the area indicated as green box in Figure 2.2.1, you can put your target gene list, one gene in a line.

### 2.2.2 Load existing gene list file



If you already have a gene list file, you can load it directly.

1. Under Gene List Input selection box, choose From File, as Figure 2.2.2 red box;

2. In Figure 2.2.2 green box, choose your gene list file through “Browse”.

**Tip:**

Gene list file should be in txt format, and one gene for a line.

Figure 2.2.2

### 2.2.3 User defined gene screening

Customer can set several criteria to get a user defined gene list

1. Under Gene List Input selection box, choose FromFilter;
2. Setting Gene function or a threshold of log2foldchange and qvalue in gene expression level
3. Click “Filter”, generated gene list file will be saved in result folder;
4. After filtering, Novofinder will tell the total number of genes and the result address as Figure 2.2.3. You can open the result file by click “Open Gene list Dir”, or close by click “OK”.

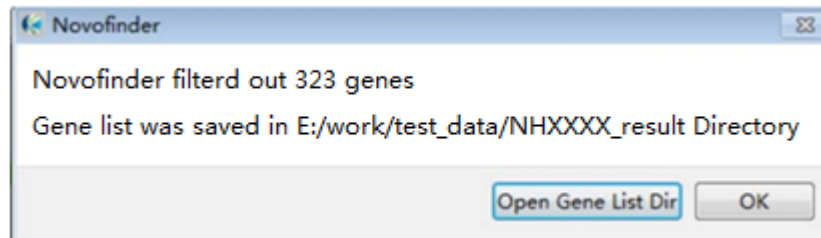


图 2.2.3

Tip: Select ALL means select all genes; Select Invert means a inverse selection.

### 2.2.3.1 Select gene according to expression level in samples

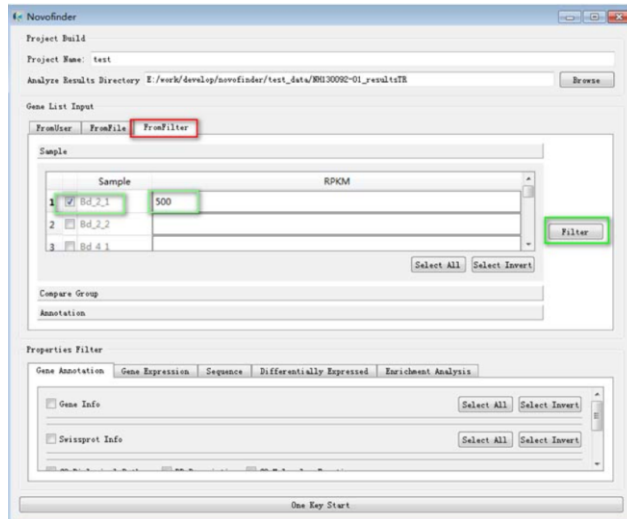


Figure 2.2.3.1

If you want get genes with certain abundance, you can set criteria on expression level

1. Under FromFilter, click Sample, filter gene according to expression, as Figure 2.2.3.1;
2. As Figure 2.2.3.1 green box, set criteria: choose sample name, and enter FPKM threshold.

As the figure: The result will be the genes of which FPKM are above 500 in sample.

### 2.2.3.2 Select gene according to log2foldchange and qvalue

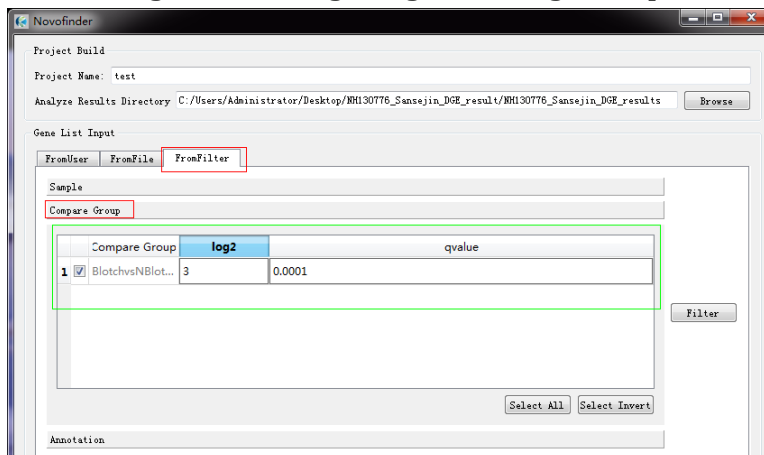


Figure 2.2.3.2

If you want get gene list with certain log2foldchang and qvalue, you can set filter as well.

1. Under FromFilter, click Compare Group, choose the group you compare, as Figure 2.2.3.2;
2. In Figure 2.2.3.2 green box area, settle down the cirteria: choose the group, enter the threshold of log2foldchange and qvalue.

As figure: The results will be the gene with  $\log_2 > 3$  and  $qvalue < 0.0001$ .

### 2.2.3.3 Select gene according to gene function

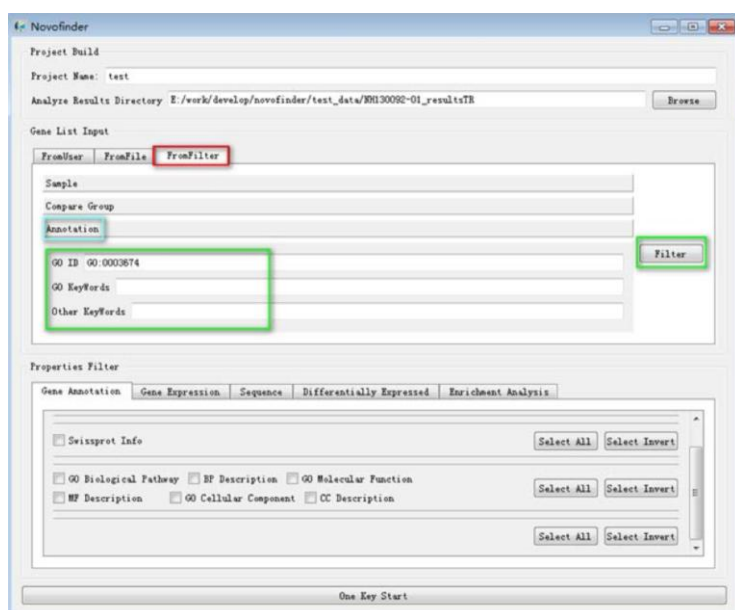


Figure 2.2.3.3

Tip: if sample、group、Annotation are selected simultaneously, the output will be genes satisfying all the criteria.

If you want to get gene with certain biological function, you can set the filter manually.

1. Under FromFilter, click Annotation, choose Annotation, as Figure 2.2.3.3 red box;
2. In Figure 2.2.3.3 green box area, you can set the criteria: Input could be target GO ID or KEGG ID, also, it can be some key words.

Tip: Be careful about key words, in case you get no output.

For example: enter “membrane”, You will get genes with description containing membrane.

## 2.3 Get results output

According to the gene list (2.2), you can obtain the sequences, the marker, the annotation, the expression level, the differential analysis result and the enrichment analysis results of genes in list.

1. In Properties Filter, choose the result terms you want (Table 2.3, the description of result term):

Table 2.3

Gene Annotation	Gene Function Annotation Result (Gene Info (location and length)/Swissprot/GO database annotation)
Gene Expression	Gene Expression Quantification Result
Sequence	Gene CDS sequence
Differentially Expressed	Results of differential analysis result
Enrichment Analysis	Functional Enrichment Result, including: GO enrichment, KEGG enrichment

2. Choose specific results under each result term;
3. Click “One key start”, Novofinder will generate the results and save it to your project result folder.
4. In the end, there will be a pop out box indicated you finished the inquiry, as figure 2.3. click Open Gene List Dir to open the result directory, click OK to close it.



Figure 2.3

### 2.3.1 Get gene annotation result

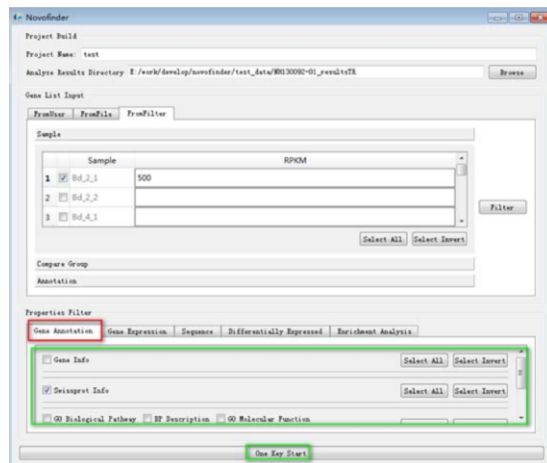


Figure 2.3.1

### 2.3.2 Get gene expression result

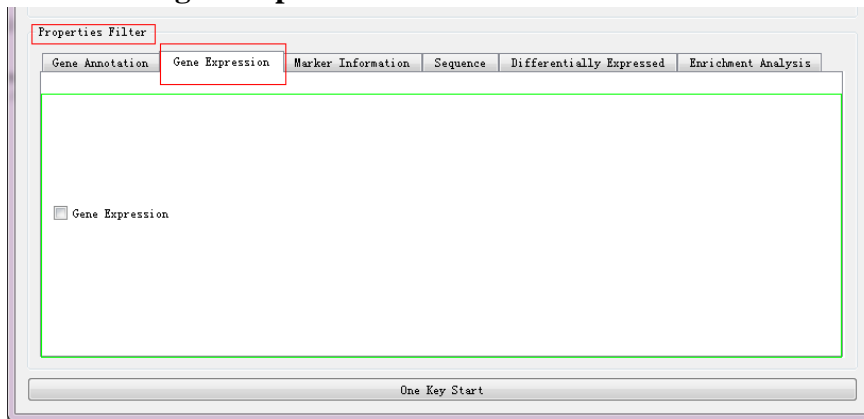


Figure 2.3.2

### 2.3.3 Get gene sequence

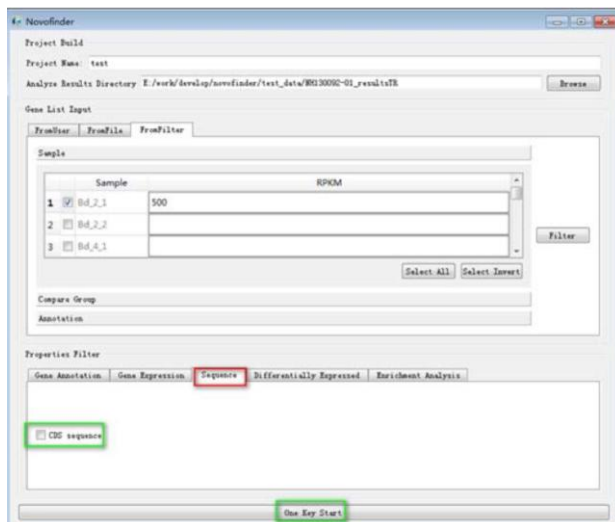


Figure 2.3.4

If customer want to get the gene annotation results:

1. Go to Properties Filter, choose the Gene Annotation, as Figure 2.3.1 red box;
2. In figure 2.3.1 green box, set the needed information, including length information and Swissprot and GO database annotation information.

If you want to get gene expression result:

1. Go to Properties Filter, click Gene Expression, as Figure 2.3.2 red box;
2. In Figure 2.3.2 green box area, Choose the gene expression information you want.

If you need the gene sequence results:

1. Go to Properties Filter, click Sequence, Figure 2.3.4 red box;
2. In Figure 2.3.4 green box area, set the needed sequence information, CDS sequence.

### 2.3.4 Get gene differential expression analysis results

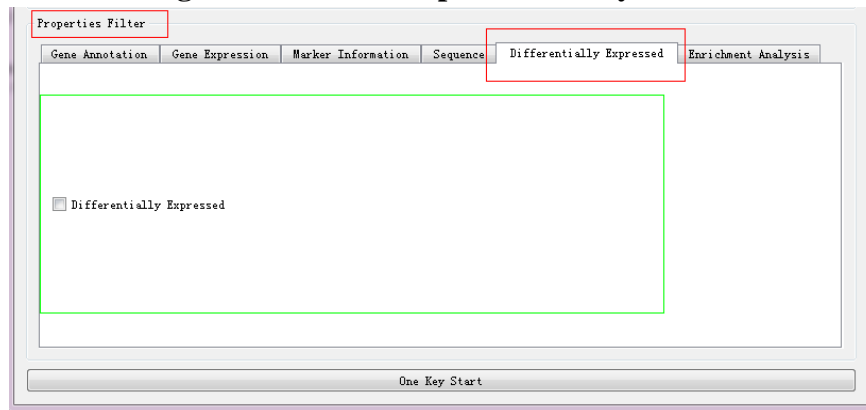


Figure 2.3.5

### 2.3.5 Get gene enrichment results

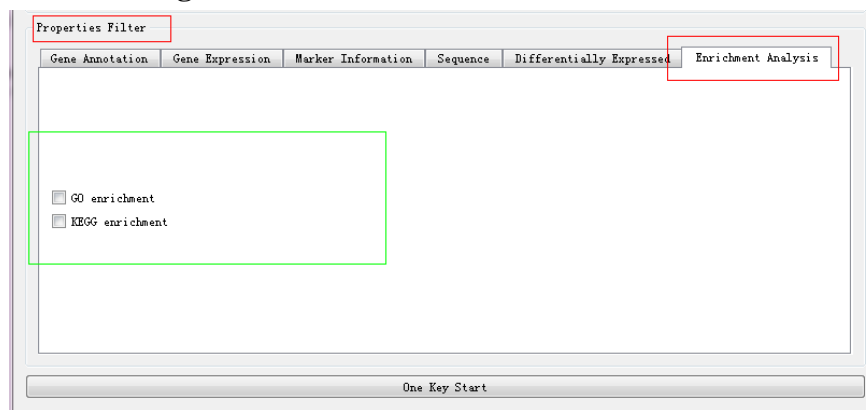


Figure 2.3.6

If you want the differential expression analysis results:

1. Go to Properties Filter, click red box Differentially Expressed, as Figure 2.3.5 red box;
2. In Figure 2.3.5 green box area, set the result you want.

**Tip:** All the comparison result will be integrated in one table.

If you want to get the gene enrichment result:

1. Go to Properties Filter, click Enrichment Analysis, as Figure 2.3.6 red box;
2. In Figure 2.3.6 green box area, choose the specific result you want, including GO enrichment and KEGG enrichment.