A Search for Novel GH9 Homologues within a Metagenomic Database

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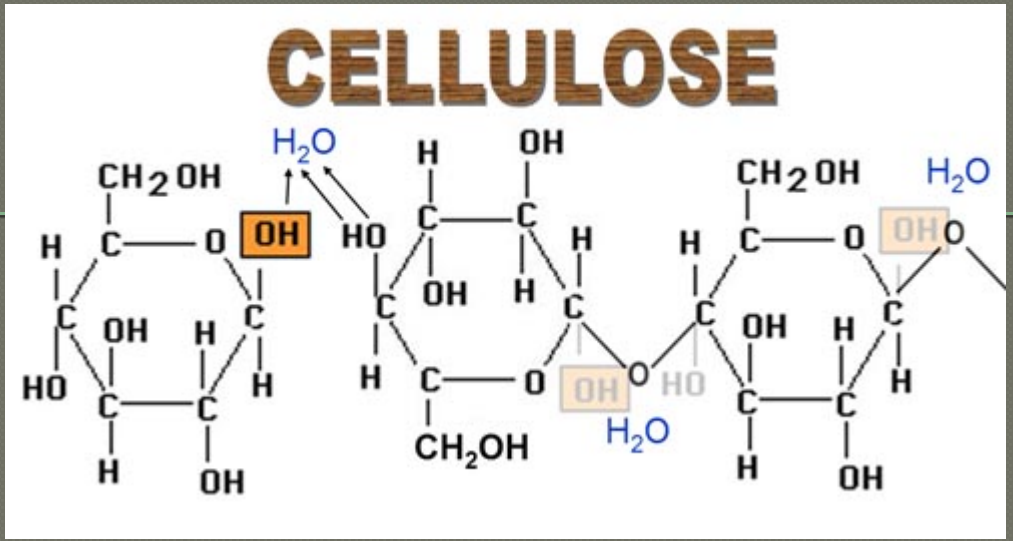
Brenda Pierson
Steve Seydell
Bill Wysocki

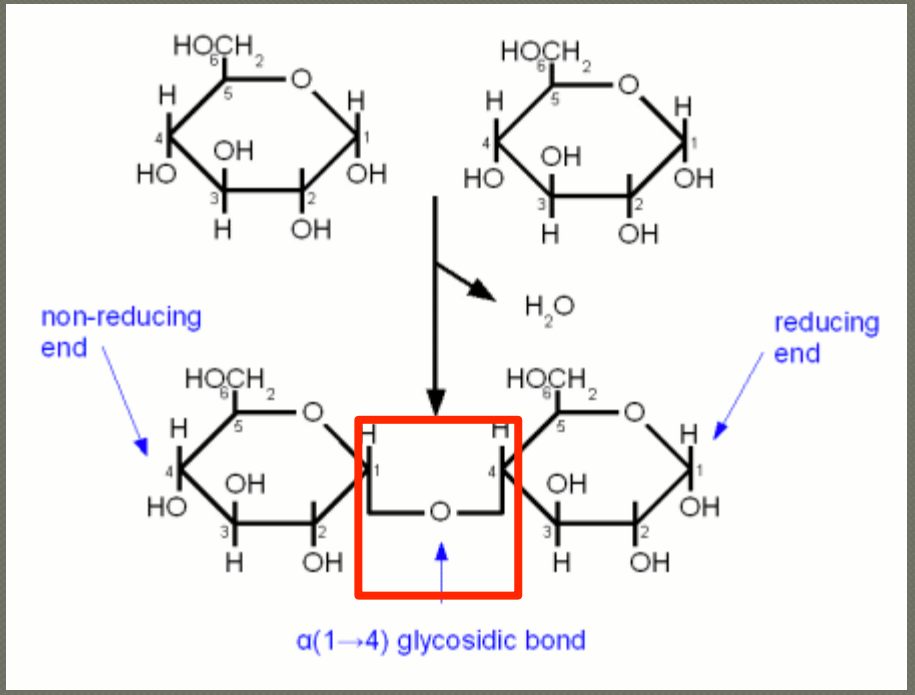


- Polysaccharide found in plant cell walls.
- Very numerous in nature
- Broken down by cellulase
  - Defined by using cellulose as a substrate.

Sukharnikov et al., 2012

Glycoside Hydrolases

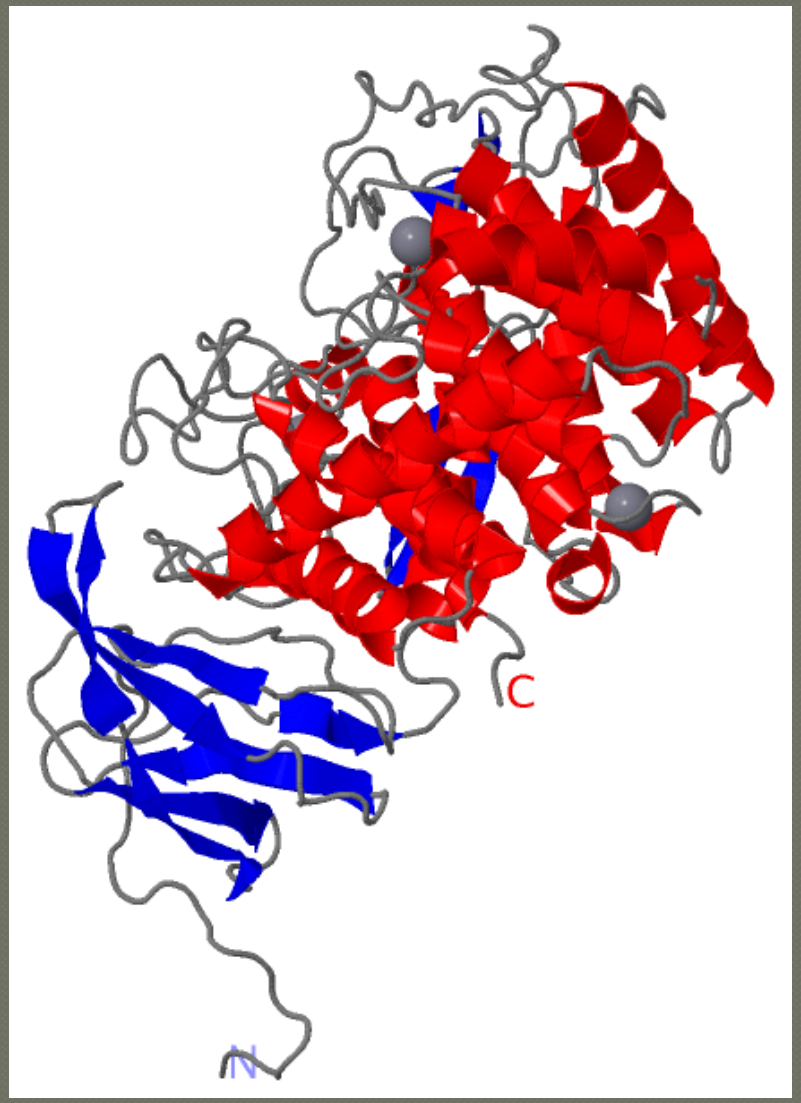
- Break glycosidic linkages
- Understanding is crucial to understanding the ‘CAZome’



Aspeborg et al., 2012

Glycoside hydrolase (GH) family 9

- All known plant cellulases belong to GH9
- 2nd largest cellulase family
- Most plant GH9 enzymes studied are cellulases



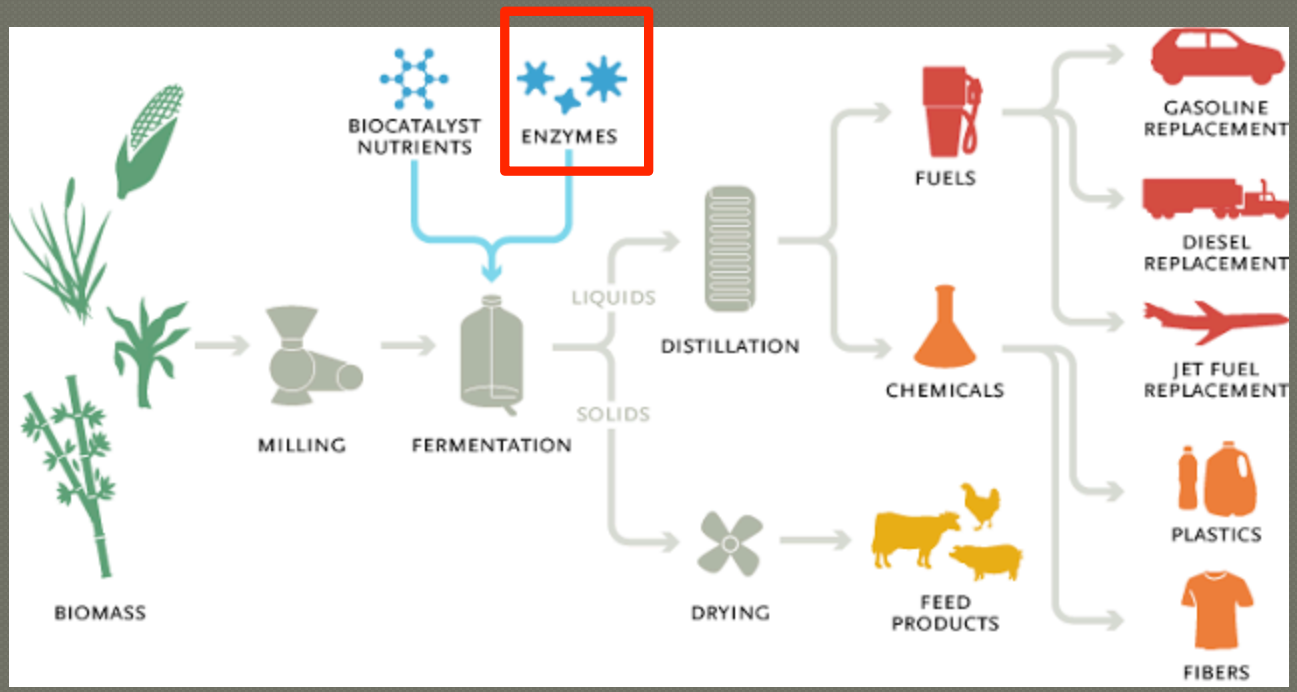
In Nature...

- Cellulases are involved in breakdown of dead plant cells

- Plants need cellulases for shedding of tissues, fruit expansion, organ development

Why do we care?

Renewable chemicals company **Gevo** is now producing a range of products from biomass cellulose-derived sugars.

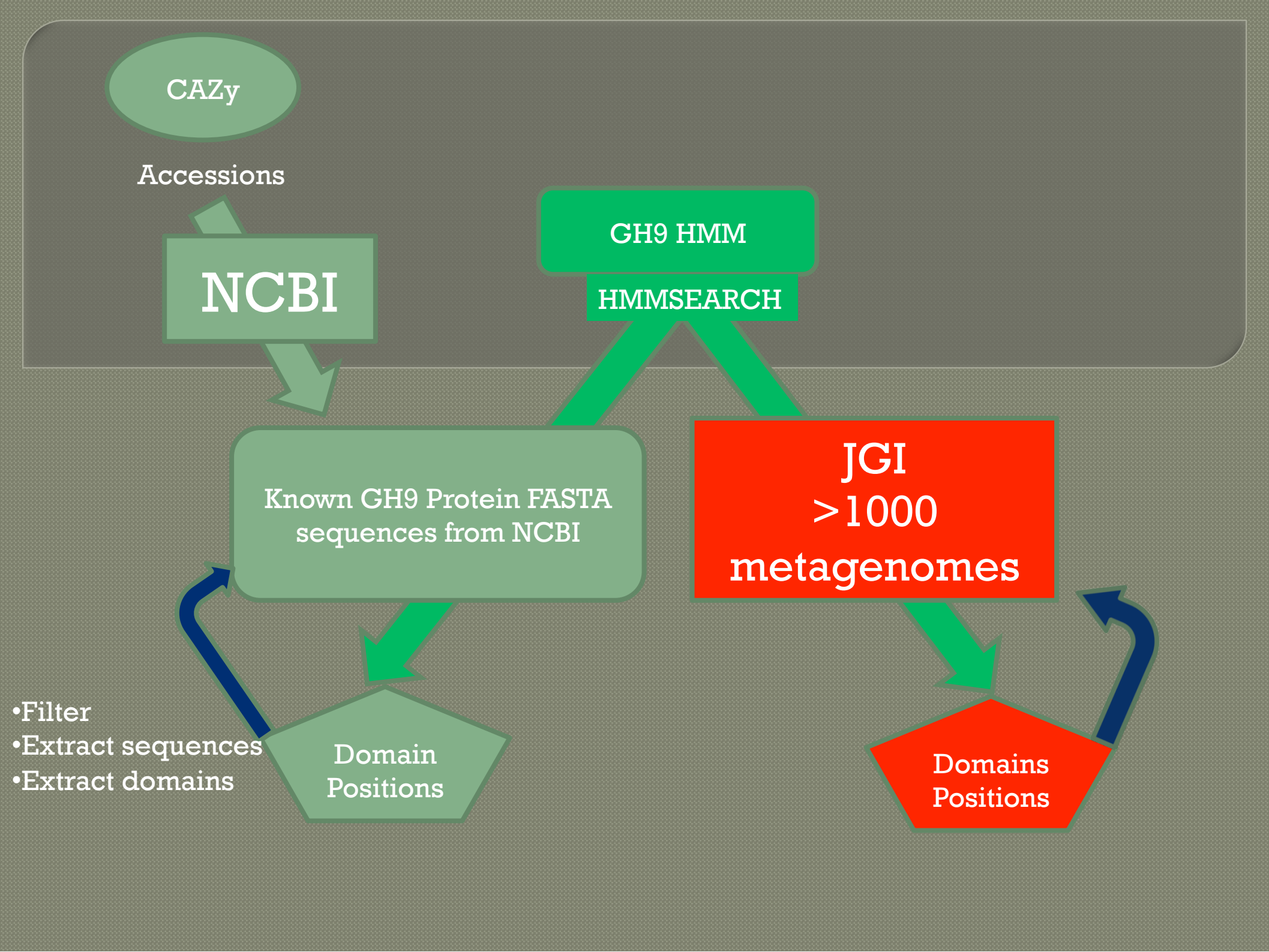


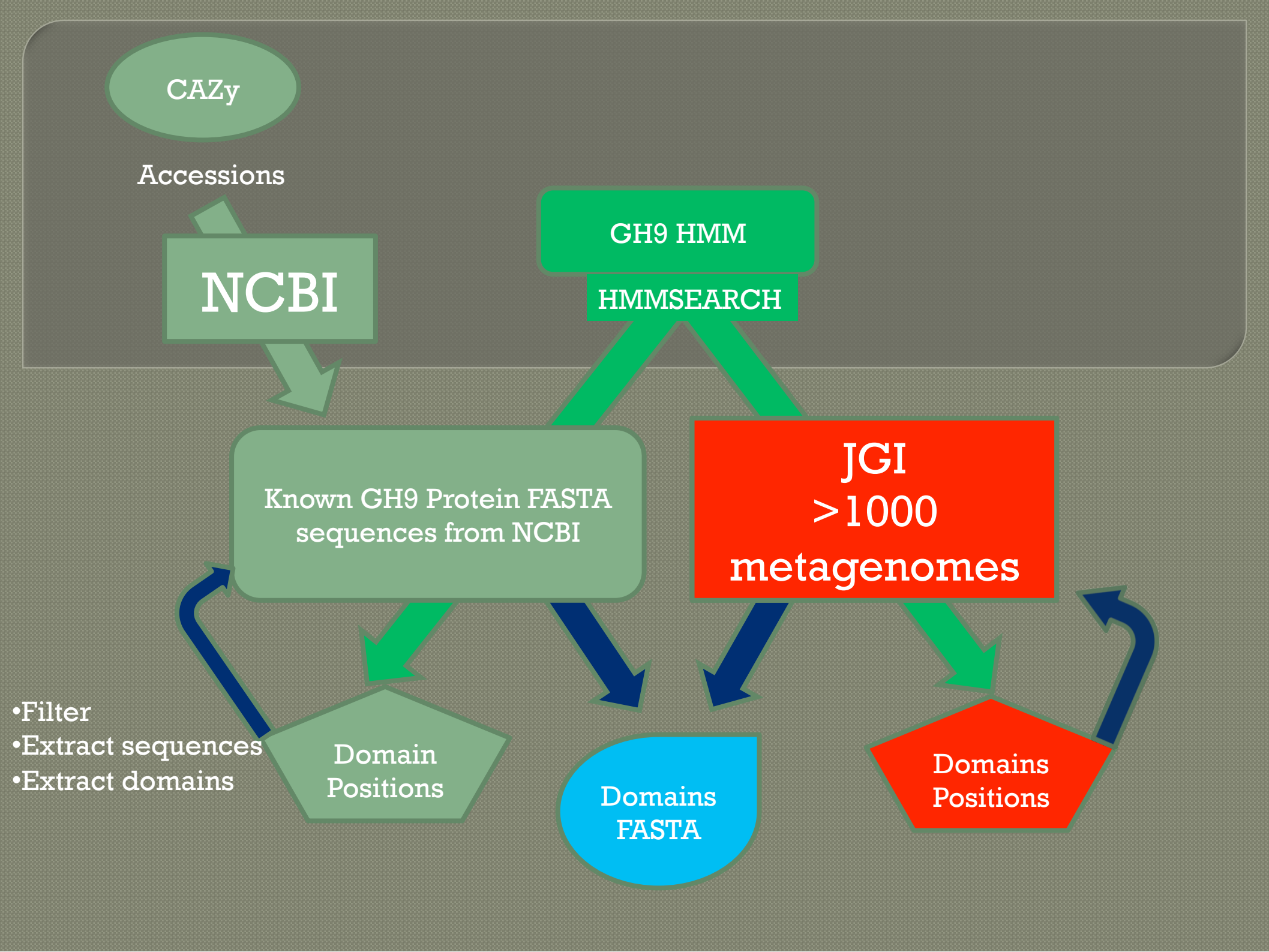
Gevo first ferments isobutanol from biomass (cellulose-derived sugars), then converts the isobutanol to other high value materials.

Project Goal:

Here we intend to identify novel GH9 cellulase domain families found in publicly-available metagenomic sample databases

PROCEDURE OUTLINE


CAZy
Accessions
NCBI
GH9 HMM
HMMSEARCH
Known GH9 Protein FASTA sequences from NCBI
JGI >1000 metagenomes
•Filter
•Extract sequences
•Extract domains
Domain Positions
Domains Positions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Domains FASTA

JGI: 1777 GH9 domains from 211 metagenomes
NCBI 1452 GH9 domains

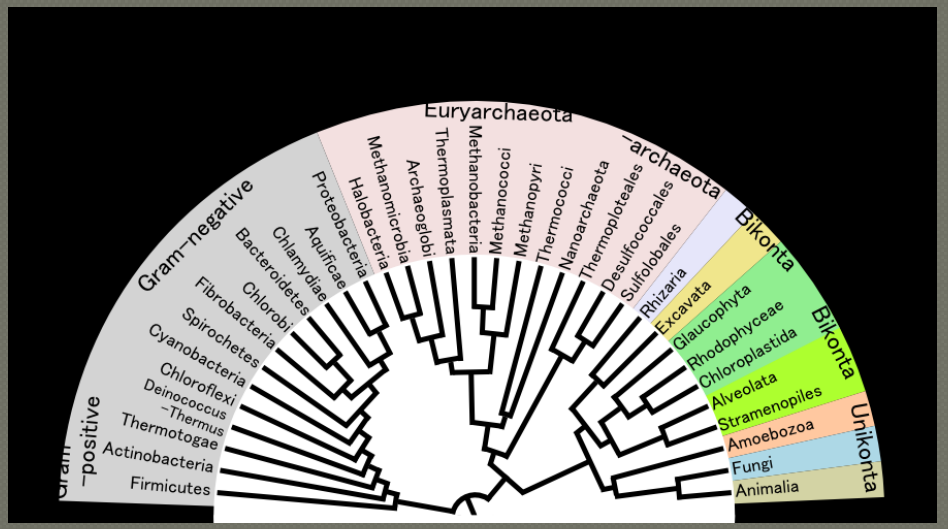
MAFFT

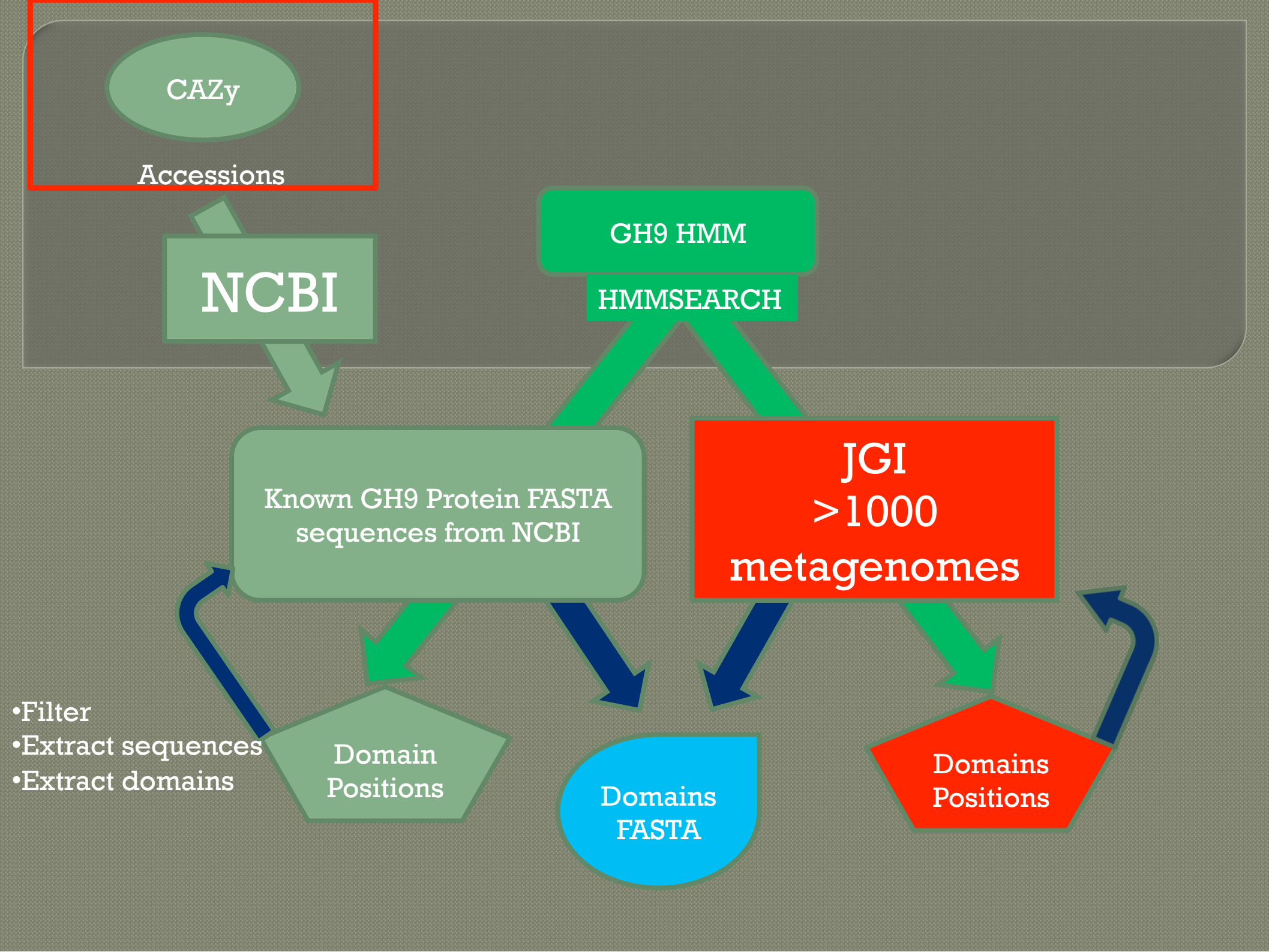
Domains Aligned

FastTree

ITOL

Phylogeny

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Dataset Download

Summary | All (1518) | Archaea (2) | Bacteria (530) | Eukaryota (779) | unclassified (207) | Structure (11) | Characterized (132)

< | 1 | 2 | >

Archaea

Protein Name	EC#	Organism	GenBank
Hmuk_0843		Halomicrobium mukohataei DSM 12286	ACV46974.1
Huta_2399		Halorhabdus utahensis DSM 12940	ACV12565.1

Bacteria

Protein Name	EC#	Organism	GenBank
AM1_2816		Acaryochloris marina MBIC11017	ABW27815.1
scaffoldin A (ScaA;CipV)		Acetivibrio cellulolyticus	AAF06064.1
endo-β-1,4-glucanase (Cel9B)	3.2.1.4	Acetivibrio cellulolyticus CD2 ATCC 33288	CAI94607.1
ACP_1014		Acidobacterium capsulatum ATCC 51196	
Acel_0970		Acidothermus cellulolyticus 11B ATCC 43068	ABK52743.1
Acel_1701		Acidothermus cellulolyticus 11B ATCC 43068	ABK53473.1
AMIS_26270		Actinoplanes missouriensis 431	BAL87847.1
AMIS_35140		Actinoplanes missouriensis 431	BAL88734.1
AMIS_48350		Actinoplanes missouriensis 431	BAL90055.1
ACPL_1206		Actinoplanes sp. SE50/110	AEV82103.1
ACPL_2890 (Cel1)		Actinoplanes sp. SE50/110	AEV83785.1
Amir_5339		Actinosynnema mirum DSM 43827	ACU39159.1
AHA_3098		Aeromonas hydrophila subsp. hydrophila ATCC 7966	ABK39743.1
β-glucosidase		Aeromonas hydrophila SUWA-9	BAF75999.1
ASA_3105		Aeromonas salmonicida subsp. salmonicida A449	ABO91099.1
B565_1044		Aeromonas veronii B565	AEB49079.1
cellulase (CelA;Aaci_2475) **(Cel9A)**	3.2.1.4	Alicyclobacillus acidocaldarius subsp. acidocaldarius DSM 446	ACV59481.1 CAC34051.1

- MS-Excel
- less GH9.accessions.raw | sed '/^$/d' | grep [0-9] > GH9.accessions.clean
- Genbank Query

Extraction from NCBI

- Batch Entrez 2092 Known Proteins

```
>gi|20415|emb|CAA39314.1| cellulase [Persea americana]
ASCGSTTVTAKNLISLAKKQVDYILGENPAKMSYMVGFGERYPQHVHHRGSSLPSVHAHPNPIPCNAGFQ
YLYSSSPNPNILVGAILGGPDSRDSFSDDRNNYQQSEPATYINAPLVGALAFFAANPVAN

>gi|20417|emb|CAA42569.1| cellulase [Persea americana]
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VDLVGGYYDAGDNLKFGLPMAFTTTMLAWGIIEFGCLMPEQVENARAALRWSTDYLLKASTATSNSLYVQ
VGEPNADHRCWERPEDMDTPRNVYKVSTQNPGSDVAAETAAALAAASIVFGDSDSSYSTKLLHTAVKVFE
FADQYRGSYSDSLGSVVCPFYCSYSGYNDELLWGASWLHRASQNASYMTYIQSNGHTLGADDDDYSFSWD
DKRVGTKVLLSKGFLQDRIEELQLYKVHTDNYICSLIPGTSSFQAQYTPGGLLYKGSASNLQYVTSTAFL
LLTYANYLNSSGGHASCGTTTVTAKNLISLAKKQVDYILGQNPAKMSYMVGFGERYPQHVHHRGSSLPSV
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PVTE

>gi|40672|emb|CAA28255.1| unnamed protein product [Clostridium thermocellum]
MSRMTLKSSMKKRVLSLLIAVVFLSLTGVFPSGLIETKVSAAKITENYQFDSRIRLNSIGFIPNHSKKAT
IAANCSTFYVVKEDGTIVYTGTATSMFDNDTKETVYIADFSSVNEEGTYYLAVPGVGKSVNFKIAMNVYE
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ITVGSMFLAWEHFKDQLEPVALEIPEKNNSIPDFLDELKYEIDWILTMQYPDGSGRVAHKVSTRNFGGFI
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PALVQSIKDSLLSTADSIVRTSQNHGYGRTLGTTYYWGCNGTVVRQTMILQVANKISPNNDYVNAALDAI
SHVFGRNYYNRSYVTGLGINPPMNPHDRRSGADGIWEPWPGYLVGGGWPGPKDWVDIQDSYQTNEIAINW
gh9.fasta
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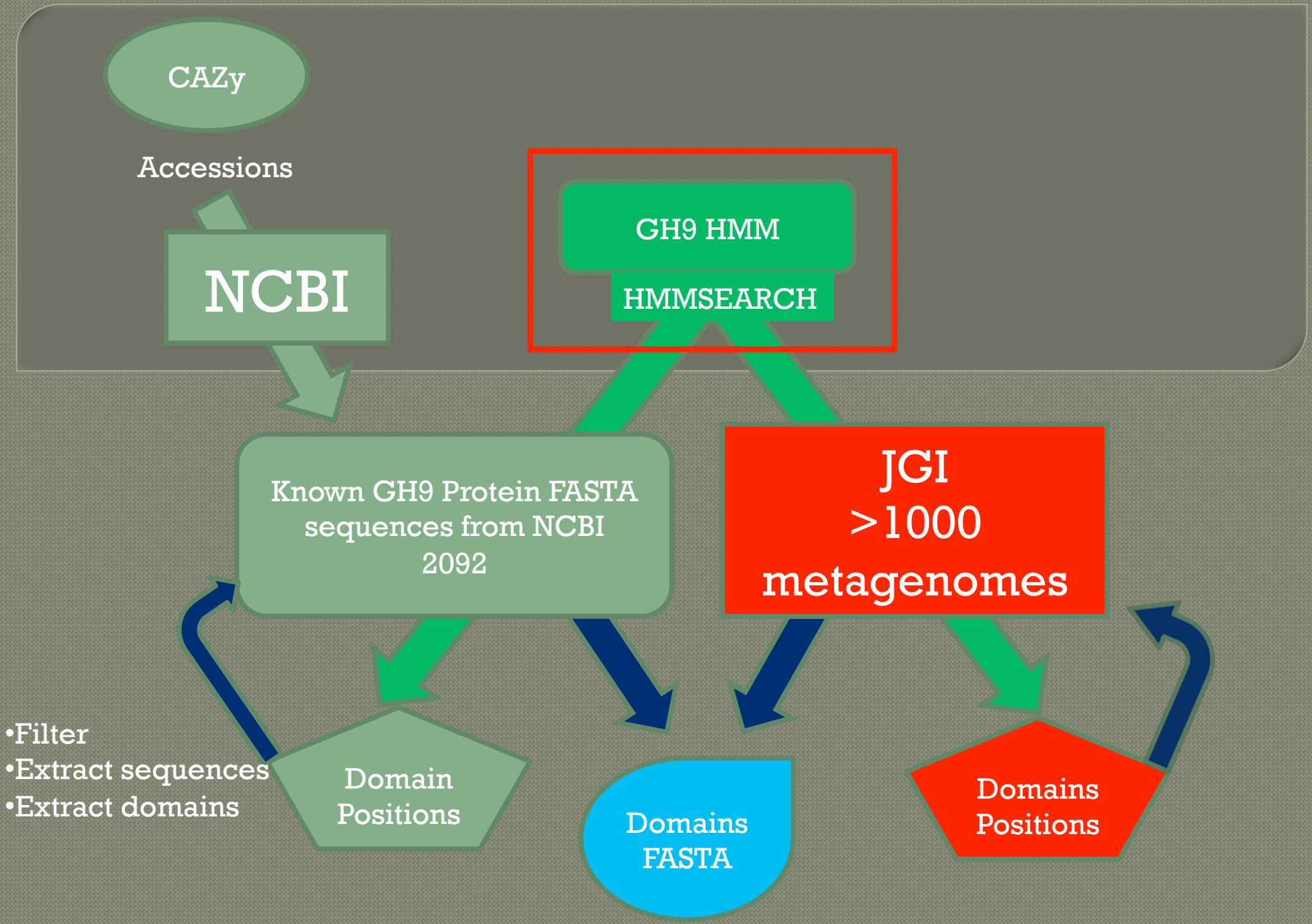
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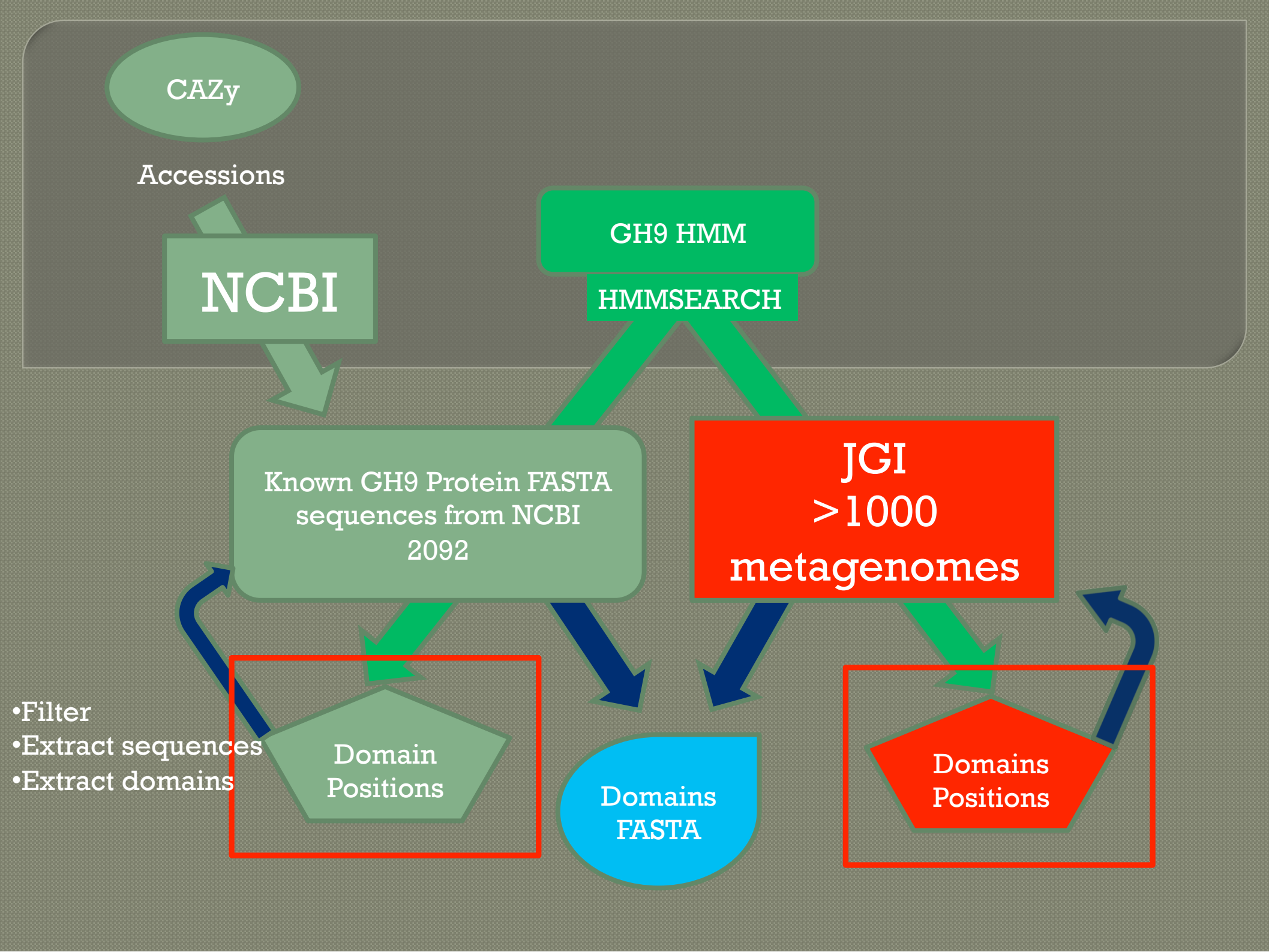
Command lines used to do homology search

hmmsearch --domtblout GH9.NCBI.HMM.out GH9.hmm.ps NCBIbatchentrez.fasta &

hmmsearch --domtblout GH9.JGI.HMM.out GH9.hmm.ps /home/yyin/db/jgi-v3.5.pr.fa &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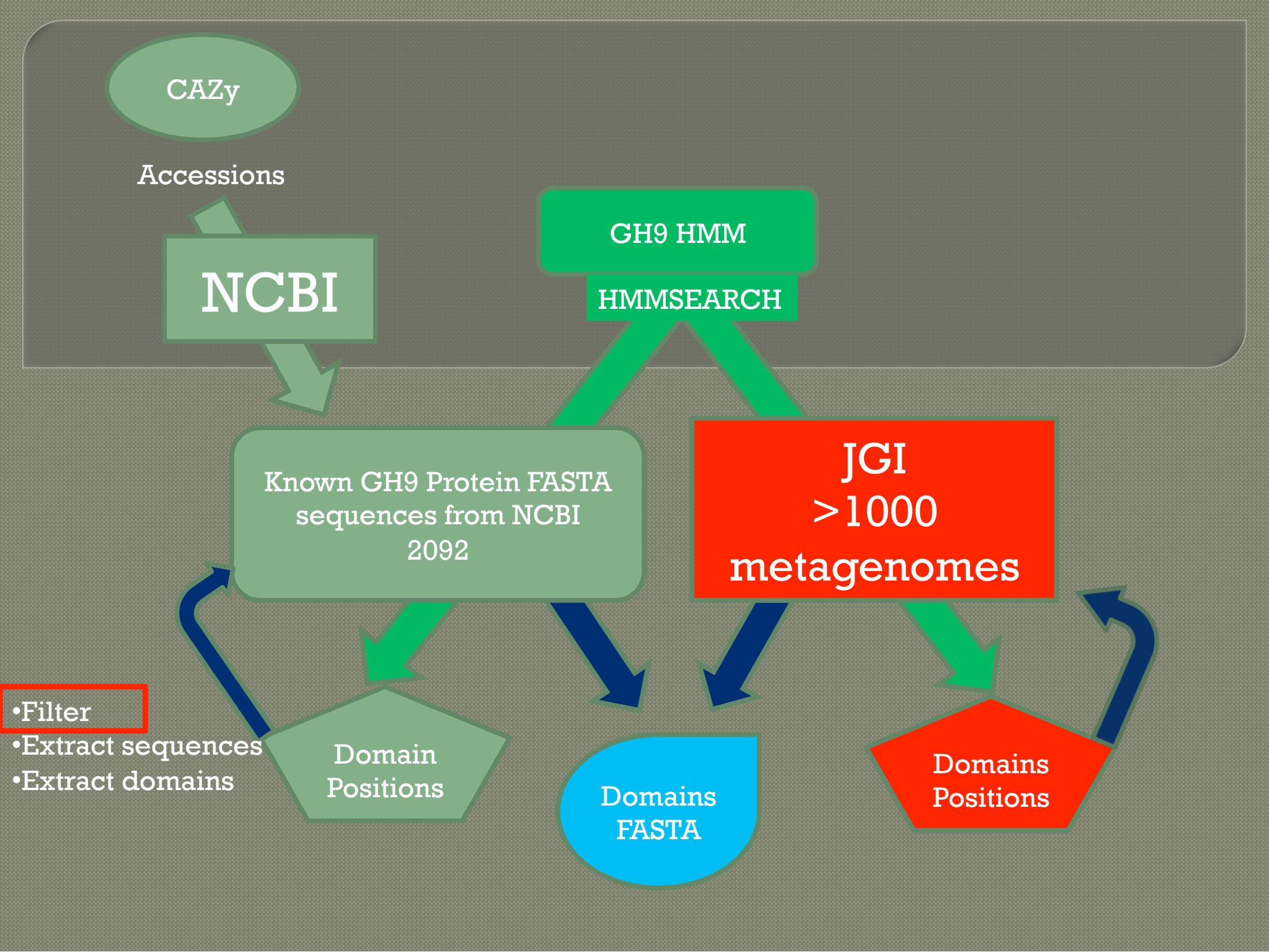
GH9 NCBI hmm – 2,102 Hits

```
                                                                                 --- full sequence --- ------------
omain -------------   hmm coord   ali coord   env coord
 target name                          accession   tlen query name           accession   qlen   E-value  score  bias   #  of  c-Ev
value  score  bias  from    to  from    to  from    to  acc description of target
                 ------------------- ---------- ----- -------------------- ---------- ----- --------- ------ ----- --- --- -----
-------- ------ ----- ----- ----- ----- ----- ----- ----- ---- ---------------------
i|42601450|gb|AAS21473.1|               -          1000 GH9.hmm              -            418  4.7e-193  639.2  21.3   1   2  3.9e
.9e-107  356.2   6.5     1   417    53   479    53   480 0.91 beta-1,4-endoglucanase 1 [Oikopleura dioica]
i|42601450|gb|AAS21473.1|               -          1000 GH9.hmm              -            418  4.7e-193  639.2  21.3   2   2   8.2
.2e-87  289.2   2.8     1   417   533   963   533   964 0.90 beta-1,4-endoglucanase 1 [Oikopleura dioica]
i|371940140|dbj|BAL45507.1|             -           653 GH9.hmm              -            418  4.7e-152  504.1  26.0   1   1  5.7e
.7e-152  503.9  18.0     1   418    41   475    41   475 0.94 glycoside hydrolase [Bacillus licheniformis]
i|264670906|gb|ACY72380.1|              -           646 GH9.hmm              -            418  1.1e-150  499.6  21.1   1   1  1.6e
.6e-150  499.1  14.6     1   418    34   467    34   467 0.93 cellulose hydrolase [Bacillus licheniformis]
i|39636954|gb|AAR29083.1|               -           633 GH9.hmm              -            418  2.4e-150  498.5  21.5   1   1  3.1e
.1e-150  498.2  14.9     1   418    21   454    21   454 0.93 cellulase [Bacillus licheniformis]
i|52003473|gb|AAU23415.1|               -           654 GH9.hmm              -            418  2.7e-150  498.3  21.5   1   1  3.4e
.4e-150  498.0  14.9     1   418    42   475    42   475 0.93 Glycoside Hydrolase Family 9 [Bacillus licheniformis DSM 13 = ATCC
i|52348141|gb|AAU40775.1|               -           654 GH9.hmm              -            418  2.7e-150  498.3  21.5   1   1  3.4e
.4e-150  498.0  14.9     1   418    42   475    42   475 0.93 cellulase EglA [Bacillus licheniformis DSM 13 = ATCC 14580]
i|4490766|emb|CAB38941.1|               -           997 GH9.hmm              -            418  1.5e-149  495.9  27.1   1   1  2.9e
.9e-149  494.9  18.8     1   418    40   479    40   479 0.95 cellulase [Paenibacillus barcinonensis]
i|37498962|gb|AAQ91573.1|               -           659 GH9.hmm              -            418  3.7e-149  494.6  19.9   1   1  4.7e
.7e-149  494.2  13.8     1   418    48   480    48   480 0.94 endoglucanase A precursor [Bacillus pumilus]
i|374343072|dbj|BAL46914.1|             -           997 GH9.hmm              -            418  4.8e-149  494.2  25.2   1   1  9.2e
.2e-149  493.3  17.5     1   418    40   479    40   479 0.95 beta-glucan hydrolase [Paenibacillus sp. WRL301]
i|188011009|gb|ACD44896.1|              -           616 GH9.hmm              -            418  1.6e-148  492.5  19.8   1   1  1.9e
.9e-148  492.2  13.7     1   418     5   437     5   437 0.94 endoglucanase [Bacillus pumilus]
i|310751795|gb|ADP09350.1|              -           659 GH9.hmm              -            418  1.6e-148  492.5  19.2   1   1  1.9e
.9e-148  492.2  13.3     1   418    48   480    48   480 0.94 endo-1,4-beta-glucanase [Bacillus pumilus]
i|148767913|gb|ABR10904.1|              -           659 GH9.hmm              -            418  1.9e-148  492.2  19.8   1   1  2.4e
.4e-148  491.9  13.7     1   418    48   480    48   480 0.94 endoglucanase [Bacillus pumilus]
i|6525242|gb|AAF15367.1|                -           659 GH9.hmm              -            418  1.9e-148  492.2  19.8   1   1  2.4e
.4e-148  491.9  13.7     1   418    48   480    48   480 0.94 endoglucanase [Bacillus pumilus]
i|122937813|gb|ABM68635.1|              -           659 GH9.hmm              -            418  1.9e-148  492.2  19.8   1   1  2.4e
.4e-148  491.9  13.7     1   418    48   480    48   480 0.94 endoglucanase A [Bacillus sp. AC-1]
i|1817723|gb|AAB42155.1|                -           880 GH9.hmm              -            418  4.9e-148  490.9  19.6   1   1  6.2e
.2e-148  490.6  13.6     1   418    51   484    51   484 0.94 beta-1,4-endoglucanase precursor [Thermobifida fusca YX]
i|71916307|gb|AAZ56209.1|               -           880 GH9.hmm              -            418  4.9e-148  490.9  19.6   1   1  6.2e
.2e-148  490.6  13.6     1   418    51   484    51   484 0.94 endoglucanase. Glycosyl Hydrolase family 9 [Thermobifida fusca YX]
```

GH9 JGI hmm – 15,428 Hits

```
#                                                                            --- full sequence --- -------------- this domain -------------   hmm coord   ali coord
env coord
# target name        accession   tlen query name           accession   qlen   E-value  score  bias   #  of  c-Evalue  i-Evalue  score  bias  from    to  from    to
from    to  acc description of target
#------------------- ---------- ----- -------------------- ---------- ----- --------- ------ ----- --- --- --------- --------- ------ ----- ----- ----- ----- -----
----- ----- ---- ---------------------
2201231693           -            883 GH9.hmm              -            418  2.9e-146  501.1  17.2   1   1  2.9e-150  3.6e-146  500.8  12.0     1   418   278   706
278   706 0.94 MRSJC2b_43491 Predicted solute binding protein [Mesophilic rice straw/compost enrichment metagenome: eDNA_1  (Mesophilic 454/Illumina Combined June 2011
assem)]
2200984066           -            756 GH9.hmm              -            418  3.9e-144  494.1  24.0   1   1  4.1e-148  5.1e-144  493.7  16.6     1   418    37   474
37   474 0.93 MRSJC2b_272891 Glycosyl hydrolase family 9./Cellulose binding domain. [Mesophilic rice straw/compost enrichment metagenome: eDNA_1  (Mesophilic 454/
Illumina Combined June 2011 assem)]
2061998357           -            768 GH9.hmm              -            418  2.1e-143  491.7  24.1   1   1    2e-147  2.6e-143  491.4  16.7     1   418    51   488
51   488 0.92 sg4i_00246050 Predicted solute binding protein [Thermophilic enrichment culture SG0.5JP960 (454-Illumina assembly) - version 2 (454-Illumina assembly v2)]
2018727539           -            880 GH9.hmm              -            418  3.6e-143  490.9  19.6   1   1  3.6e-147  4.5e-143  490.6  13.6     1   418    51   484
51   484 0.94 PFMN_291642 hypothetical protein [Sample 267]
2201115235           -           1283 GH9.hmm              -            418  6.8e-141  483.4  15.8   1   1  7.6e-145  9.5e-141  482.9  11.0     1   418   666  1094
666  1094 0.94 MRSJC2b_32941 Uncharacterized protein conserved in bacteria [Mesophilic rice straw/compost enrichment metagenome: eDNA_1  (Mesophilic 454/Illumina
Combined June 2011 assem)]
2200446424           -           1001 GH9.hmm              -            418  4.9e-140  480.6  17.9   1   1  6.3e-144  7.9e-140  479.9  12.4     1   417    95   528
95   529 0.94 TRSJC2b_5223 Cellobiohydrolase A (1,4-beta-cellobiosidase A) [Thermophilic rice straw/compost enrichment metagenome: eDNA_2  (Thermophilic 454/Illumina
Combined  June 2011 assem)]
2078025289           -           1028 GH9.hmm              -            418  4.6e-139  477.4  22.8   1   1  5.9e-143  7.4e-139  476.7  15.8     1   418    73   503
73   503 0.93 MA55A_00355410 Cellulose binding domain./Glycosyl hydrolase family 9./Domain of unknown function. [Mixed alcohol bioreactor microbial communities from
Texas A&M University, sample 55C (55 degree reactor, August 2010 assembly)]
2029678661           -            569 GH9.hmm              -            418  1.5e-138  475.7  21.2   1   1  1.4e-142  1.8e-138  475.4  14.7     1   417    41   474
41   475 0.95 GBAN_2580090 Cellulose binding domain./Glycosyl hydrolase family 9. [Compost Minireactor Metagenome (final assembly)]
2201670307           -            668 GH9.hmm              -            418  3.1e-138  474.6  21.3   1   1    3e-142  3.7e-138  474.4  14.8     1   418    30   469
30   469 0.94 MRSJC2b_190371 Glycosyl hydrolase family 9./Cellulose binding domain. [Mesophilic rice straw/compost enrichment metagenome: eDNA_1  (Mesophilic 454/
Illumina Combined June 2011 assem)]
2150562325           -            513 GH9.hmm              -            418  1.9e-137  472.0  28.9   1   1  1.8e-141  2.2e-137  471.8  20.1     1   418    36   466
36   466 0.91 GBSCES77_01292830 Glycosyl hydrolase family 9. [Sediment microbial communities from Great Boiling Spring, Nevada, sample from Cellulolytic enrichment S
77C (Cellulolytic enrichment S 77C sediment)]
2150657657           -            513 GH9.hmm              -            418  1.9e-137  472.0  28.9   1   1  1.8e-141  2.2e-137  471.8  20.1     1   418    36   466
36   466 0.91 GBSCES77_02246150 Glycosyl hydrolase family 9. [Sediment microbial communities from Great Boiling Spring, Nevada, sample from Cellulolytic enrichment S
77C (Cellulolytic enrichment S 77C sediment)]
2150468268           -            640 GH9.hmm              -            418  3.3e-137  471.3  28.9   1   1  3.6e-141  4.6e-137  470.8  20.1     1   418    36   466
36   466 0.91 GBSCES77_00352260 Cellulose binding domain./Glycosyl hydrolase family 9. [Sediment microbial communities from Great Boiling Spring, Nevada, sample from
Cellulolytic enrichment S 77C (Cellulolytic enrichment S 77C sediment)]
2150635057           -            640 GH9.hmm              -            418  3.3e-137  471.3  28.9   1   1  3.6e-141  4.6e-137  470.8  20.1     1   418    36   466
36   466 0.91 GBSCES77_02020150 Cellulose binding domain./Glycosyl hydrolase family 9. [Sediment microbial communities from Great Boiling Spring, Nevada, sample from
Cellulolytic enrichment S 77C (Cellulolytic enrichment S 77C sediment)]
2150588393           -            641 GH9.hmm              -            418  3.3e-137  471.3  28.9   1   1  3.7e-141  4.6e-137  470.8  20.1     1   418    36   466
36   466 0.91 GBSCES77_01553510 Cellulose binding domain./Glycosyl hydrolase family 9. [Sediment microbial communities from Great Boiling Spring, Nevada, sample from
```

Plain Text ▾ Tab Width: 8 ▾ Ln 1, Col 1 INS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Perl script to:

extract hit id,e-value, bit score,query start, query end, hit start, hit end as a tabular file.

Filter by E-values <1X10^{-5}, Coverage over 80%

```
#!/usr/bin/perl -w

#extract the hit id, e-value, bit score, query start, query end, hit
#start, and hit end as a tabular file

open(OUT,">GH9.ncbi.hmm.out.4.1.1");
open(IN,$ARGV[0]);
print OUT "Hit ID\t\t\t\te-value\t\tbit score\tquery start\t query end\thit start\thit end\n";
while(<IN>){
if($_=~/#/){
$_=~s/^$//; #first if statement removes the header lines of the .out #file
}
else{
@line = $_; #stores all the lines into an array
}

foreach(@line){
#for each line there is an array created for columns which are #separated by whitespaces
@col=split(/\s+/,$_);

#check to see if e-value is less than 1e-5 and also has a coverage >0.8
if($col[6]<1e-5&&(($col[16]-$col[15])/$col[5])>0.8){
#if true, prints out the required values.
print OUT $col[0], "\t", $col[6], "\t", $col[7], "\t\t\t", $col[15], "\t", $col[16], "\t", $col[17], "\t", $col[18], "\n";
}
}
}
```

GH9 JGI hmm out tabluar format

GH9.jgi.hmm.out.4.1(1).1

it ID		e-value	bit score	query start	query end	hit start	hit end
201231693	2.9e-146	501.1	1	418	278	706	
200984066	3.9e-144	494.1	1	418	37	474	
061998357	2.1e-143	491.7	1	418	51	488	
018727539	3.6e-143	490.9	1	418	51	484	
201115235	6.8e-141	483.4	1	418	666	1094	
200446424	4.9e-140	480.6	1	417	95	528	
078025289	4.6e-139	477.4	1	418	73	503	
029678661	1.5e-138	475.7	1	417	41	474	
201670307	3.1e-138	474.6	1	418	30	469	
150562325	1.9e-137	472.0	1	418	36	466	
150657657	1.9e-137	472.0	1	418	36	466	
150468268	3.3e-137	471.3	1	418	36	466	
150635057	3.3e-137	471.3	1	418	36	466	
150588393	3.3e-137	471.3	1	418	36	466	
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150525080	3.3e-137	471.3	1	418	36	466	
150601450	3.3e-137	471.3	1	418	36	466	
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150498305	3.3e-137	471.3	1	418	36	466	
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077991057	2.8e-135	464.9	1	418	53	483	
150675743	3e-134	461.5	1	418	42	460	
150675975	3e-134	461.5	1	418	42	460	
150635401	3e-134	461.5	1	418	42	460	
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150447302	3.1e-134	461.5	1	418	42	460	
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150471155	3.1e-134	461.5	1	418	42	460	
150524468	3.1e-134	461.5	1	418	42	460	
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018770236	3.4e-126	435.0	1	418	30	451	

GH9 JGI hit id's 1777

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2018691481

Plain Text

GH9 NCBI hmm out tabular format

it ID	e-value	bit score	query start	query end	hit start	hit end
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i|52003473|gb|AAU23415.1|	2.7e-150	498.3	1	418	42	475
i|52348141|gb|AAU40775.1|	2.7e-150	498.3	1	418	42	475
i|4490766|emb|CAB38941.1|	1.5e-149	495.9	1	418	40	479
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i|374343072|dbj|BAL46914.1|	4.8e-149	494.2	1	418	40	479
i|188011009|gb|ACD44896.1|	1.6e-148	492.5	1	418	5	437
i|310751795|gb|ADP09350.1|	1.6e-148	492.5	1	418	48	480
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i|1817723|gb|AAB42155.1|	4.9e-148	490.9	1	418	51	484
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i|157681096|gb|ABV62240.1|	5.8e-148	490.7	1	418	5	437
i|2897802|dbj|BAA24918.1|	1.2e-147	489.6	1	415	29	458
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i|7208809|emb|CAB76932.1|	1.2e-147	489.6	1	418	77	509
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i|359824960|gb|AEV67733.1|	1.1e-145	483.2	1	418	30	460
i|99644508|emb|CAK22316.1|	1.5e-145	482.7	1	418	31	463
i|3600052|gb|AAC35539.1|	1.6e-145	482.7	1	418	26	487
i|4850284|emb|CAB43040.1|	1.6e-145	482.7	1	418	26	487
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i|22136600|gb|AAM91619.1|	1.6e-145	482.7	1	418	26	487
i|30681638|ref|NP_192843.2|	1.6e-145	482.7	1	418	26	487
i|332657566|gb|AEE82966.1|	1.6e-145	482.7	1	418	26	487
i|125713287|gb|ABN51779.1|	2.1e-145	482.3	1	418	31	461
i|316940717|gb|ADU74751.1|	2.1e-145	482.3	1	418	31	461
i|581006|emb|CAA43035.1|	2.1e-145	482.3	1	418	31	461
i|334812465|gb|AEH04391.1|	6.6e-145	480.6	1	418	51	484
i|6179388|emb|CAB59900.1|	3.8e-144	478.1	1	418	32	488

GH9 NCBI hit id's 1452

Close document

```
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|42601[illegible]3.1|
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|52003473|gb|AAU23415.1|
|52348141|gb|AAU40775.1|
|4490766|emb|CAB38941.1|
|37498962|gb|AAQ91573.1|
|374343072|dbj|BAL46914.1|
|188011009|gb|ACD44896.1|
|310751795|gb|ADP09350.1|
|148767913|gb|ABR10904.1|
|6525242|gb|AAF15367.1|
|122937813|gb|ABM68635.1|
|1817723|gb|AAB42155.1|
|71916307|gb|AAZ56209.1|
|157681096|gb|ABV62240.1|
|2897802|dbj|BAA24918.1|
|144808|gb|AAA20892.1|
|125712789|gb|ABN51281.1|
|7208809|emb|CAB76932.1|
|316941198|gb|ADU75232.1|
|353441419|gb|AEQ94264.1|
|219544539|gb|ACL26277.1|
|451784663|gb|AGF55631.1|
|372100184|gb|AEX68682.1|
|141603871|gb|ABO88214.1|
|125713488|gb|ABN51980.1|
|316940505|gb|ADU74539.1|
|359824960|gb|AEV67733.1|
|99644508|emb|CAK22316.1|
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|4850284|emb|CAB43040.1|
|7267803|emb|CAB81206.1|
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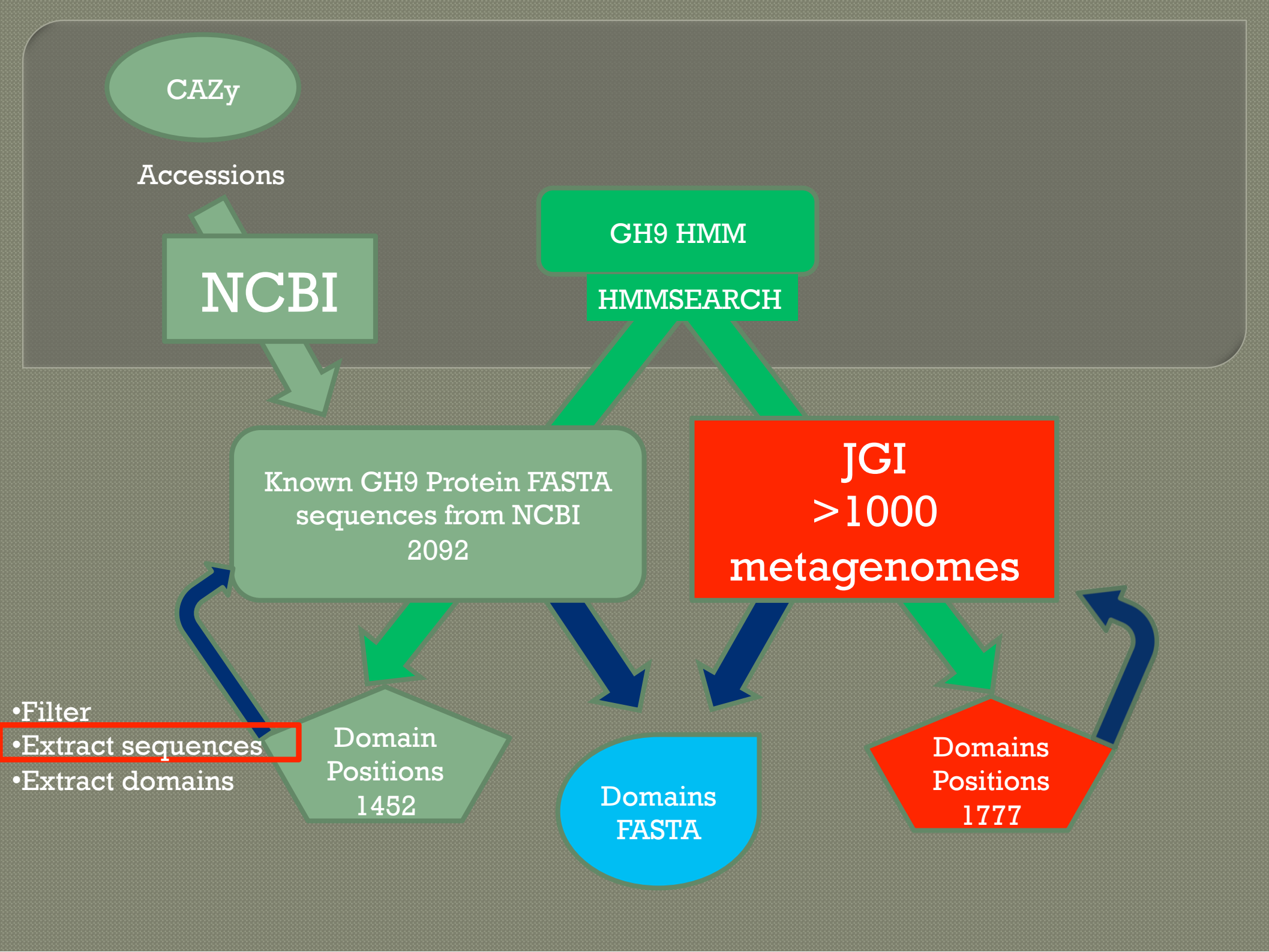
|30681638|ref|NP_192843.2|
|332657566|gb|AEE82966.1|
|125713287|gb|ABN51779.1|
|316940717|gb|ADU74751.1|
|581006|emb|CAA43035.1|
|334812465|gb|AEH04391.1|
|6179388|emb|CAB59900.1|
|1655545|emb|CAA65828.1|
```


CAZy
Accessions
NCBI
GH9 HMM
HMMSEARCH
Known GH9 Protein FASTA sequences from NCBI 2092
JGI >1000 metagenomes
•Filter
•Extract sequences
•Extract domains
Domain Positions 1452
Domains FASTA
Domains Positions 1777

Perl script to extract full length protein FASTA sequences

get_sequence.pl retrieve_fasta.pl

```
#!/usr/bin/perl -w

# retrieve_fasta

# Author: Steven Seydell
# Date: 4/13/2013

# This program will read in a file of unsorted sequence IDs, and locate the
# fasta format sequences in a sorted sequence library.
#
# Usage: perl retrieve_fasta.pl <input file> <database file> <output file>


# Use the BioPerl SeqIO library and a general utility library
use Bio::SeqIO;
use List::MoreUtils qw(uniq);

# Read the sequence IDs and sort them
if (open(INPUT, $ARGV[0]) == 0){
        die "unable to open input file $ARGV[0]";
}
@input_seq=<INPUT>;
@dup_seq = sort { $a <=> $b } @input_seq; # Sort numerically assending
@sorted_seq = uniq(@dup_seq); # remove duplicates

# Work through the database file, saving the sequence info as we go
$seqio_obj = Bio::SeqIO->new(-file => $ARGV[1], -format => "fasta" );
$seqio_obj2 = Bio::SeqIO->new(-file => ">$ARGV[2]", -format => "fasta" );
$current_index = 0;
$end_of_db = 0;

do { # loop for each value in the input file
        do { # loop to go through database until we find a target seq
                if (!($seq_obj = $seqio_obj->next_seq)) {
                        $end_of_db = 1;
                        next;
```

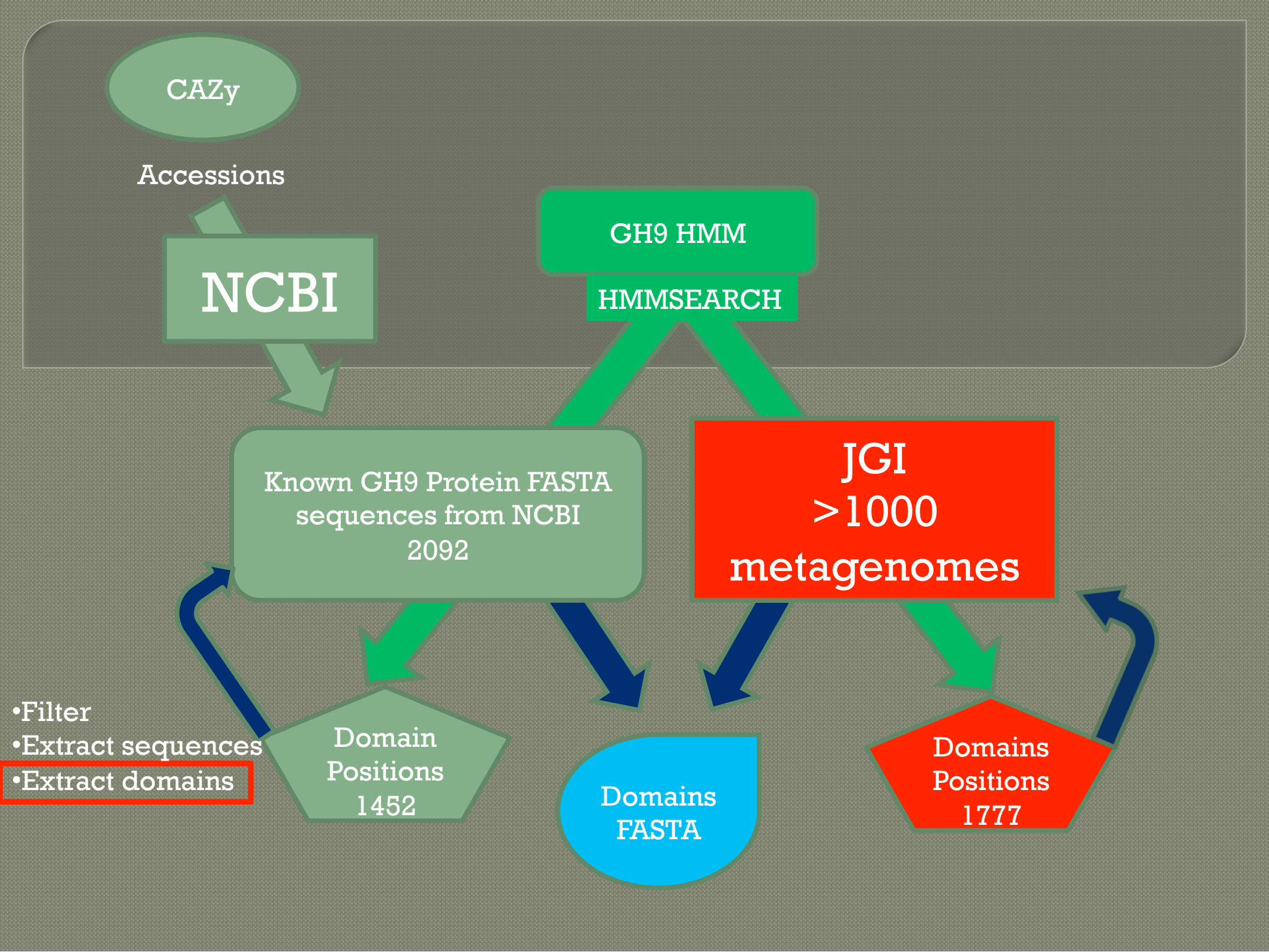
Perl script continued

```
                }
        } until (($seq_obj->display_id() == $sorted_seq[$current_index]) || $end_of_db);
        next if $end_of_db;

        # print the sequence out to the output file
        $seqio_obj2->write_seq($seq_obj);
        $current_index++;

} until (($current_index == $#sorted_seq + 1) || $end_of_db);
if ($end_of_db) {
        print "ERROR: end of database reached without finding all data.\n";
}

close INPUT;
```


CAZy
Accessions
NCBI
GH9 HMM
HMMSEARCH
Known GH9 Protein FASTA sequences from NCBI 2092
JGI >1000 metagenomes
•Filter
•Extract sequences
•Extract domains
Domain Positions 1452
Domains FASTA
Domains Positions 1777

Python script to retrieve domain regions while simultaneously creating a color code file

```
import re
from Bio import SeqIO

#Parsing FASTA into Biopython
FASTA= SeqIO.parse('GH9.jgi.hmm.out.4.1.1.fa', 'fasta')

#Opening HMM output
hitid = open('GH9.jgi.hmm.out', 'r')

#These are the files that will be written to
o = open('ncbi.output', 'a')
c = open('colors.output', 'a')

#Creating a list for all of the numbers
bank = []
#Creating a list for all rows in HMM output
bigfile = []

#Filling these lists.
for hit in hitid:
        bigfile.append(hit)
        preacc = hit.find(' ')
        accession = hit[:preacc]
        bank.append(accession)

#Actually getting sequences from the FASTA file
for sequence in FASTA:
#This step is to figure out if the FASTA id
#is in the HMM output
    if sequence.id in bank:
        if bank.count(sequence.id)>1:
                ind = bank.index(sequence.id)
                cnt=bank.count(sequence.id)
                add =[]
                while cnt !=0:
```

Python cont.

```
                    end = ''.join(preend)
                    end = int(end)

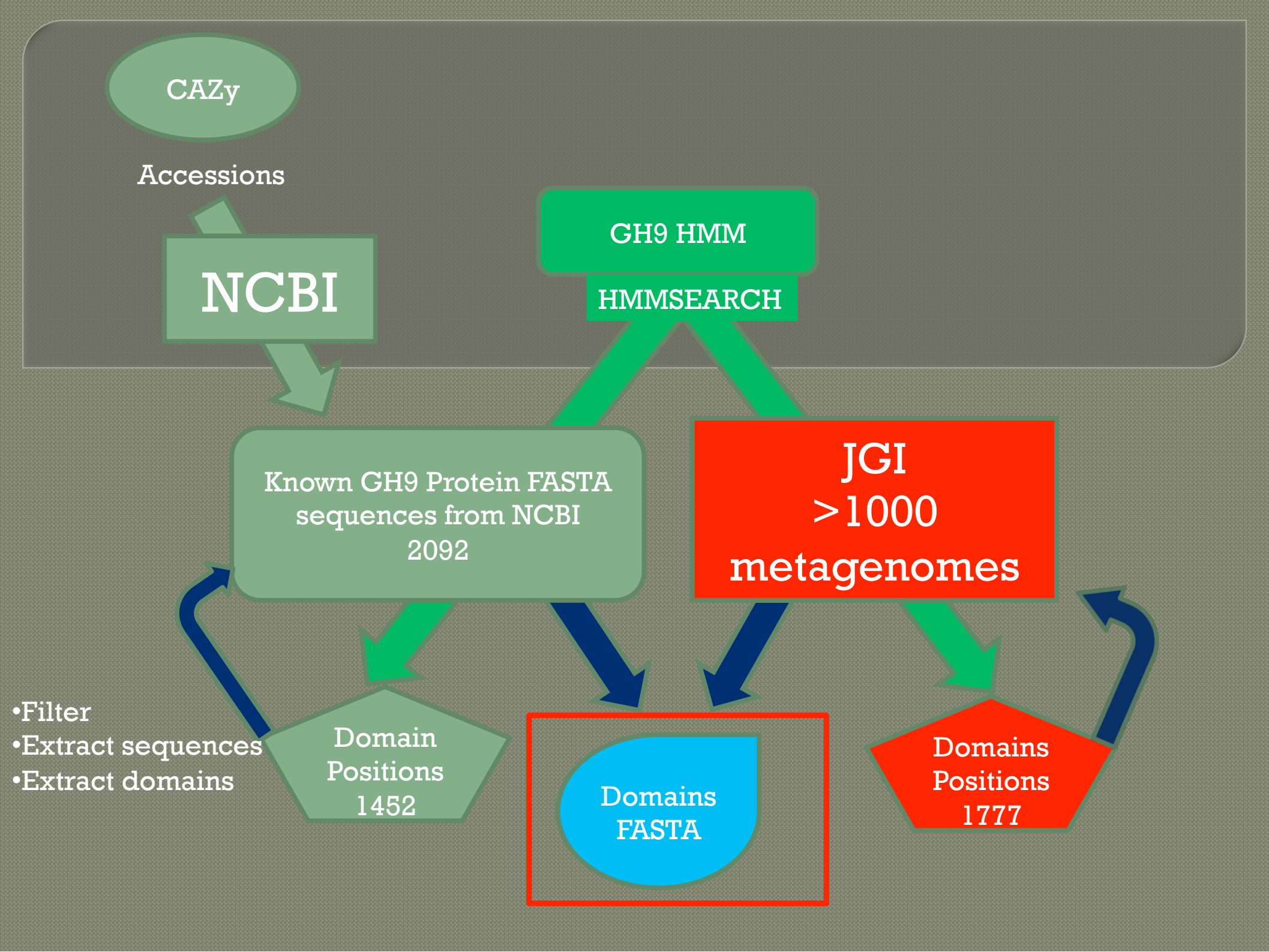
                    print '>',sequence.id,'\n'
                    o.write('>%s|%s|%s|\n' % (sequence.id, start+1, end+1))
                    c.write('%s|%s|%s|\t#FF0000\n' % (sequence.id, start+1, end+1))
                    print sequence.seq[start+1:end+1],'\n'

                    o.write('%s\n' % sequence.seq[start+1:end+1])
                    ###the +1 was added to offset the Python indices starting at zero.
                inds = []
        else:
                ind = bank.index(sequence.id)
                useline = bigfile[ind]
                prestart = re.findall('[0-9]', useline[153:158])
                start = ''.join(prestart)
                start = int(start)

                preend = re.findall('[0-9]', useline[158:164])
                end = ''.join(preend)
                end = int(end)

                print '>',sequence.id,'\n'
                o.write('>%s|%s|%s|\n' % (sequence.id, start+1, end+1))
                c.write('%s|%s|%s|\t#FF0000\n' % (sequence.id, start+1, end+1))
                print sequence.seq[start+1:end+1],'\n'
                o.write('%s\n' % sequence.seq[start+1:end+1])


hitid.close()
o.close()
c.close()
```


CAZy
Accessions
NCBI
GH9 HMM
HMMSEARCH
Known GH9 Protein FASTA sequences from NCBI 2092
JGI >1000 metagenomes
•Filter
•Extract sequences
•Extract domains
Domain Positions 1452
Domains FASTA
Domains Positions 1777

All Domains in FASTA format

```
>gi|20417|emb|CAA42569.1||31|486|
DALEKSILFFEGQRSGKLPTNQRLTWRGDSGLSDGSSYHVDLVGGYYDAGDNLKFGLPMAFTTTMLAWGIIEE
>gi|40672|emb|CAA28255.1||140|570|
DAFKTAMLGMYLLRCGTSVSATYNGIHYSHGPCHTNDAYLDYINGQHTKKDSTKGWHDAGDYNKYVVNAGITV
>gi|45504|emb|CAA31082.1||126|596|
AAFYDALKYFYHNRSGIAIETPYTGGGRGSYASHSRWSRPAGHLNQGANKGDMNVPCWSGTCNYSLNVTKGWY
>gi|144416|gb|AAA23086.1||39|473|
AEALQKSMFFYQAQRSGDLPADFPVSWRGDSGLTDGADVGKDLTGGWYDAGDHVKFGFPMAFSATMLAWGAIE
>gi|144808|gb|AAA20892.1||78|510|
GEALQKAIFFYECQRSGKLDPSTLRLNWRGDSGLDDGKDAGIDLTGGWYDAGDHVKFNLPMSYSAAMLGWAVY
>gi|166947|gb|AAA32912.1||31|486|
DALEKSILFFEGQRSGKLPTNQRLTWRGDSGLSDGSSYHVDLVGGYYDAGDNLKFGLPMAFTTTMLAWGIIEE
>gi|167883|gb|AAA52077.1||27|452|
CSLLENALMFYKMNRAGRLPDNDIPWRGNSALNDASPNSAKDANGDGNLSGGYFDAGDGVKFGLPMAYSMTMI
>gi|296791|emb|CAA39010.1||32|462|
GEALQKAIMFYEFQRSGKLPENKRDNWRGDSGLNDGADVGLDLTGGWYDAGDHVKFNLPMAYSQTMLAWAAYE
>gi|305112|gb|AAA68129.1||103|551|
SNIKSFYYQRSGVELPERLAGIWARPAAHLDDKLEFHPTMERAGLWNAHGGWYDAGDYGKYIVNGGVSVATLM
>gi|310897|gb|AAC06387.1||278|746|
ELRVDALSFYYPQRSGIEILDSIAPGYGRPAGHIGVPPNQGDTDVPCAPGTCDYSLDVSGGWYDAGDHGKYVV
>gi|349601|gb|AAA02563.1||41|489|
ADALAKAILFFEGQRSGKLPSSQRVKWREDSALSDGKLQNVNLMGGYYDAGDNVKFGWPMAFSTSLLSWAAVE
>gi|530014|emb|CAA56918.1| 309 811
QMKYDALAFFYHKRSGIPIEMPYAGGEQWTRPAGHIGIEPNKGDTNVPTWPQDDEYAGIPQKNYTKDVTGGWY
```

Domains FASTA

JGI: 1777 GH9 domains from 211 metagenomes
NCBI 1452 GH9 domains

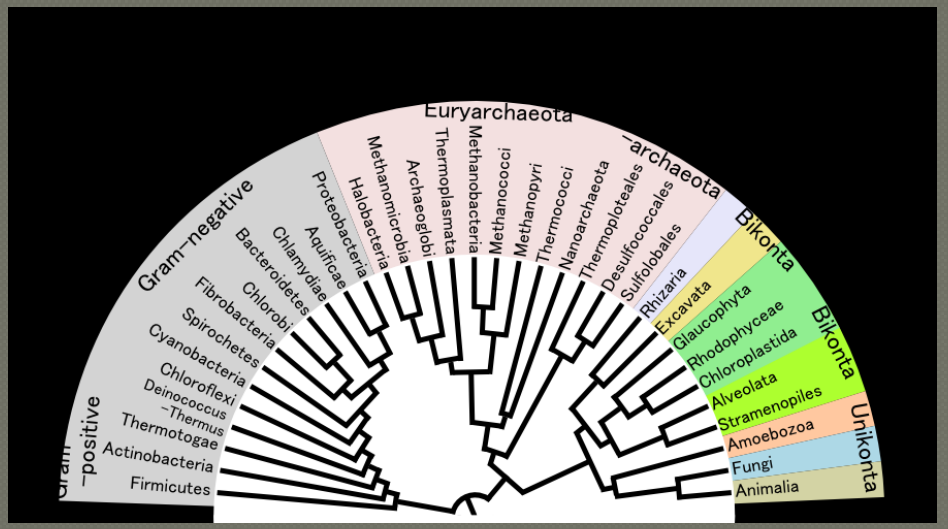
MAFFT

Domains Aligned

FastTree

ITOL

Phylogeny



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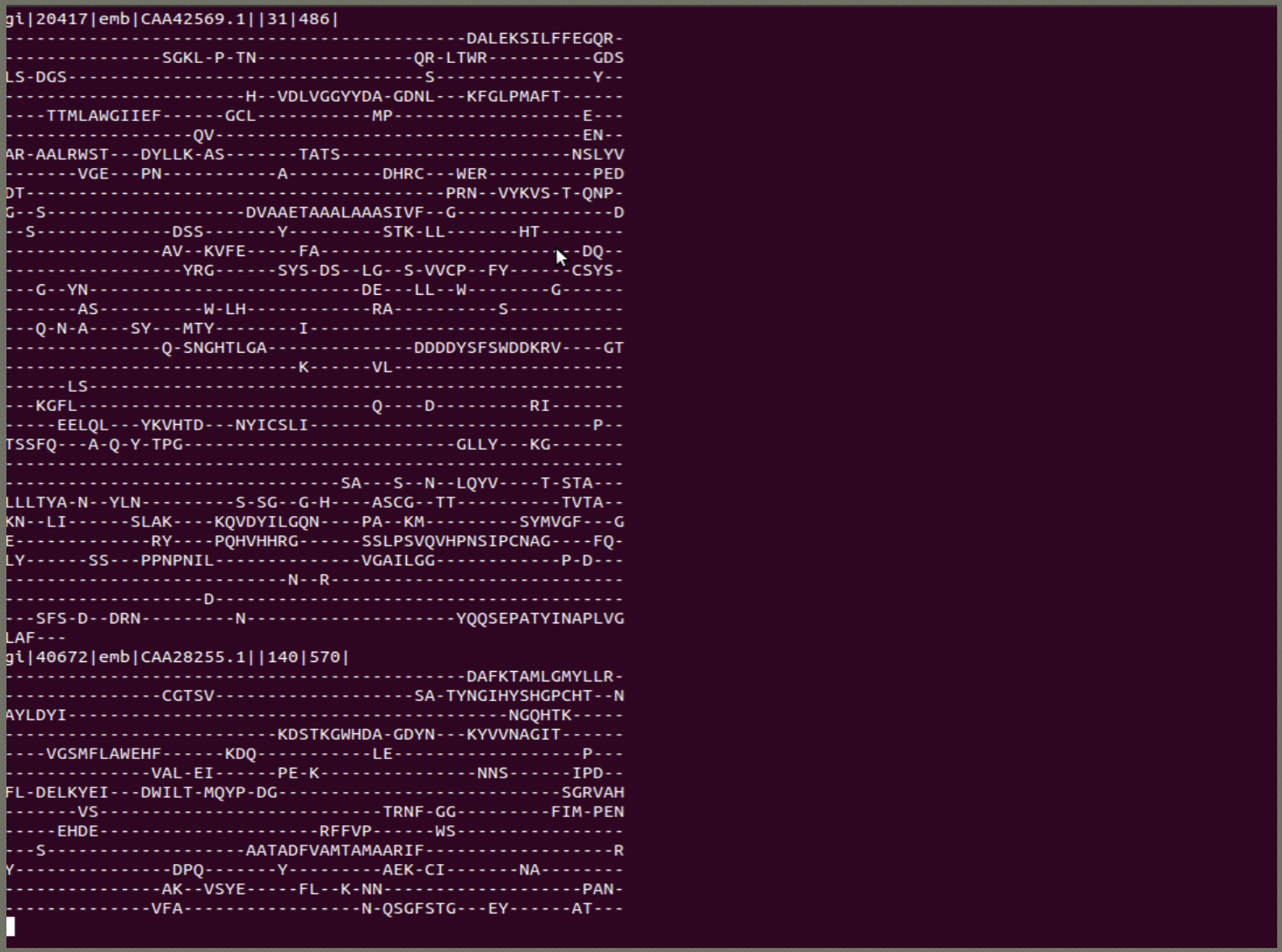


Domains FASTA

JGI: 1777 GH9 domains from 211 metagenomes
NCBI 1452 GH9 domains

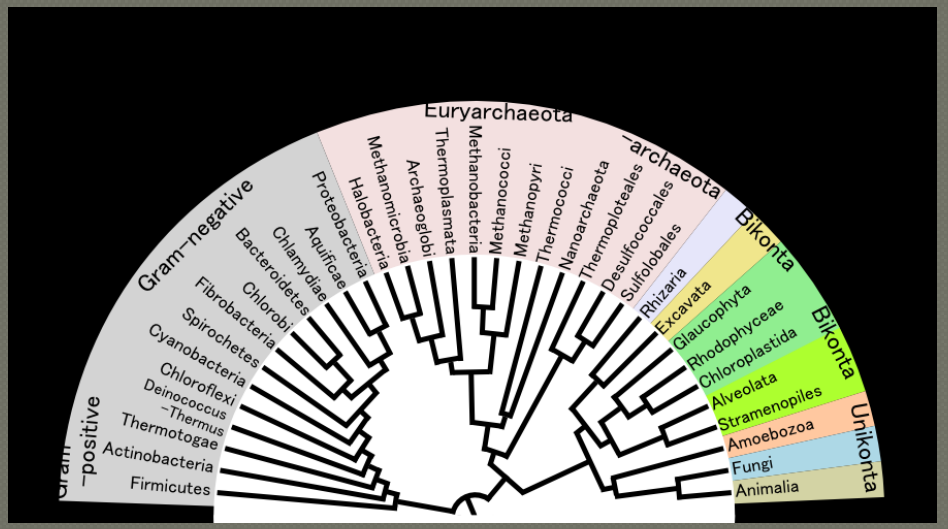
MAFFT

Domain s Aligned

FastTree

ITOL

Phylogeny



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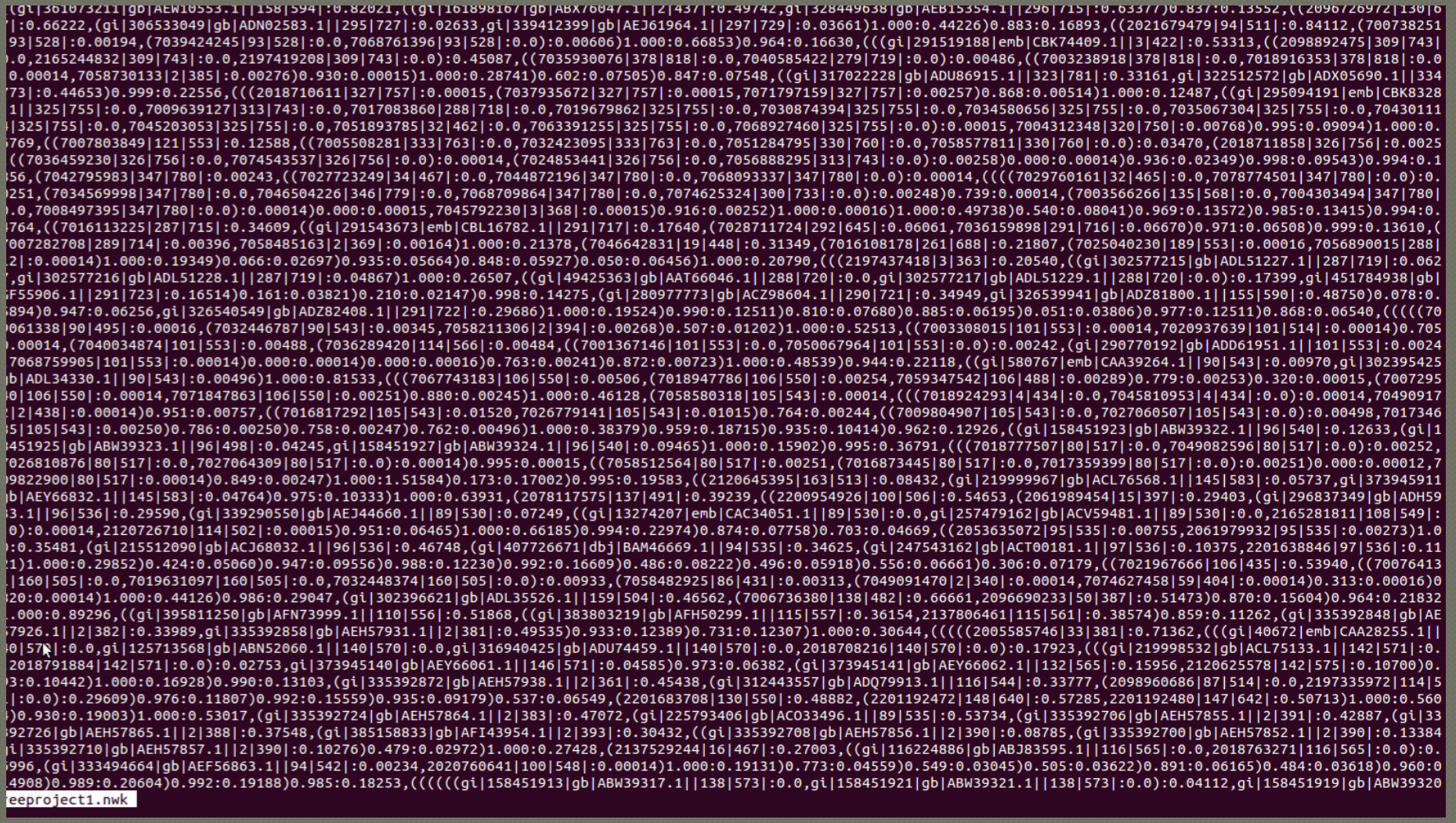


Domains FASTA

JGI: 1777 GH9 domains from 211 metagenomes
NCBI: 1452 GH9 domains

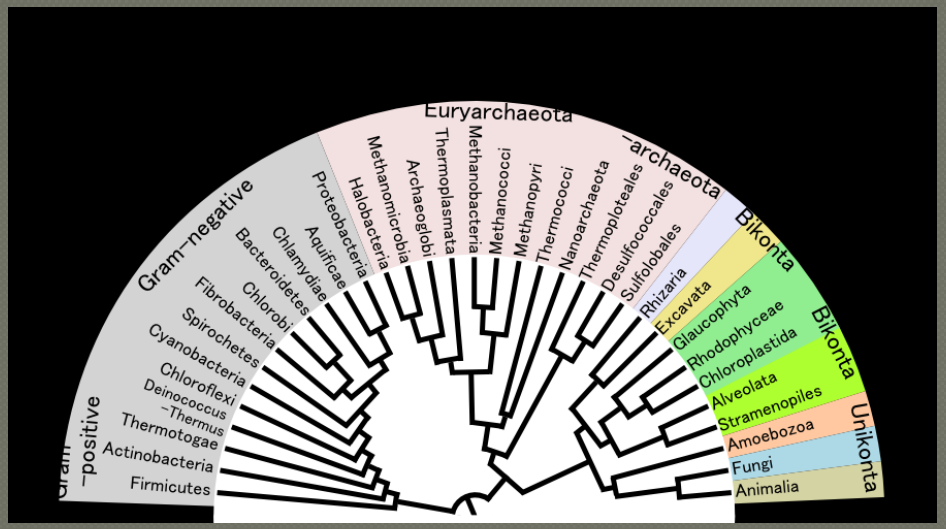
MAFFT

Domain s Aligned

FastTree

ITOL

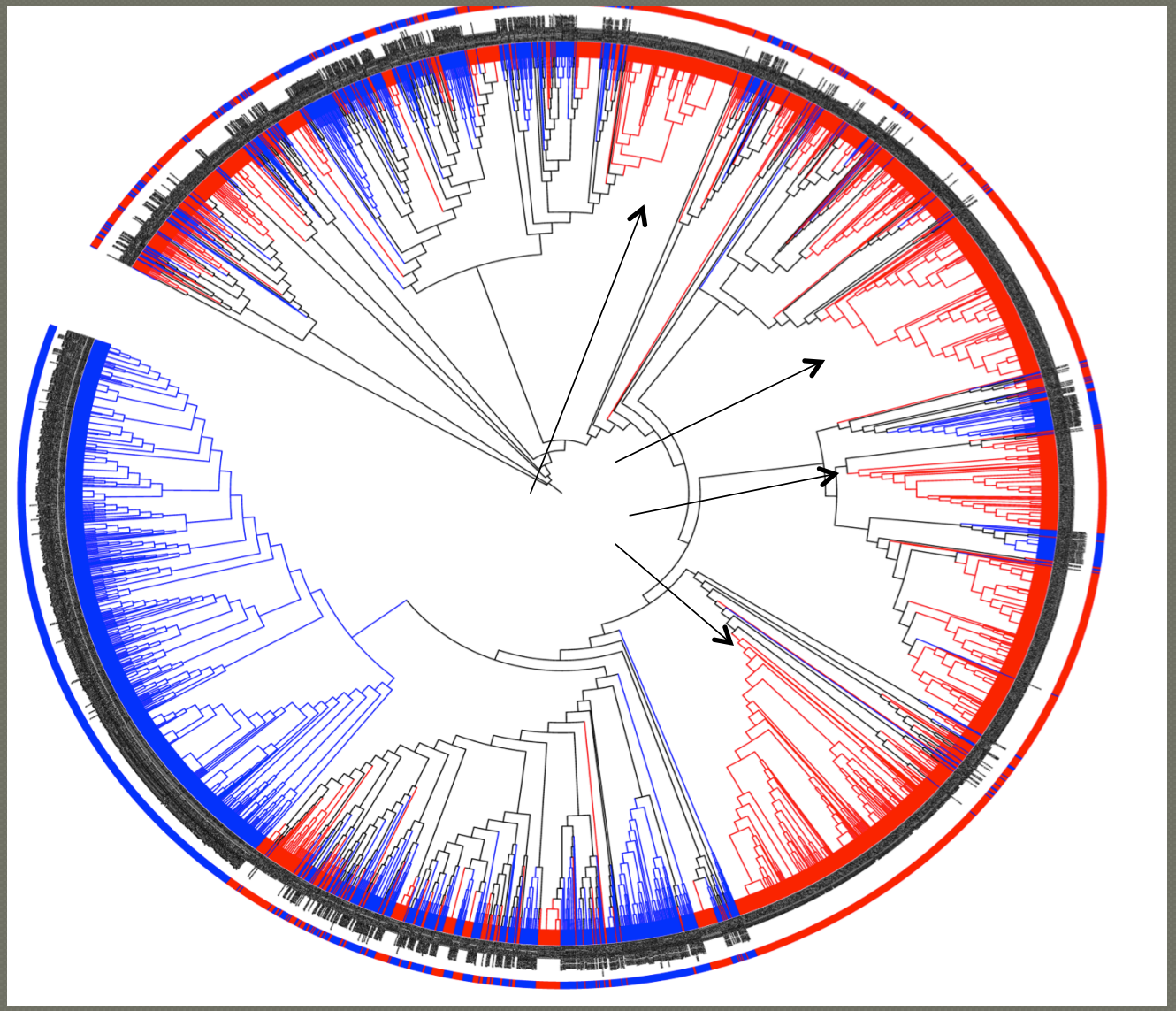
Phylogeny

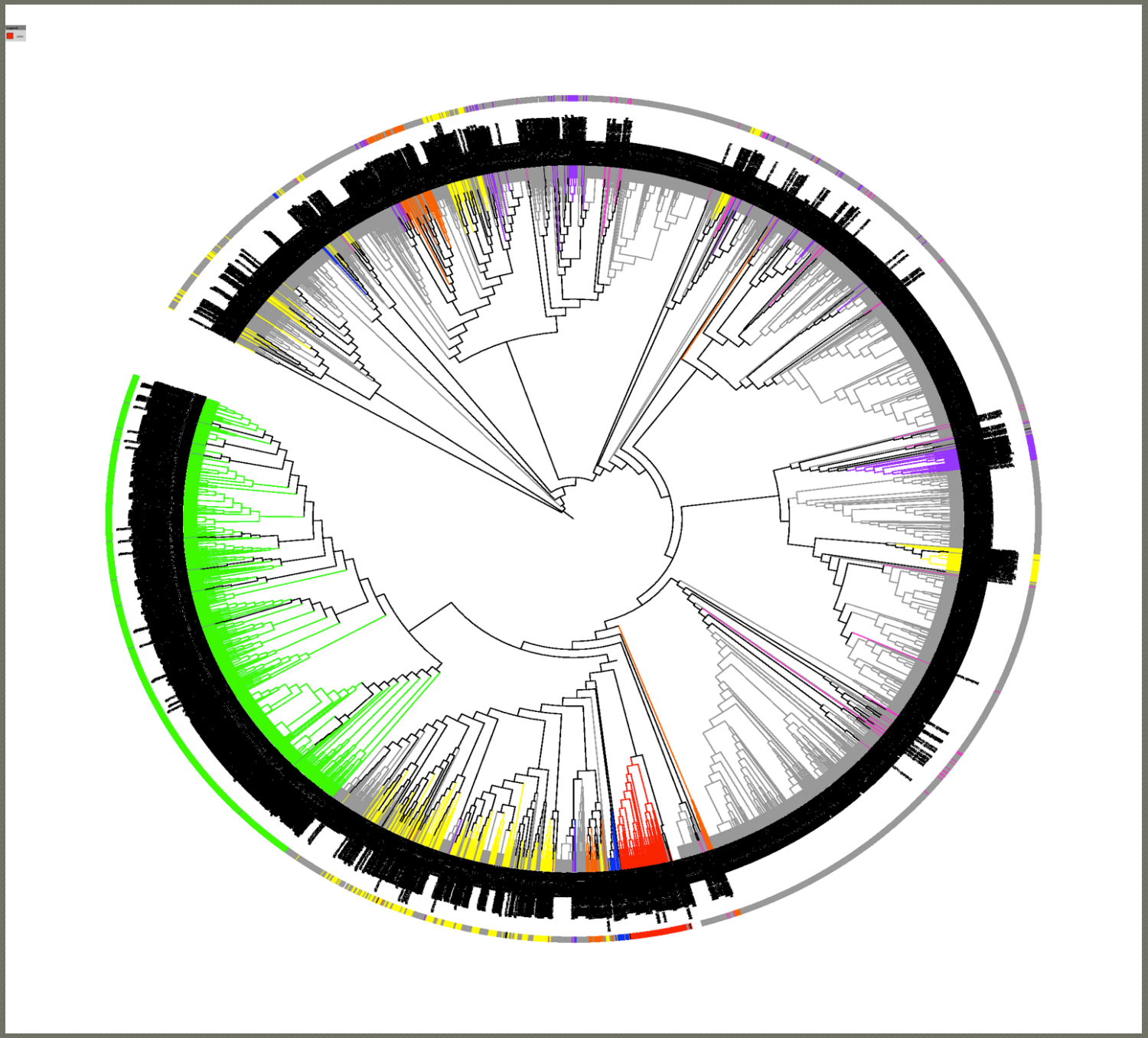


Tree created in iTOL-

Red: JGI

Blue: NCBI





Misc. Bacteria

Fungi

Cyanobacteria

Bacteroidetes

Metazoa

Actinobacteria

Proteobacteria

Viridiplante

Firmicutes

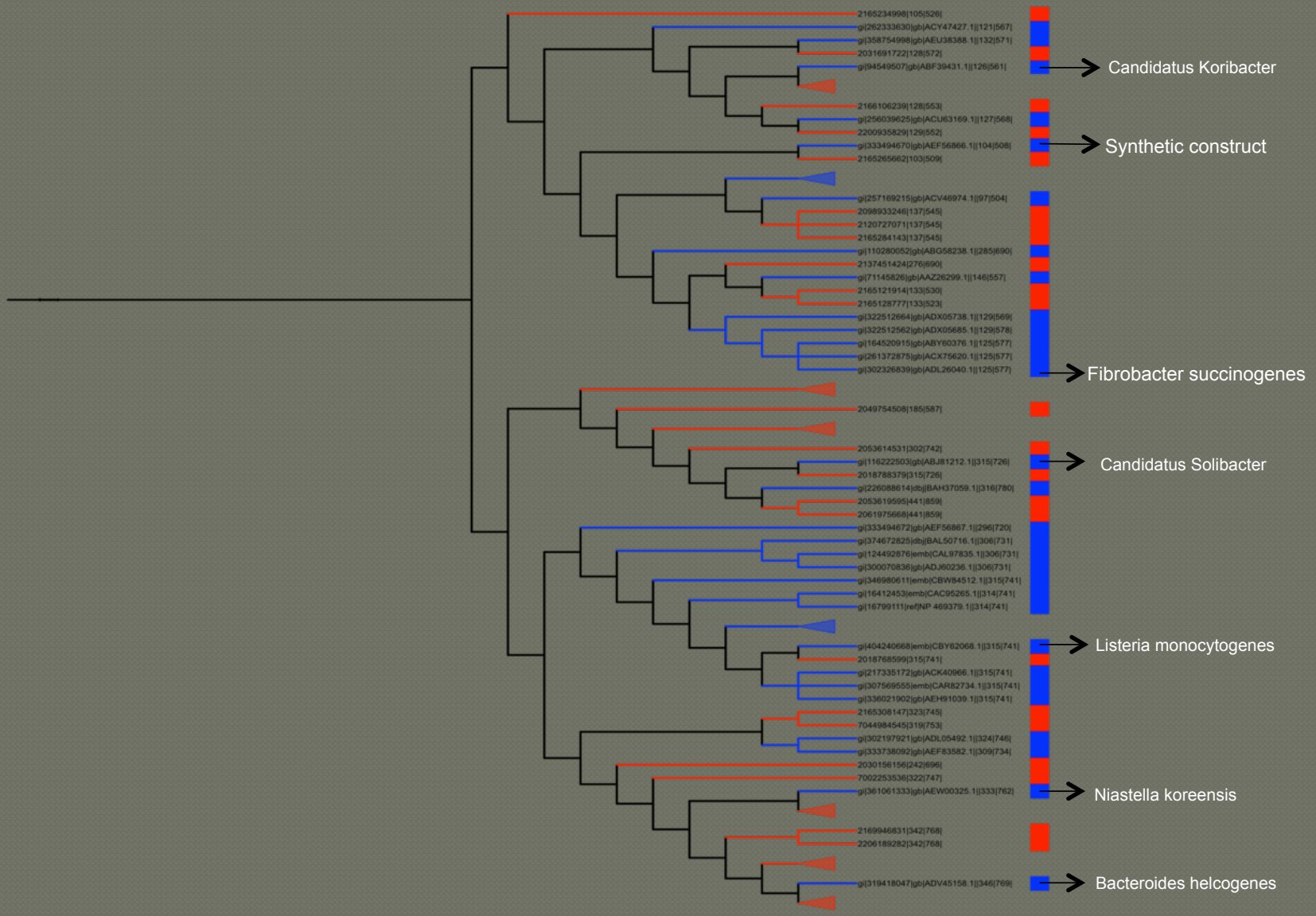
Protists

Stramenopiles

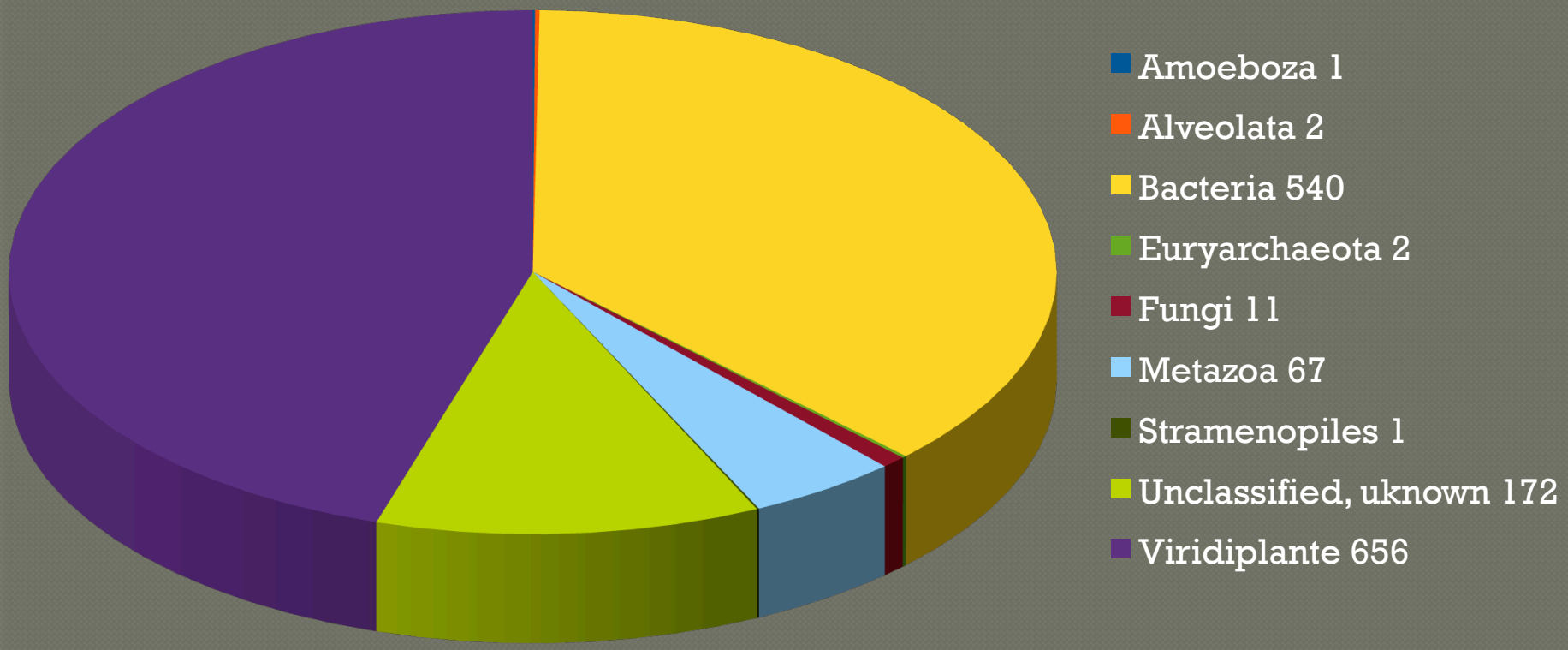
Euryarchaeota

All unknown organisms - Gray

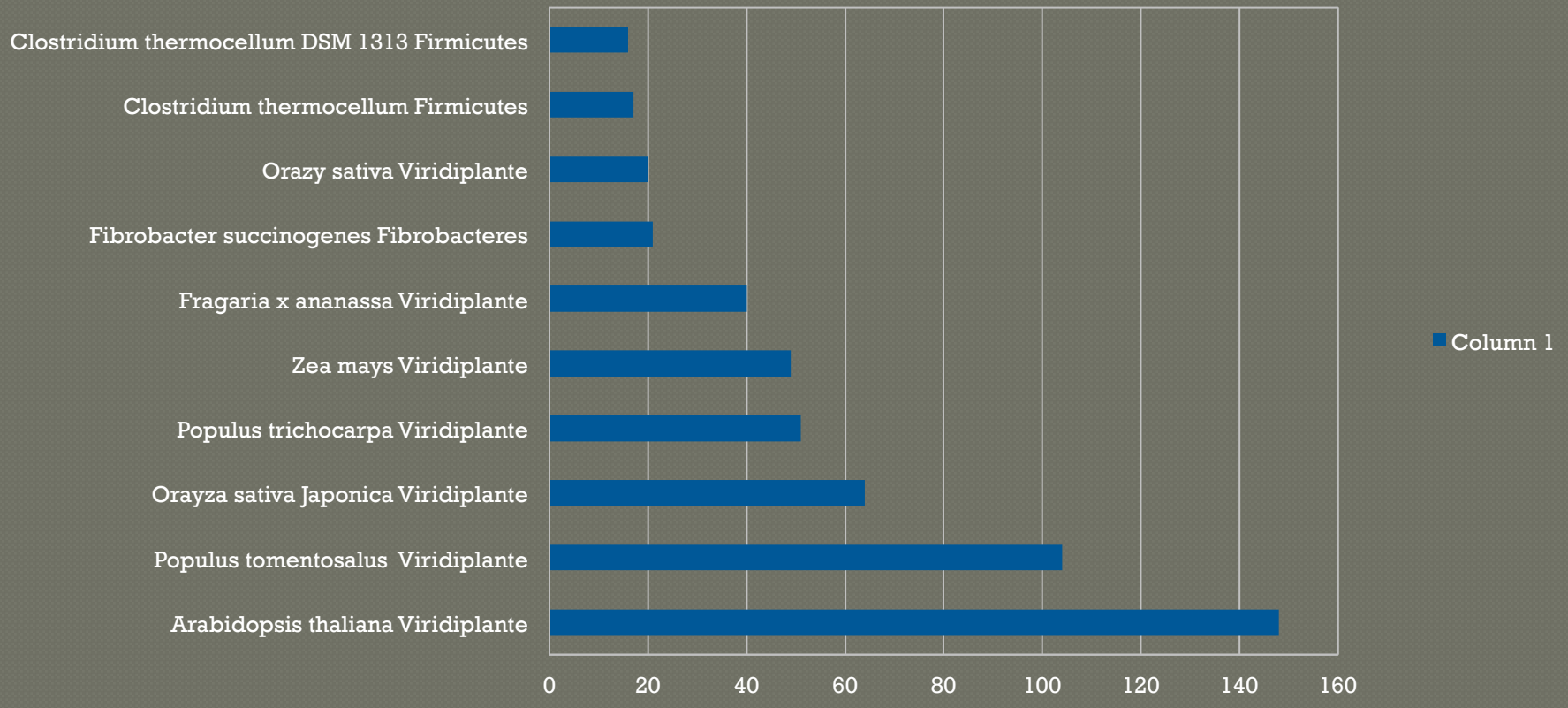
Un-banked GH9 proteins



Phyletic distribution of 1452 GH9 sequences in NCBI That contain biochemically confirmed cellulases

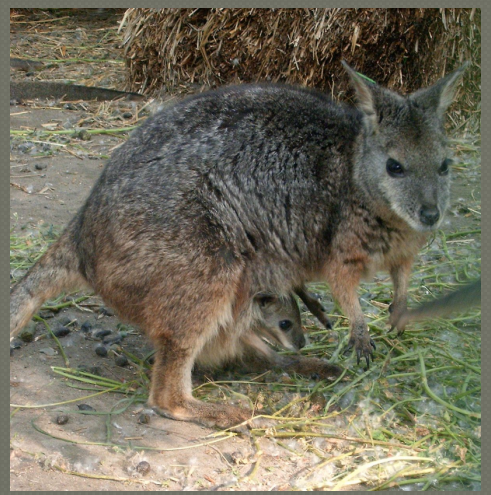


Top 10 organisms with multiple GH9 domains



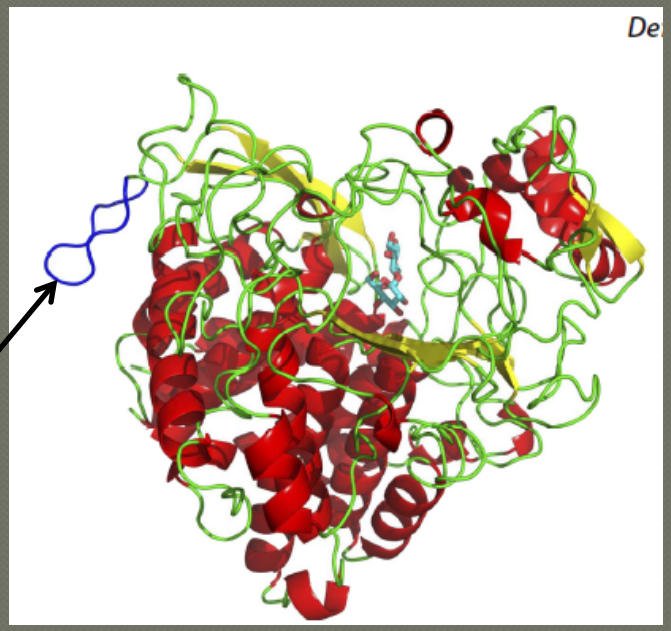
Examples of metagenomic sources of GH9 domains:

- Underneath the Human Tongue
- *Bankia setacea* gills
- Rice straw compost
- Hot springs
- Reindeer rumen
- *Macropus eugenii* forestomach



Novel GH9? What Next?

- Locate, Isolate, Experimentally Test
  - Computational identification is not reliable
  - Efficiency
    - Synergy with GH48
  - Working Conditions
  - 3D Structure



Sukharnikov et al., 2012

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