转基因棉花的差异表达蛋白分析

第6小组

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研究背景

差异表达蛋白的获得

UniProt数据库检索

Blast2GO软件分析

致谢

研究背景

- 转Bt基因抗虫棉：

  Bt基因：来源于苏云金芽孢杆菌（Bacill
  thuringiensis），编码产生一种毒蛋白，
  鳞翅目昆虫有很强的杀伤作用。

  转Bt基因农作物抵抗病虫害的能力大大提高。

- 转基因技术是一把"双刃剑"：

通过导入外源基因获得需要的遗传性状	VS	安全性问题：环境安全性 食品安全性

- 探究转Bt基因对陆地棉（Gossypium hirsutum）蛋白质组的影响。

研究背景

蛋白质组学方法：2-DE，MS

UniProt数据库，Blast2GO软件

转Bt基因抗虫棉

差异表达蛋白

蛋白功能分析

转基因对陆地棉蛋白质组的影响

差异表达蛋白的获得

- 利用蛋白质组学的方法（2-DE，MS）获得转Bt基因陆地棉的差异表达蛋白共18种：

Protein No.	Gene product and protein name	Accession No.in NCBI	Plant species
1	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	331690237	Hiraea wiedeana
2	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	2502002	Theobroma cacao
3	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	112408854	Heisteria densifrons
4	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	331690145	Doplopterys nutans
5	ribulose-1,5-bisphosphate carboxylase/oxygenase activase 1	12620881	Gossypium hirsutum
6	ribulose-1,5-bisphosphate carboxylase/oxygenase activase 2	12620883	Gossypium hirsutum
7	ribulose-bisphosphate carboxylase	4995197	Hildegardia barteri
8	ATP synthase CF1 alpha subunit	91208887	Gossypium hirsutum
9	ATP synthase CF1 alpha subunit	114107118	Daucus carota
10	ATP synthase beta chain	332105877	Gossypium arboreum
11	ATP synthase beta subunit	13236756	Reinhardtia simplex
12	ATP synthase beta subunit	7688411	Utricularia gibba
13	putative glutamine synthase 2	193290696	Capsicum annuum
14	hypothetical protein VITISV_027630	147791852	Vitis vinifera
15	hypothetical protein VOLCADRAFT_76573	302847369	Volvox carteri f.nagariensis
16	RecName: Full=Ribulose bisphosphate carboxylase small chain, chloroplastic; Short=RuBisCO small subunit; Flags: Precursor	1352821	
17	predicted protein	224138546	Populus trichocarpa
18	predicted protein	224157625	Populus trichocarpa

UniProt数据库检索

- 使用UniProt数据库对这18种蛋白质的相关信息进行检索：

UniProt数据库检索

Protein No.	Gene product and protein name	Accession No.in NCBI	Plant species
1	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	331690237	Hiraea wiedeana

- 以第一个蛋白为例:

UniProt数据库检索

- 重点关注biological process、molecular function和cellular component方面的信息：

UniProt数据库检索

- 18种差异表达蛋白的相关信息整理：

| Gene product and protein name	Plant species	biological process	molecular function	cellular component
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Hiraea wiedeana	Photosynthesis	magnesium ion binding/ribulose-bisphosphate carboxylase activity	chloroplast
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Theobroma cacao	Photosynthesis	magnesium ion binding/ribulose-bisphosphate carboxylase activity	chloroplast
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Heisteria densifrons	Photosynthesis	magnesium ion binding/ribulose-bisphosphate carboxylase activity	chloroplast
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	Doplopterys nutans	Photosynthesis	magnesium ion binding/ribulose-bisphosphate carboxylase activity	chloroplast
ribulose-1,5-bisphosphate carboxylase/oxygenase activase 1	Gossypium hirsutum		ATP binding	
ribulose-1,5-bisphosphate carboxylase/oxygenase activase 2	Gossypium hirsutum		ATP binding	
ribulose-bisphosphate carboxylase	Hildegardia barteri	Photosynthesis /carbon fixation	magnesium ion binding/ribulose-bisphosphate carboxylase activity	chloroplast
ATP synthase CF1 alpha subunit	Gossypium hirsutum	ATP synthesis/Hydrogen ion transport/Ion transport/Transport	Hydrolase	CF/Chloroplast/Membrane/Plastid/Thylakoid

UniProt数据库检索

Gene product and protein name	Plant species	biological process	molecular function	cellular component
ATP synthase CF1 alpha subunit	Daucus carota	ATP synthesis/Hydrogen ion transport/Ion transport/Transport	Hydrolase	CF/ChloroplastMembrane/Plastid/Thylakoid
ATP synthase beta chain	Gossypium arboreum	ATP synthesis/Hydrogen ion transport/Ion transport/Transport	Hydrolase	CF/ChloroplastMembrane/Plastid/Thylakoid
ATP synthase beta subunit	Reinhardtia simplex	ATP synthesis/Hydrogen ion transport/Ion transport/Transport	Hydrolase	CF/ChloroplastMembrane/Plastid/Thylakoid
ATP synthase beta subunit	Utricularia gibba	ATP synthesis/Hydrogen ion transport/Ion transport/Transport	ATP binding/hydrogen ion transporting ATP synthase activity, rotational mechanism/hydrogen-exporting ATPase activity, phosphorylative mechanism/proton-transporting ATPase activity, rotational mechanism	CF/ChloroplastMembrane/Plastid/Thylakoid
putative glutamine synthase 2	Capsicum annuum	glutamine biosynthetic process	ATP binding/glutamate-ammonia ligase activity	Cytoplasm
Hypothetical protein	Vitis vinifera	photosynthesis/photosystem II stabilization	calcium ion binding	extrinsic to membrane/oxygen evolving complex
hypothetical protein	Volvox carteri f.nagariensis	malate metabolic process/carbohydrate metabolic process	malate dehydrogenase (NADP+) activity/nucleotide binding	

UniProt数据库检索
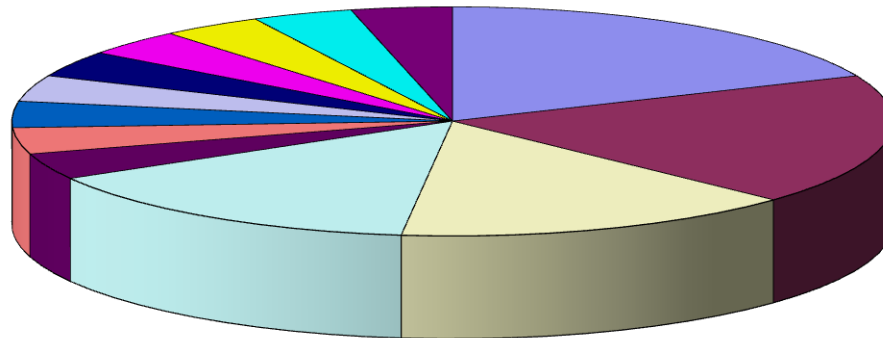
- biological process分布分析：

biological process distribution
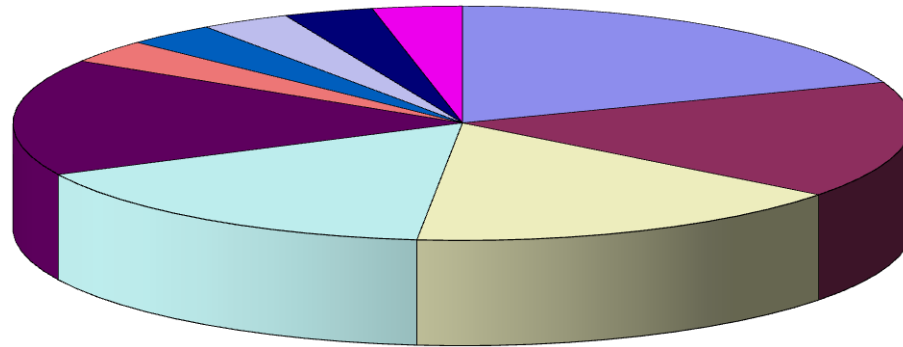
■ photosynthesis	■ ATP synthesis
□ hydrogen ion transport	□ ion transport
■ transport	■ carbon fixation
■ glutamine biosynthetic process	■ photosystem II stabilization
■ malate metabolic process	■ carbohydrate metabolic process

UniProt数据库检索

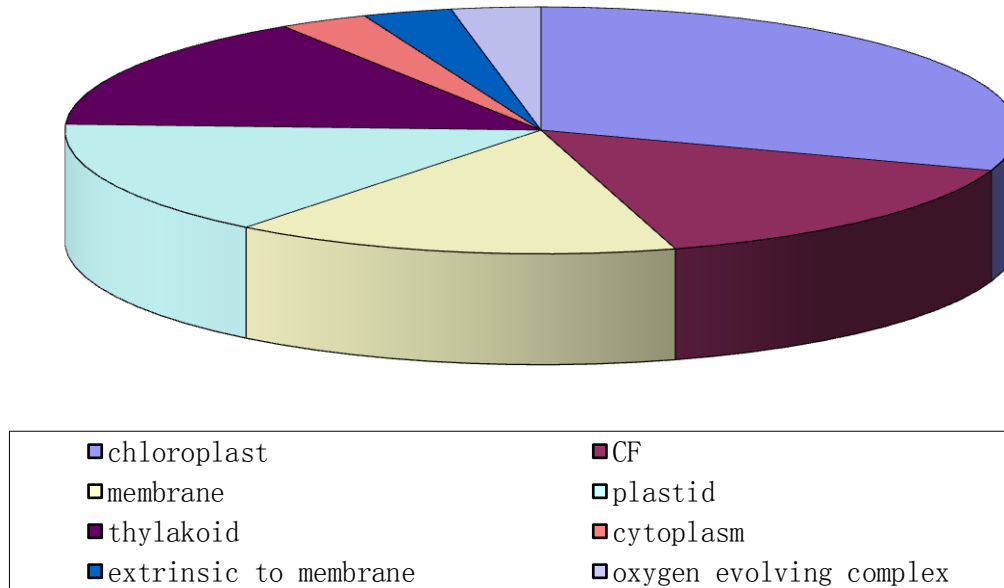- molecular function分布分析：

molecular funcion distribution

- ■ magnesium ion binding
- ■ ribulose-bisphosphate carboxylase activity
- ☐ ATP binding
- ☐ hydrolase
- ■ hydrogen ion transporting ATP synthase activity
- ■ hydrogen-exporting ATPase activity
- ■ proton-transporting ATPase activity
- ☐ rotational mechanism
- ■ phosphorylative mechanism
- ■ glutamate-ammonia ligase activity
- ■ malate dehydrogenase activity
- ■ calcium ion binding
- ■ nucleotide binding

UniProt数据库检索

- cellular component分布分析：

cellular component distribution

☐chloroplast	☐CF
☐membrane	☐plastid
☐thylakoid	☐cytoplasm
☐extrinsic to membrane	☐oxygen evolving complex

Blast2GO软件分析

- Blast2GO: Online GO Tool
- Blast2GO® is an ALL in ONE tool for functional annotation of (novel) sequences and the analysis of annotation data.

  Blast2GO是一个综合型的序列注释分析软件。

- User-friendly, High-throughput and interactive, Highly configurable, Data mining, Vocabularies.

  Blast2GO的优点：易使用，高通量，各部分相互协作，可以自主进行调配，可以按照需要对数据进行整理统计并做成想要的形式，还支持GO、KEGG maps、Interpro和 Enzyme Codes等数据库。
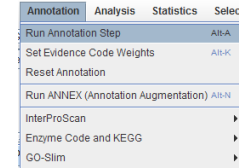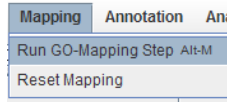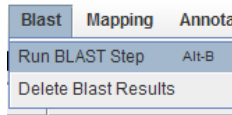
Blast2GO软件分析

- Blast2GO的基本使用方法通常分为五个步骤：
  BLASTing、mapping、annotation、statistics analysis和 visualization。

Blast2GO软件分析

- Blast2GO主页网址：

- http://www.blast2go.com/b2ghome
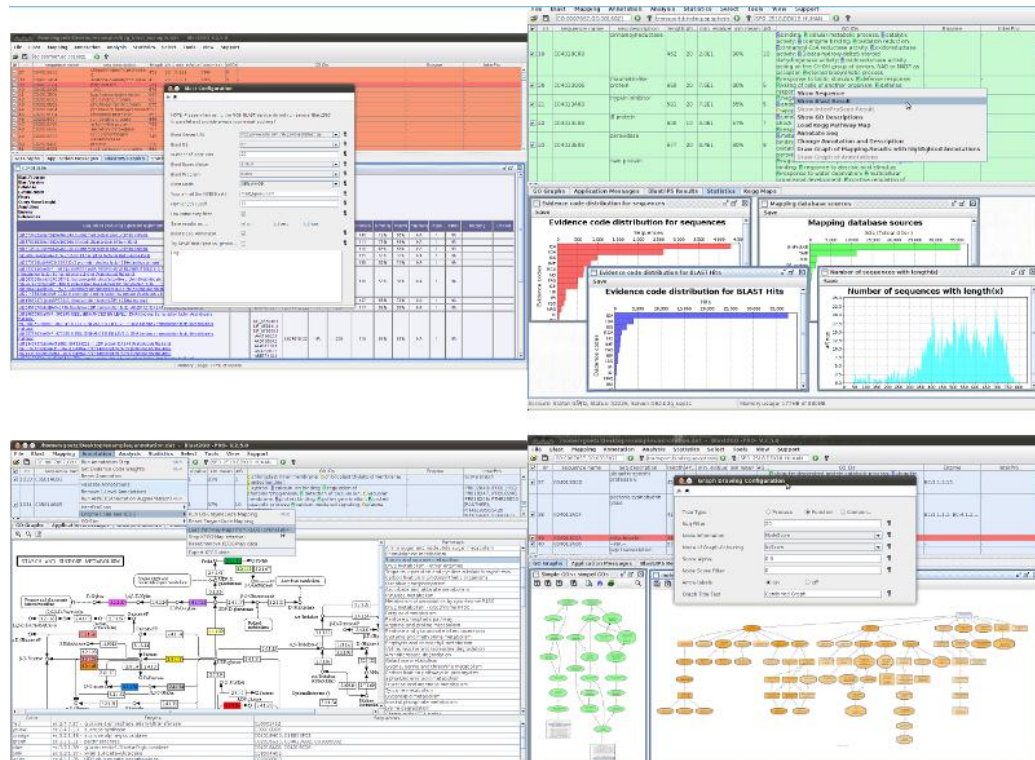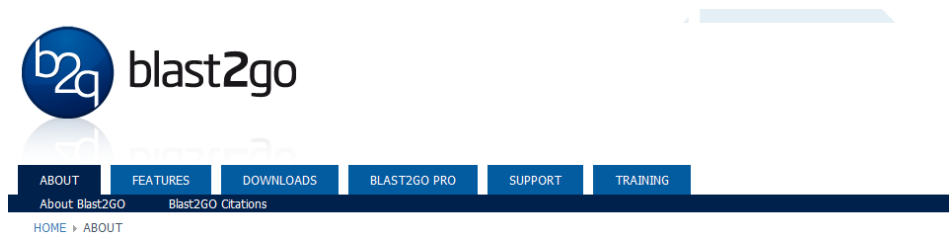
- **从以下网址可以下载并安装Blast2GO软件：**

- http://www.blast2go.com/b2glaunch/start-

Start Blast2GO by Java Web Start

Select the amount of java-memory 500 ▼ MB
To run Blast2GO via Java Web Start
please click here
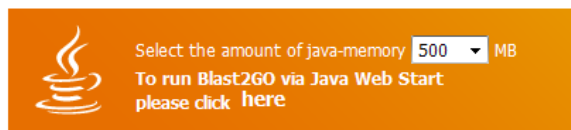
Please select the max. amount of system memory (RAM) you allow JAVA
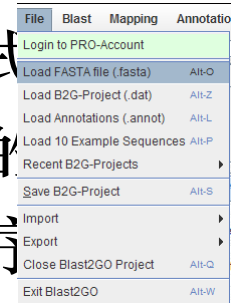to use to improve the performance of Blast2GO:

Blast2GO软件分析
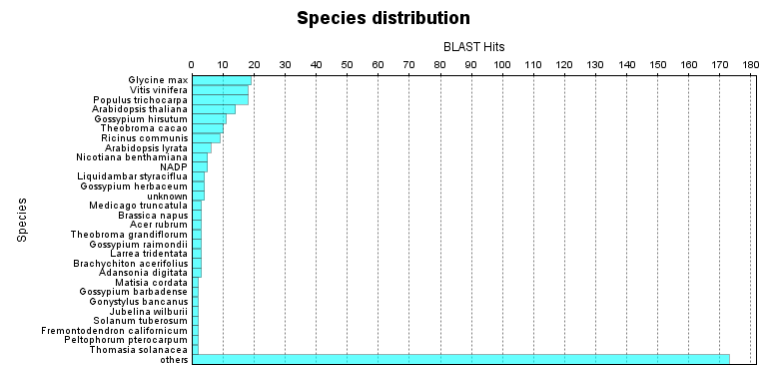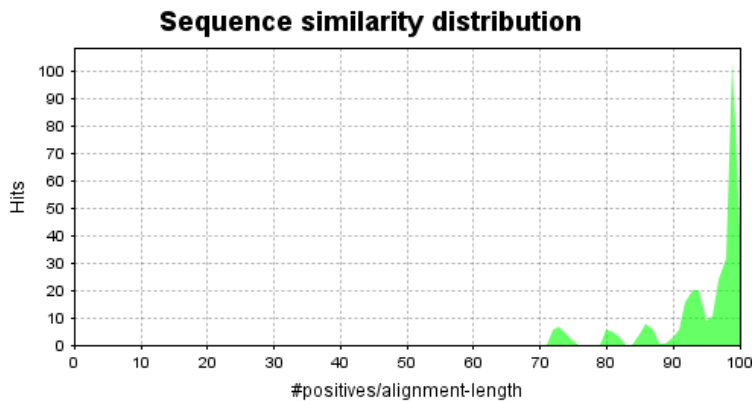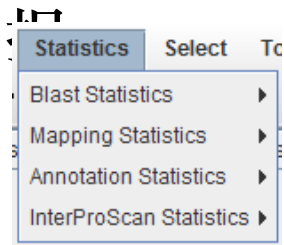
- Blast2GO软件主界面：
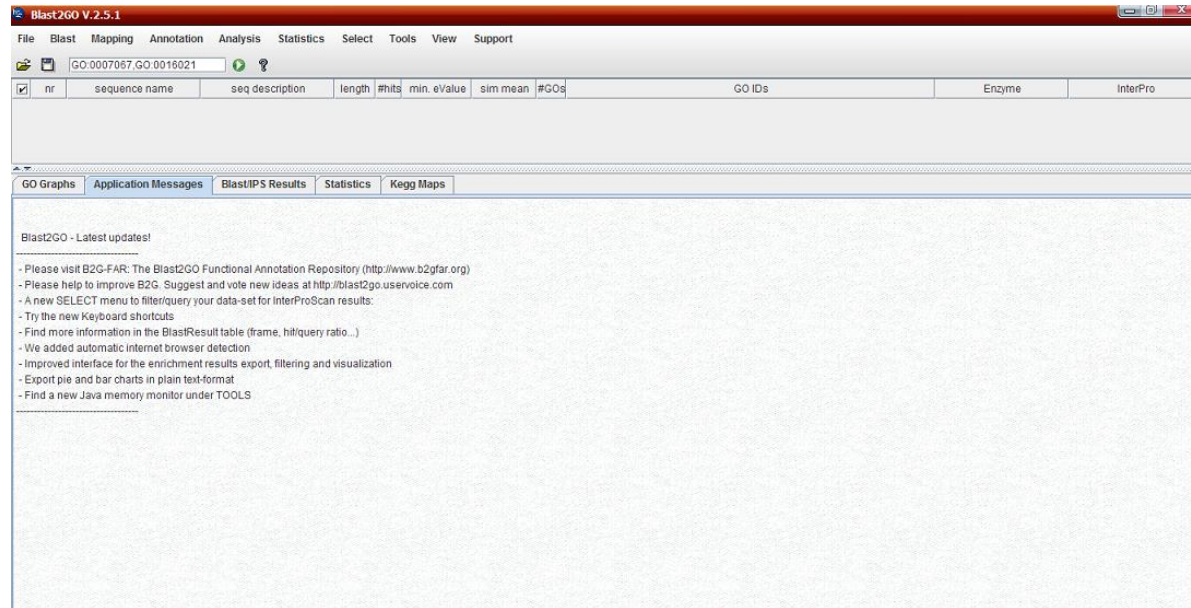
Blast2GO软件分析

- 下载18种差异表达蛋白序列的fasta格式
  并合成一个fasta文件，点击File中的
  Load FASTA file(.fasta)输入蛋白序

- 依次点击Blast中的Run BLAST Step、Mapping中的
  Run GO-Mapping Step、Annotation中的Run
  Annotation Step、Run InterProScan(online)、
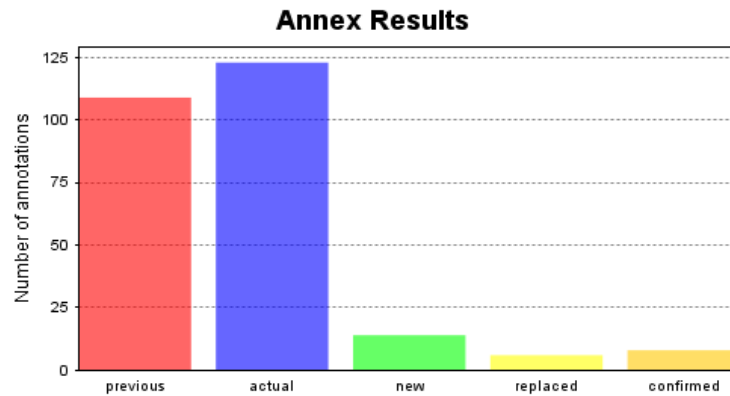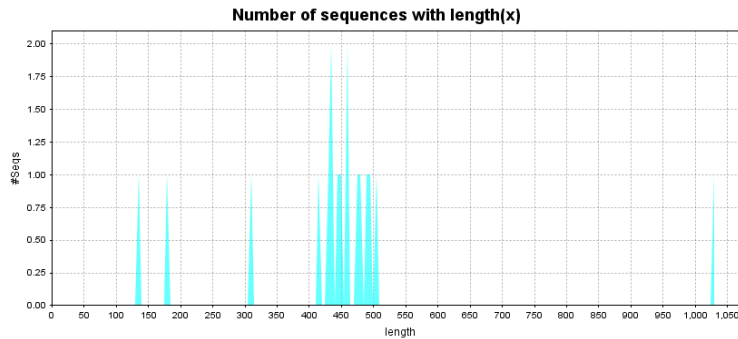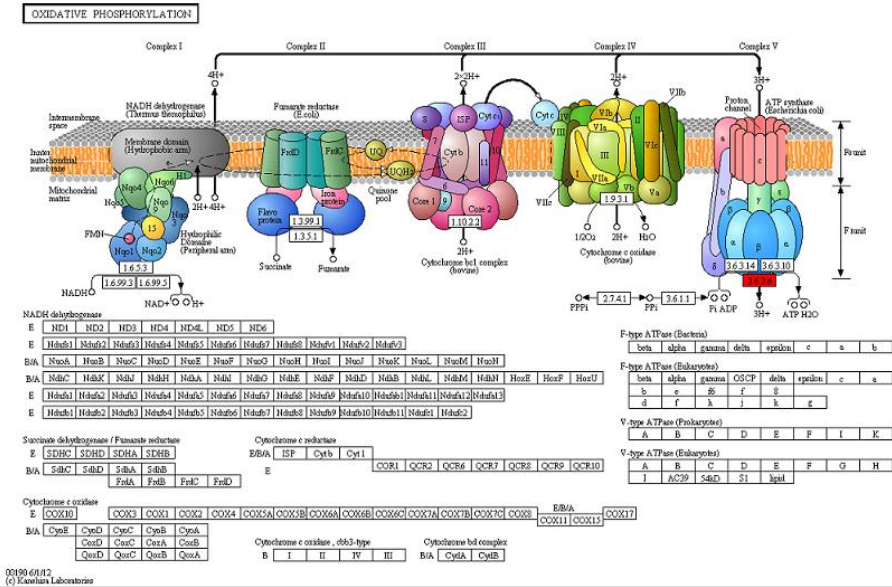 Run GO-Enzyme Code Mapping、Run GO-
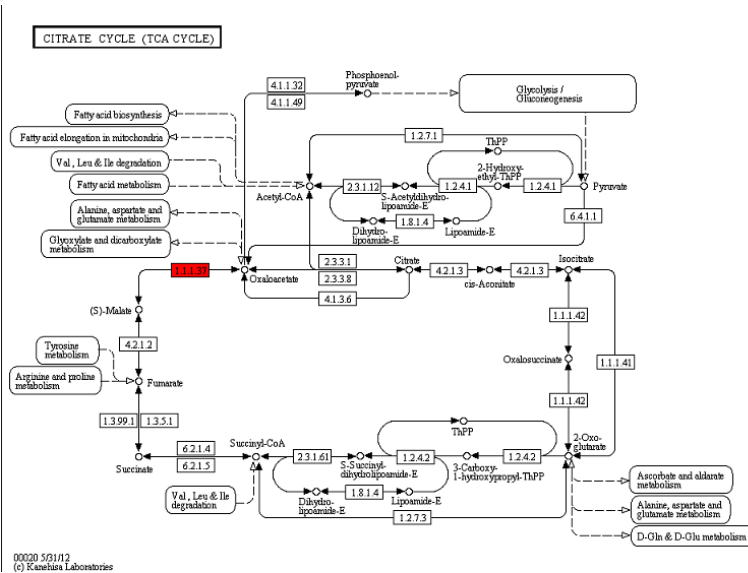 Slim(online)运行程序。
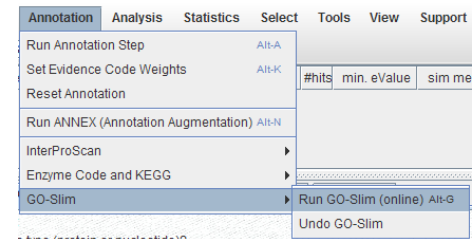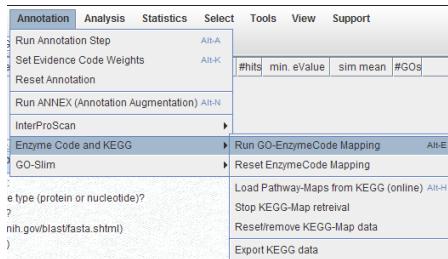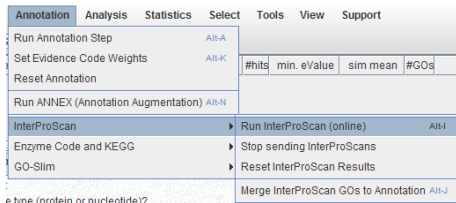
Blast2GO软件分析

- 通过点击Statistics中的各项获得相关的统计学数据

Sequence similarity distribution

Species distribution

Blast2GO软件分析
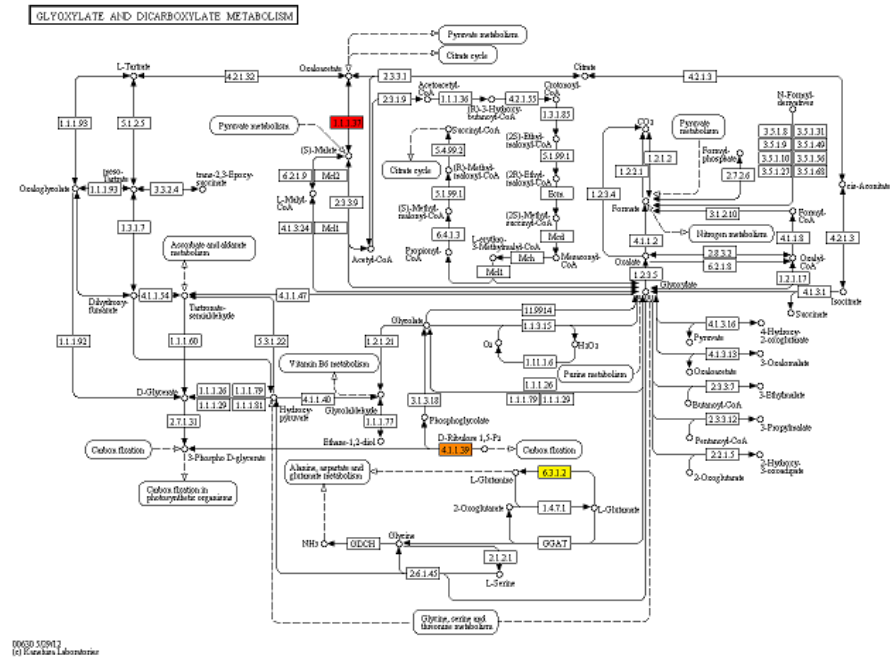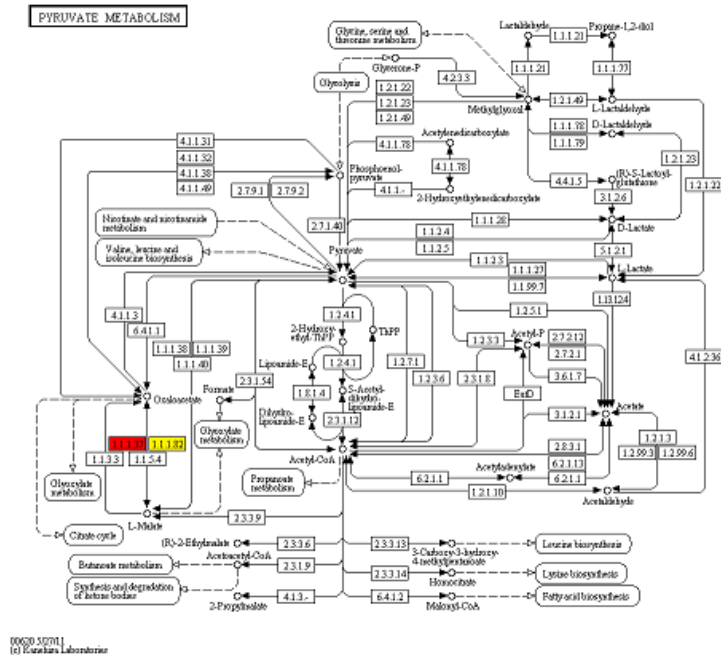

Number of sequences with length(x)

Annex Results

Blast2GO软件分析

1.1.1.37: hypothetical protein VOLCADRAFT_76573
3.6.3.6: ATP synthase beta chain/subunit

Blast2GO软件分析

6.3.1.2: putative glutamine synthase 2

Blast2GO软件分析
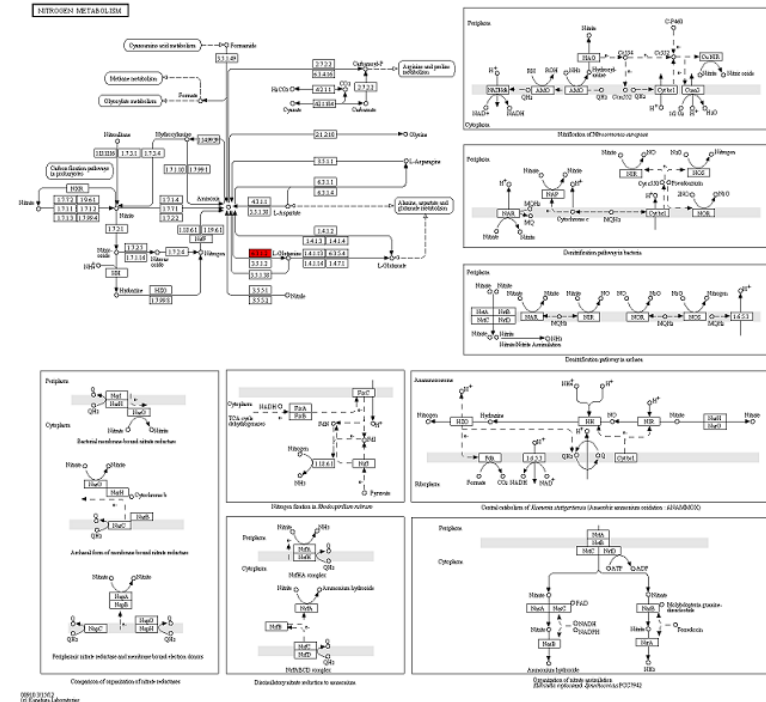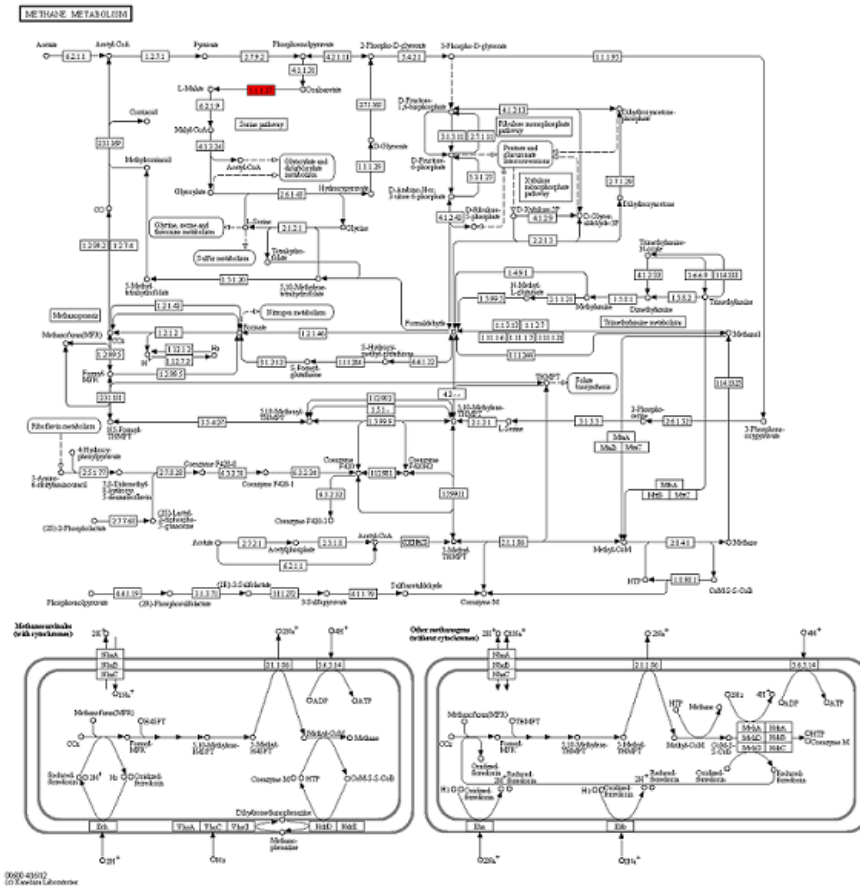

1.1.1.37: hypothetical protein VOLCADRAFT_76573
1.1.1.82: hypothetical protein VOLCADRAFT_76573
4.1.1.39: ribulose-1,5-bisphosphate carboxylase/oxygenase
large subunit ; ribulose-bisphosphate carboxylase ;
Ribulose bisphosphate carboxylase small chain
6.3.1.2: putative glutamine synthase 2
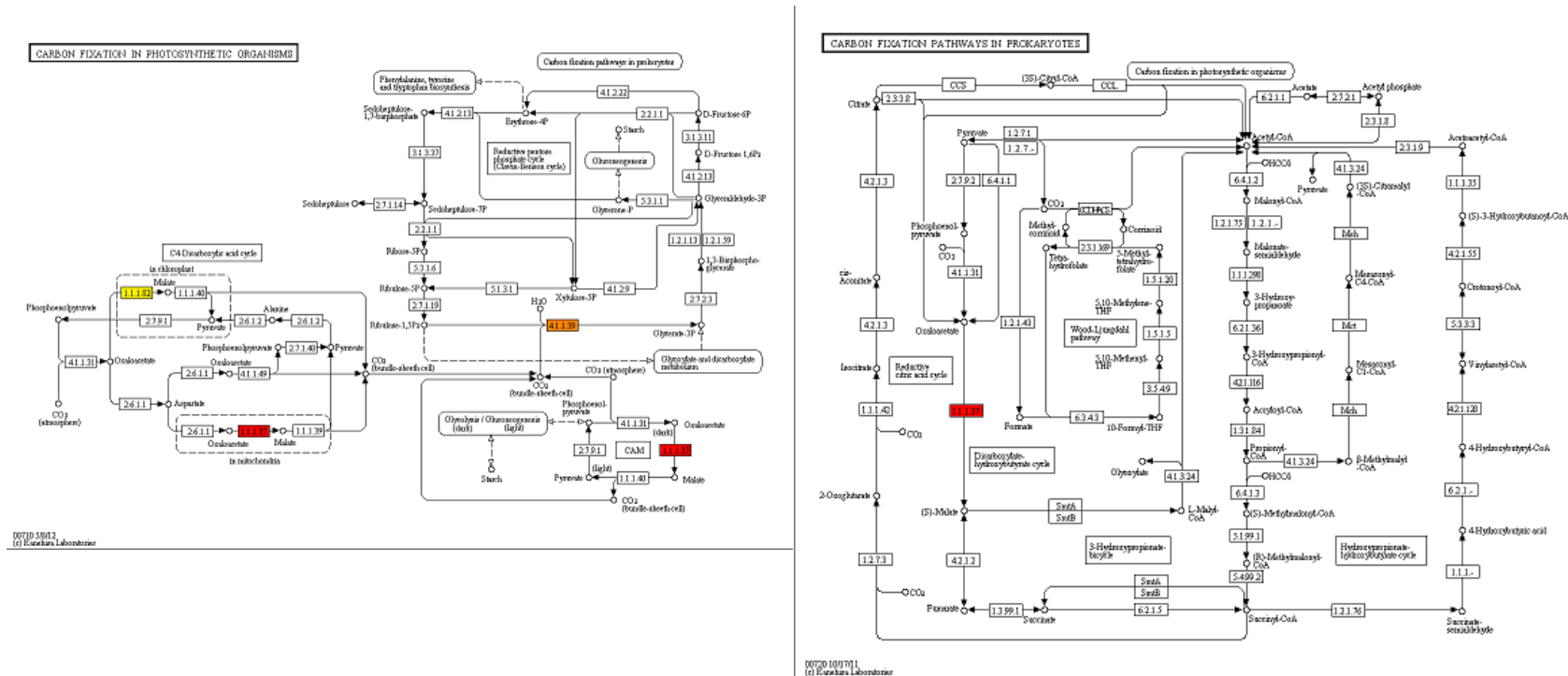
Blast2GO软件分析

1.1.1.82: hypothetical protein VOLCADRAFT_76573
4.1.1.39: ribulose-1,5-bisphosphate
carboxylase/oxygenase large subunit ; ribulose-
bisphosphate carboxylase ; Ribulose bisphosphate
carboxylase small chain
1.1.1.37: hypothetical protein VOLCADRAFT_76573

Blast2GO软件分析

1.1.1.37: hypothetical protein VOLCADRAFT_76573
6.3.1.2: putative glutamine synthase 2

Blast2GO软件分析

- 以上代谢途径的总体关系图：

致谢

感谢一个学期以来

- 罗老师对我们的悉心指导
- 助教们认真负责的工作
- 全体组员的共同努力

谢谢！