

BCP-MG: A Web Server for Predicting Bacterial Community of Metagenome

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Method Article

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Abstract

Introduction

The researchers have been paying more attention to culturing individual microorganisms for a long time, but the majority of microorganisms found in natural ecosystems cannot be easily cultured in the laboratory¹. Metagenomics analysis of genetic material obtained directly from the environment without isolating and culturing the species in a laboratory, which is not possible with traditional methods since only a tiny fraction of all microorganisms (~1%) can be cultivable using standard techniques². The metagenomics analysis employs techniques that enable researchers to obtain, and sequence the genomic content of microbial communities directly, thereby bypassing the need for prior cultivation of all individual organisms present in the sample^{3,4}. The metagenomics offers scientists the method to study the structure of the microorganism community. The current research of metagenome focuses on gene annotation⁵⁻⁷, reconstruction of the metabolism network⁸, analysis the diversity of the microorganisms community^{9,10} and so on, which are mainly based on the known genetic fragments¹¹⁻¹³. Since diversity in metagenomic samples is often too large to provide a high sequencing coverage of single species¹⁴⁻¹⁶, these methods cannot give a microscopy view of the microbial community structure from a metagenomic sample data. So, we present a tool which can predict a bacterial community from a metagenomic sample data according to the enzyme information extracted from the sample or the metabolism network reconstructed from this data. The predicted community not only covers all the known metabolic functions, but also can be used to discover unknown functions of the metagenomic samples. This tool can help researcher to form an overall understanding and find new functions in the metagenomic sample data. The webaddress for this tool is: "<http://bioinfo.ustc.edu.cn/software/bcp-mg/>":<http://bioinfo.ustc.edu.cn/software/bcp-mg/>

Equipment

A computer with access to the internet and a web browser. **Data** Your input is a flat file which contains the enzyme information extracted from the metagenomic sample data or the reactions reconstructed from this data. Each enzyme or reaction takes one line. **Programs** In practice, BCP-MG server process query data using a computationally intensive bioinformatics protocol. (A detailed flow-chart of the protocol is provided in Figure 1.) BCP-MG is a php web server, and the core background program was written in C++ which uses an improved set-covering algorithm to predict the bacterial community.

Procedure

1. Choose the data type you used for representing the metagenomic sample data. The data type includes enzyme and reaction. You can use the enzyme information extracted from the sample to predict the bacterial community; reaction information reconstructed from the sample can be used also.
2. upload the

data file The data file must be a text file. \! CAUTION Uploaded file must be a plain text file \ (generally using ASCII or Unicode schemes) while other rich text formats like those produced by most text editing tools e.g. Microsoft Word , cannot be processed by BCP-MG server. It may take a little time to upload this file depends on the size of the file you want to upload and the bandwidth limit. 3.Choose the metabolic database to use There are two metabolic databases that you can use, BioCyc and KEGG. Individual databases or the union can be applied. Please note that there are many differences between these two databases. One is that the organisms' number and kinds are not identical in the two databases. The other is that even the same organism in the two databases will have different metabolic network for they have used diverse pathway reconstruction algorithms and curative methods. 4.Choose the organisms selecting strategy There are two organisms selecting strategies in use: all-organisms and base-set-organisms. If all-organisms is chosen, the predict algorithm considers all organisms without predilection which may introduce some organisms with same strain. Reversely, the base-set-organisms strategy eliminates these organisms with same strain and reserves one typical organism. 5.Check for any messages. If your data were uploaded successfully to the BCP-MG server, you can see the message "upload successfully", and then a preprocess script will be employed to check whether your data is complied with the request. Depending on the success or failure of enzymes or reactions information submission different messages will be displayed. If you have not given required information, an error message, "Incorrect file content. Please make sure the content of the file uploaded are enzymes or reactions\!" will be displayed, you need to recheck and fill the required information for successful submission. 6.Obtain the results. Once your submission is successful, the data will be processed by the server and the results can be accessed by pressing the "Show Results" button which is available on the neck of the process finished. Time required for processing of your data highly depends on the size of your query and on the parameters you have chosen \ (please see the section on Time Taken to obtain an indication). 7.Interpret the results. The results are displayed using a table, see Figure 3. The table displays the size of the predicted bacterial community and the microorganisms with details \ (Domain, Phylum, Class, Order, Genus and Population according to the KEGG)

Timing

Time taken highly depends on the size of the enzymes or reactions information of the metagenomic sample data, the metabolism database and the other parameters chosen by the user \ (more details are shown on Figure 2). For example, the GS000a Shotgun - Open

Troubleshooting

If the server does not accept the input data for prediction, the error might be caused by one of the following reasons: 1.Input file is not a txt file 2.The content of the input file is not enzymes or reactions 3.The format of the input file is incorrect. 4.The content of the input file does not match the data type you have selected. Error information will be given if you set data type as enzyme but upload a data file with reactions information.

Anticipated Results

The analysis of metagenome samples by BCP-MG protocol provides a quick and conservative – but reliable - prediction of bacterial community conditions. Here we use Acid Mine Drainage Biofilm (4441137.3) as an example, which has been well studied¹⁷⁻¹⁹. The predicted bacterial community, with 22 organisms, is smaller than the community generated by MG-RAST which includes 69 organisms. The comparison detail is shown in Table. 1 with different taxonomic ranks considered (phylum, class and order). At the rank of phylum, 17 out of 22 organisms in the predicted community correspond to the community generated by MG-RAST, while 15 at the class level and 4 at the order level.

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Figures

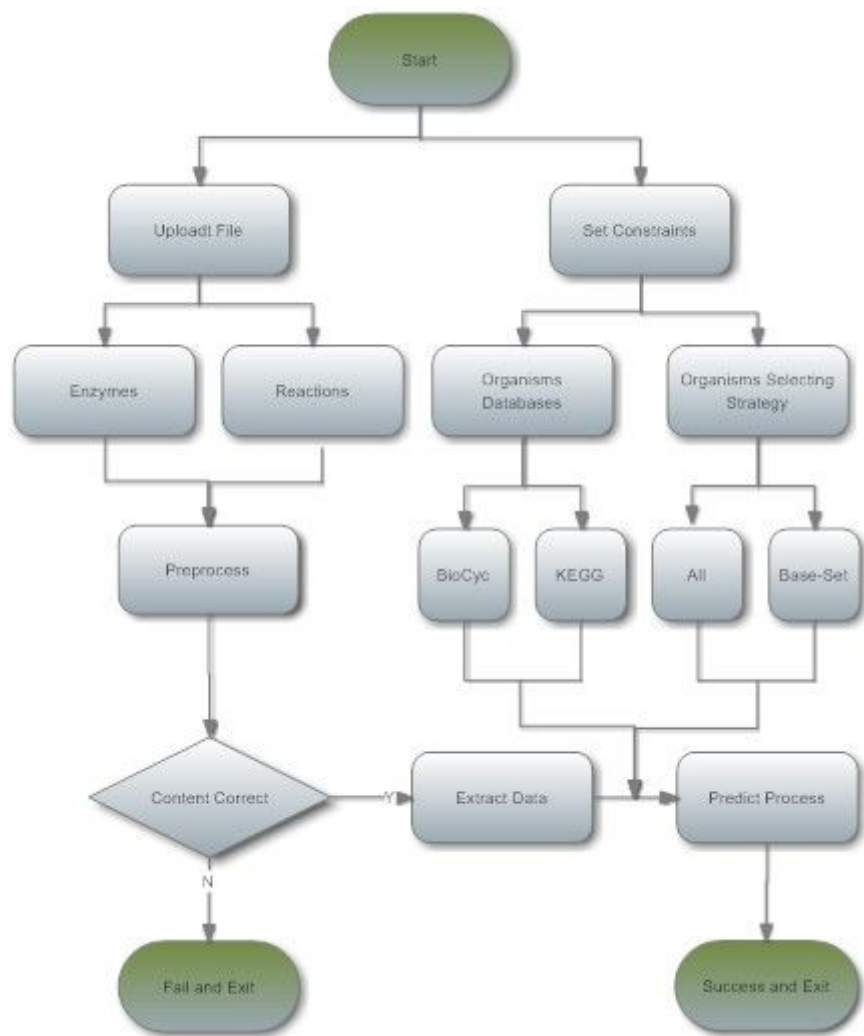


Figure 1

Flowchart illustrating the methodology

Predict Configurations

Select the data type reactions list ec list

* The data that you upload for predict is either a list of reactions or ec numbers.

Upload your data (Example)

Select the database KEGG BioCyc Both

* Select the database that you want to use for predict. There may be some differences in organisms number or metabolite networks of each organism between the two databases.

Organisms Limits Using All Base Set of the organisms

* Predict the Bacterial Community using all or the base set of the organisms that exist in our database.

Figure 2

BCP-MG input options

Task Result

Number	Details (name) (Domain Phylum Class Order Genus Population)
	pchl-425-01cyc (Parachlamydia sp) (Prokaryotes Bacteria Verrucomicrobia)
	yeastcyc (S. cerevisiae S288C) (Prokaryotes Archaea Crenarchaeota)
	meme246198cyc (Mycobacterium smegmatis MC2 155) (Prokaryotes Bacteria Actinobacteria)
	avar240292cyc (Anabaena variabilis ATCC 29413) (Prokaryotes Bacteria Cyanobacteria)
	rzph272943cyc (Rhodobacter sphaeroides 2.4.1) (Prokaryotes Bacteria Proteobacteria Alphatheta)
	ecocyc (Escherichia coli K12) (Prokaryotes Bacteria Proteobacteria GammaEnterobacteria)
	lpla220668cyc (Lactobacillus plantarum WCFS1) (Prokaryotes Bacteria Firmicutes Lactobacillales)
	ecoo157cyc (Escherichia coli O157:H7 EDL933) (Prokaryotes Bacteria Proteobacteria GammaEnterobacteria)
	snub309607cyc (Salinibacter ruber DSM 13855) (Prokaryotes Bacteria Bacteroides)
	bisp36773cyc (Burkholderia sp. 383) (Prokaryotes Bacteria Proteobacteria Beta)
	ecol354106cyc (Escherichia coli UT89) (Prokaryotes Bacteria Proteobacteria GammaEnterobacteria)
	brme358391cyc (Brucella melitensis biovar Abortus) (Prokaryotes Bacteria Proteobacteria Alphaproteobacteria)
Predicted Bacterial Community	25
	agroocyc (Agrobacterium tumefaciens C58) (Prokaryotes Bacteria Proteobacteria Alphaproteobacteria)
	rhru269796cyc (Rhodospirillum rubrum ATCC 11170) (Prokaryotes Bacteria Proteobacteria Alphaproteobacteria)
	bcer228900cyc (Bacillus cereus ATCC 14579) (Prokaryotes Bacteria Firmicutes Bacillales)
	cvio243365cyc (Chromobacterium violaceum ATCC 12472) (Prokaryotes Bacteria Proteobacteria Beta)
	mhun323259cyc (Methanospirillum hungatei JF-1) (Prokaryotes Archaea Euryarchaeota)
	rden375451cyc (Roseobacter denitrificans OCh 114) (Prokaryotes Bacteria Proteobacteria Alphaproteobacteria)
	lnt-130-01cyc (Leptospira interrogans L1-130) (Prokaryotes Bacteria Spirocheta)
	asp76114cyc (Azotarcus sp. EbN1) (Prokaryotes Bacteria Proteobacteria Beta)
	mmaz192952cyc (Methanosarcina mazei Go1) (Prokaryotes Archaea Euryarchaeota)
	bxen266265cyc (Burkholderia xenovorans LB400) (Prokaryotes Bacteria Proteobacteria Beta)
	frantcyc (Francisella tularensis tularensis SCHU S4) (Prokaryotes Bacteria Proteobacteria GammaAlphaproteobacteria)
	vohocyc (Vibrio cholerae O1 biovar eltor str. N16961) (Prokaryotes Bacteria Proteobacteria GammaAlphaproteobacteria)
	hsom205914cyc (Haemophilus somnus 126PT) (Prokaryotes Bacteria Proteobacteria GammaAlphaproteobacteria)

Figure 3

BCP-MG result

		BCP-HG	MG-RAST
Actinobacteria		Mycobacterium smegmatis	Acidimicrobium
			Brevibacterium
			unclassified_Microbacteriales
			Arthrobacter
			Rhodococcus
			Amycolatopsis
			unclassified_Actinomycetales
			unclassified_Actinobacteria
Firmicutes	Cyanobacteria	Anabaena variabilis	Oscillatoria
		Cyanospora sp. PCC 7424	unclassified_Family 4.1
		Synechococcus sp. WH7803	unclassified_Deferribacteriales
	Bacilli	Bacillus amyloquelificans	Bacillus
			unclassified_Bacillaceae
			Paenibacillus
	Mollicutes		unclassified_Bacillales
			Acholeplasma
			Unclassified Mollicutes
Clostridia		unclassified_Mollicutes	
		Caloramprobacter	
		Clostridium difficile	
		Haloalkalibacterium modesticaldum	
		Anaerofium	
		unclassified_Lachnospiraceae	
		Dehalobacter	
		Pelotomaculum	
		unclassified_Syntrophomonadaceae	
		unclassified_Clostridiales	
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified_Clostridia
			unclassified_Methylocystaceae
		Rhodospirillales	Rhizobium
			Magnetospirillum
			Magnetospirillum
	Betaproteobacteria	Sphingomonadales	
			Glucanacetobacter
			Erythrobacter
			Massilia
			Acidovorax avenae
			Azoarcus sp. BH72
			unclassified_Burkholderiales
			Aromatobacterium aromaticum
			Dechloromonas
			Bordetella pertussis
	Deltaproteobacteria		Desulfobacterium autotrophicum
			Desulfobacterium
			unclassified_Deltaproteobacteria
			Desulfobacterales psychrophila
	Gammaproteobacteria	enterobacteria	Candidatus
			Biochmannia pennsylvanicus
			Yersinia enterocolitica
			unclassified_Enterobacteriaceae
		Atheromonadales	
			Pseudalteromonas
		Shewanella	
		Thalassomonas	
		unclassified_Atheromonadaceae	
		Methylobacter	
	Oceanospirillales		
	Oceanospirillum		
	unclassified_Oceanospirillum		
	Pseudomonas		
	unclassified_Xanthomonadaceae		
	unclassified_Gammaproteobacteria		
Basidiomycetes	Cryptosporidium neoformans JEC1		
Chlorobacteria	Chlorobium chlorochromatii		
Aquificae		Sulfurhydrogenibium	
Bacteroidetes		Prevotella	
		Psychroserpens	
		Tenacibaculum	
		unclassified_Flavobacteriaceae	
		Chlorohaga	
		Hymenobacter	
		unclassified_Flavobacteriaceae	
		Halicomonas	
		unclassified_Bacteroidetes	
	Denitrosococcus-Thermus		Thermus
Genera_Incertae_sedis_OPF10		OPF10	
Nitrospira		Leptospirillum	
		Thermodesulfobacterium	
Flanctomyxetes		Prebulla	
Spiraeobacterales		Trogonema	
Thermotogae		Thermotoga	
unclassified_Bacteria		unclassified_Bacteria	

Figure 4

Table 1 The predicted bacterial community contrast with MG-RAST data

Task Result

	Number	Details (name) (Domain Phylum Class Order Genus Population)
Predicted Bacterial Community	25	pchl-e25-01cyc (Parachlamydia sp) (Prokaryotes Bacteria Verrucomicrobia)
		yeastcyc (S. cerevisiae S288C) (Prokaryotes Archaea Crenarchaeota)
		msme246196cyc (Mycobacterium smegmatis MC2 155) (Prokaryotes Bacteria Actinobacteria)
		avar240292cyc (Anabaena variabilis ATCC 29413) (Prokaryotes Bacteria Cyanobacteria)
		rsph272943cyc (Rhodobacter sphaeroides 2.4.1) (Prokaryotes Bacteria Proteobacteria Alpha/others)
		ecocyc (Escherichia coli K12) (Prokaryotes Bacteria Proteobacteria Gamma/enterobacteria)
		lpla220668cyc (Lactobacillus plantarum WCFS1) (Prokaryotes Bacteria Firmicutes Lactobacillales)
		ecoo157cyc (Escherichia coli O157:H7 EDL933) (Prokaryotes Bacteria Proteobacteria Gamma/enterobacteria)
		srub309807cyc (Salinibacter ruber DSM 13855) (Prokaryotes Bacteria Bacteroides)
		bsp36773cyc (Burkholderia sp. 383) (Prokaryotes Bacteria Proteobacteria Beta)
		ecol364106cyc (Escherichia coli UTI89) (Prokaryotes Bacteria Proteobacteria Gamma/enterobacteria)
		bmel359391cyc (Brucella melitensis biovar. Abortus) (Prokaryotes Bacteria Proteobacteria Alpha/rhizobacteria)
		agrocyc (Agrobacterium tumefaciens C58) (Prokaryotes Bacteria Proteobacteria Alpha/rhizobacteria)
		rrub269796cyc (Rhodospirillum rubrum ATCC 11170) (Prokaryotes Bacteria Proteobacteria Alpha/others)
		bcer226900cyc (Bacillus cereus ATCC 14579) (Prokaryotes Bacteria Firmicutes Bacillales)
		cvio243365cyc (Chromobacterium violaceum ATCC 12472) (Prokaryotes Bacteria Proteobacteria Beta)
		mhun323259cyc (Methanospirillum hungatei JF-1) (Prokaryotes Archaea Euryarchaeota)
		rden375451cyc (Roseobacter denitrificans OCh 114) (Prokaryotes Bacteria Proteobacteria Alpha/others)
		lint-130-01cyc (Leptospira interrogans L1-130) (Prokaryotes Bacteria Spirochete)
		asp76114cyc (Azoarcus sp. EbN1) (Prokaryotes Bacteria Proteobacteria Beta)
		mmaz192952cyc (Methanosarcina mazei Go1) (Prokaryotes Archaea Euryarchaeota)
		bxen266265cyc (Burkholderia xenovorans LB400) (Prokaryotes Bacteria Proteobacteria Beta)
		frantcyc (Francisella tularensis tularensis SCHU S4) (Prokaryotes Bacteria Proteobacteria Gamma/others)
		vchocyc (Vibrio cholerae O1 biovar eltor str. N16961) (Prokaryotes Bacteria Proteobacteria Gamma/others)
		hsom205914cyc (Haemophilus somnus 129PT) (Prokaryotes Bacteria Proteobacteria Gamma/others)

Figure 5

Figure 3

		BCP-MG	MG-RAST	
Actinobacteria		Mycobacterium smegmatis	Acidimicrobium	
			Brevibacterium	
			unclassified_Microbacteriaceae	
			Arthrobacter	
			Rhodococcus	
			Amycolatopsis	
			unclassified_Actinomycetales	
Firmicutes	Cyanobacteria	Anabaena variabilis	Oscillatoria	
		Cyanothece sp. PCC 7424	unclassified_Family 4.1	
		Synechococcus sp. WH7803	unclassified_Deferritobacterales	
	Bacilli	Bacillus amyloquelificans	Bacillus	
			unclassified_Bacillaceae	
			Paenibacillus	
	Mollicutes		unclassified_Bacillales	
			Acholeplasma	
			Ureaplasma	
			unclassified_Mollicutes	
Clostridia		Clostridium difficile	Caloramrobacter	
		Helio bacterium modesticaldum	Anaerofilum	
			unclassified_Lachnospiraceae	
			Dehalobacter	
			Pectonaculum	
			unclassified_Syntrophomonadaceae	
			unclassified_Clostridiales	
			unclassified_Clostridia	
	Proteobacteria	Alpha	Rhizobiales	Bredyrhizobium japonicum
			Rhodospirillales	Magnetospirillum magneticum
Sphingomonadales				
Beta			Acidovorax avenae	unclassified_Methylocystaceae
			Azoarcus sp. B422	unclassified_Burkholderiales
			Aromatobacter aromaticum EB1	Dactylomonas
			Bordetella pertussis	
			Desulfotobacterium autotrophicum	Desulfactinrum
			Desulfotalea psychrophila	unclassified_Deltaproteobacteria
			Candidatus Blochmannia pennsylvanicus	unclassified_Enterobacteriaceae
Gamma		Enterobacteriales	Yersinia enterocolitica	Pseudoalteromonas
				Shewanella
		Alteromonadales		Thalassomonas
				unclassified_Alteromonadaceae
		Methylococcales		Methylobacter
		Oceanospirillales		Claspira
		Pseudomonadales		unclassified_Oceanospirillales
		Xanthomonadales		Pseudomonas
unclassified_Gammaproteobacteria			unclassified_Xanthomonadaceae	
			unclassified_Gammaproteobacteria	
Vibrionales			Vibrio vulnificus CMCP-6	
				unclassified_Proteobacteria
			Sulfurospirillum	
Euryarchaeota		Picrophilus torridus		
		Cryptococcus neoformans JEC21		
Basidiomycetes		Cryptococcus neoformans JEC21		
Chlorobia		Chlorobium chlorochromatii		
Aquificae			Sulfhydrogenibium	
Bacteroidetes			Prevotella	
			Psychroserpens	
			Tenacibaculum	
			unclassified_Flavobacteriaceae	
			Chitinophaga	
			Hymenobacter	
			unclassified_Flexibacteraceae	
			Haloscomenobacter	
			unclassified_Bacteroidetes	
Deinococcus-Thermus			Thermus	
Genera incertae sedis, OP10			OP10	
Nitrospirae			Leptospirillum	
Planctomycetes			Thermodesulfobacterium	
Spirochaetes			Frutilla	
Thermotogae			Treponema	
unclassified_Bacteria			Thermotoga	
			unclassified_Bacteria	

Figure 6

Table 1

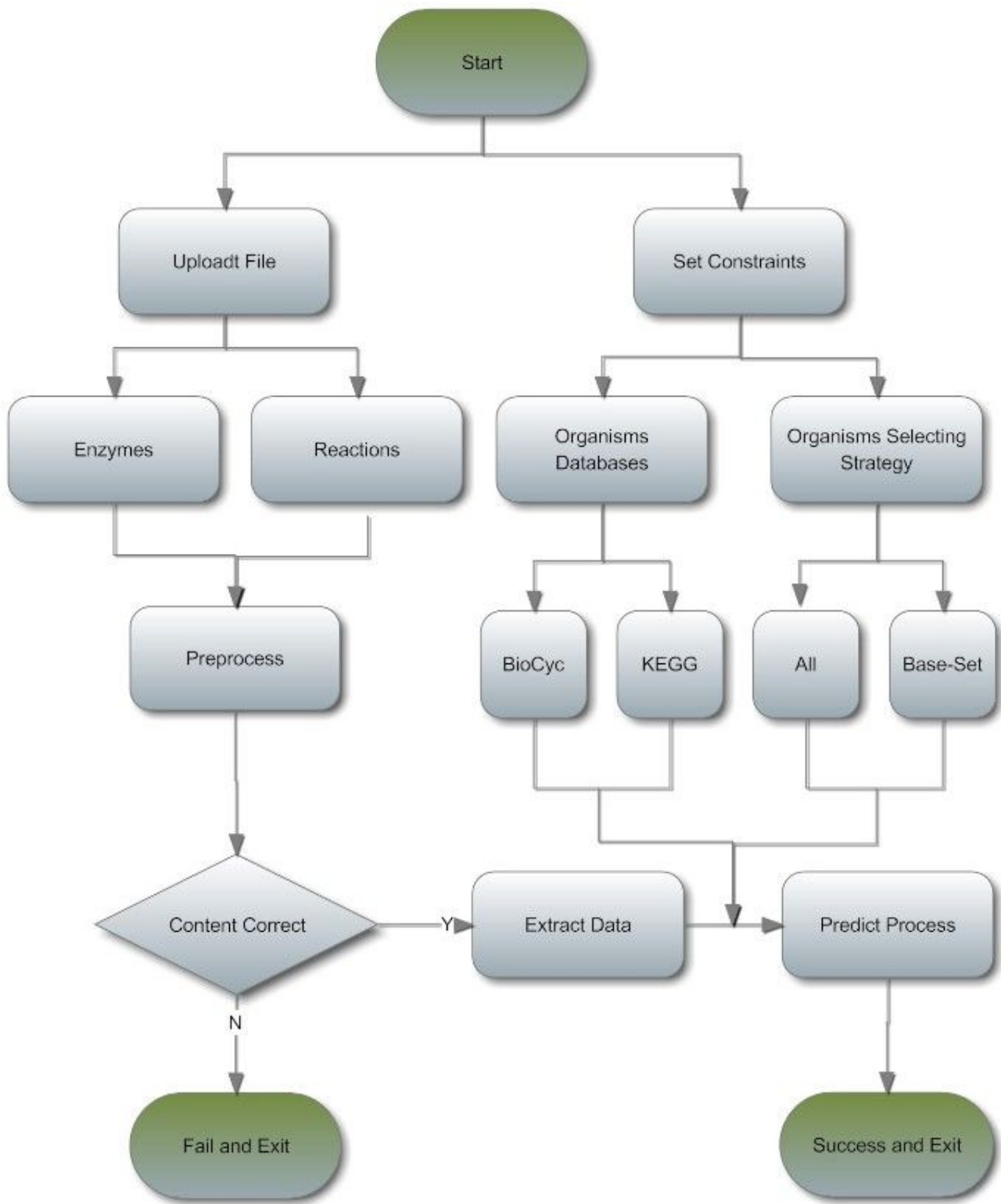


Figure 7

Figure 1

Predict Configurations

Select the data type reactions list ec list

* The data that you upload for predict is either a list of reactions or ec numbers.

Upload your data (Example)

Select the database KEGG BioCyc Both

* Select the database that you want to use for predict. There may be some differences in organisms number or metabolite networks of each organism between the two databases.

Organisms Limits Using All Base Set of the organisms

* Predict the Bacterial Community using all or the base set of the organisms that exist in our database.

Figure 8

Figure 2