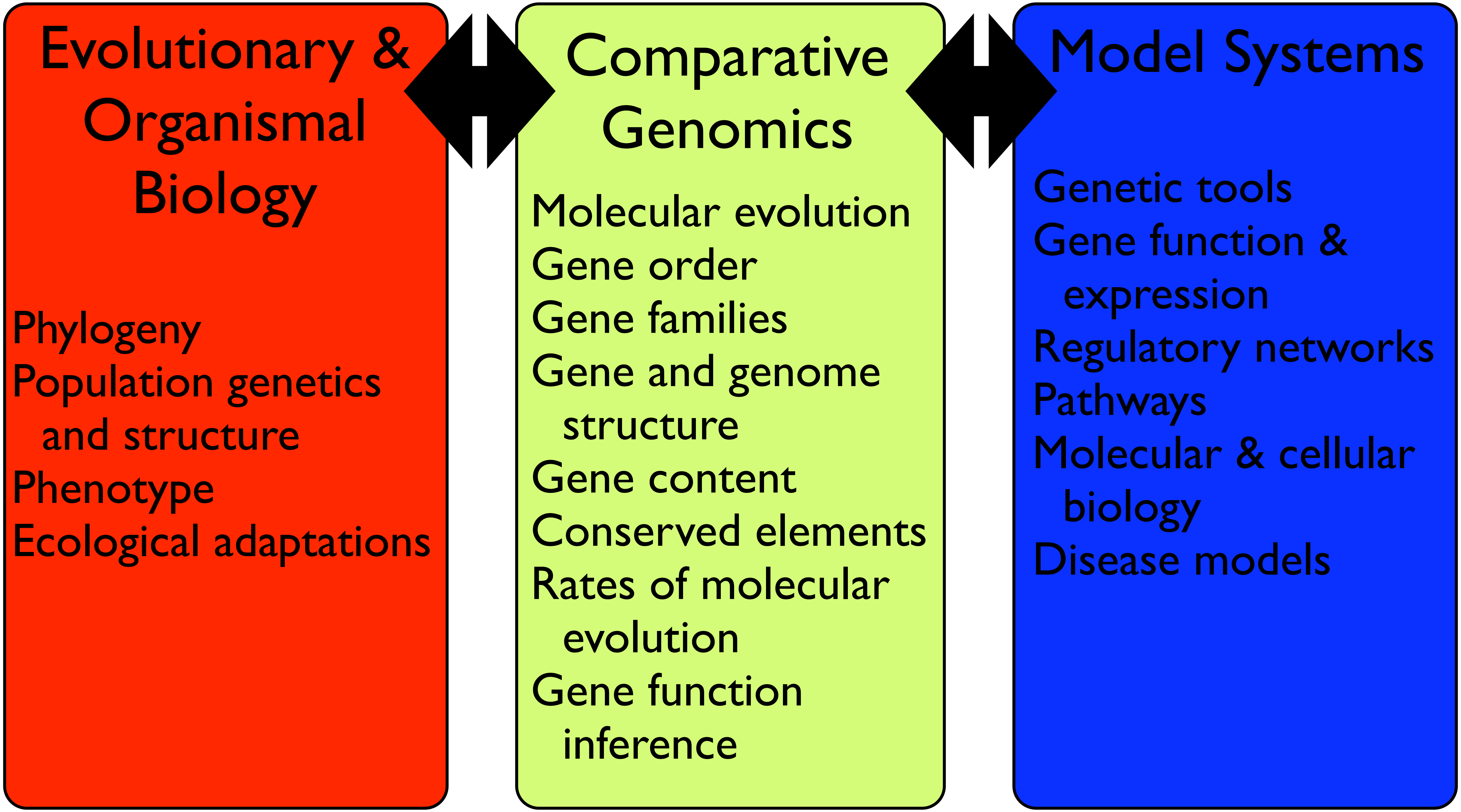
FUNGAL COMPARATIVE GENOMICS

Jason Stajich
Duke University
UC Berkeley (Aug 2006)

Evolutionary genomics



Evolutionary & Organismal Biology

- Phylogeny
- Population genetics and structure
- Phenotype
- Ecological adaptations

Comparative Genomics

- Molecular evolution
- Gene order
- Gene families
- Gene and genome structure
- Gene content
- Conserved elements
- Rates of molecular evolution
- Gene function inference

Model Systems

- Genetic tools
- Gene function & expression
- Regulatory networks
- Pathways
- Molecular & cellular biology
- Disease models

Industrial uses of fungi

- Bread, beer, wine - *Saccharomyces cerevisiae*
- Sake and soy sauce - *Aspergillus oryzae*
- Dairy - *Penicillium roqueforti*, *Kluyveromyces lactis*
- Citric acid - *Aspergillus niger*
- Riboflavin - *Ashbya gossypii*
- Stonewashed jeans - *Trichoderma reesei*
- Penicillin antibiotic - *Penicillium notatum*
- Button Mushrooms - *Agaricus bisporus*

Agricultural impact of fungi

- Two-thirds of plant disease is caused by fungi
  - Wheat blight (*Fusarium*)
  - Strawberry grey mold (*Botrytis*)
  - Leaf rusts (*Puccinia*)
  - Wheat and maize smuts (*Ustilago*).
  - Also deposit mycotoxins - *e.g.* ergot
- Mycorrhizal fungi provide nutrient exchange and nitrogen fixation

www.gov.mb.ca

USDA

A.G. Bölker

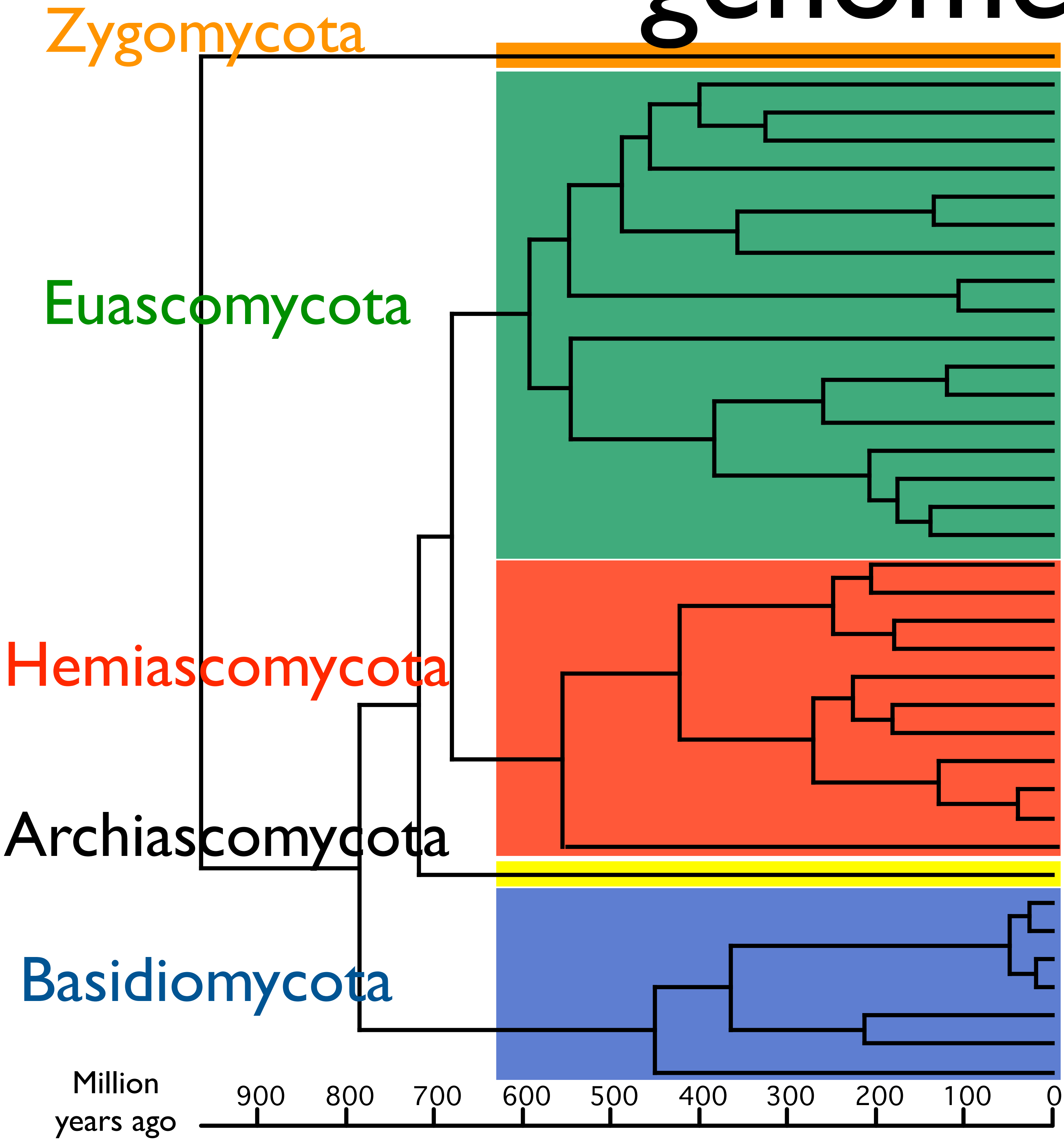
FUNGAL COMPARATIVE GENOMICS

- Problems
  - Many fungal genomes
  - No central place for annotations, interlinking homolog information
  - Want to visual gene structures and genome context
  - Need system for good database system for scripting genome questions

GETTING THE DATA IN

- GFF3 as the data transfer format
- Write GenBank -> GFF3 scripts
- Read in data from genome Centers (Broad, Sanger, WashU, JGI, SGD)
- Pipeline for Genome Annotatation

37 Fully sequenced fungal genomes


Zygomycota

Euascomycota

Hemiascomycota

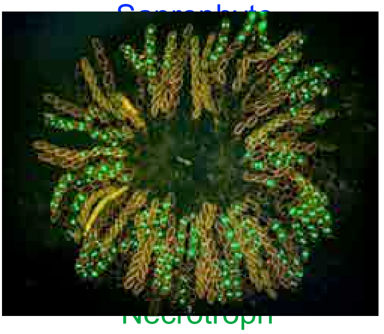
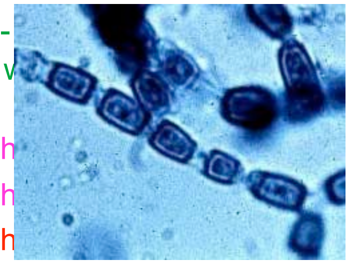
Archiascomycota

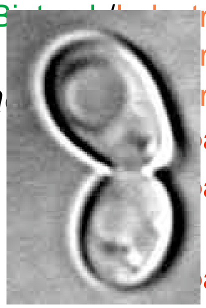
Basidiomycota

Million years ago

900 800 700 600 500 400 300 200 100 0

Rhizopus oryaze	Bread mold, Opp Hum pathogen
Neurospora crassa	Saprophyte
Podospora anserina	
Chaetomium globosum	
Magnaporthe grisea	
Fusarium verticillioides	H
Fusarium graminearum	H
Trichoderma reesei	
Sclerotinia sclerotiorum	Necrotroph
Botrytis cinerea	Necrotroph -
Stagonospora nodor	- v
Uncinocarpus reesii	
Coccidioides immitis	ath
Histoplasma capsula	ath
Aspergillus fumigatu	ath
Aspergillus nidulans	rophyte
Aspergillus terreus	athogen
Aspergillus oryzae	Saprophyte/Industrial uses
Ashbya gosspyii	Bi rial uses
Kluyveromyces lactis	rial uses
Saccharomyces cerevisia	rial uses
Candida glabrata	athogen
Candida lusitaniae	athogen
Debaryomyces hansenii	
Candida guilliermondii	athogen
Candida tropicalis	Opp Hum pathogen
Candida albicans	Opp Hum pathogen
Candida dubliniensis	Opp Hum pathogen
Yarrowia lipolytica	Industrial uses
Schizosaccharomyces pombe	
Cryptococcus neoformans	O
Cryptococcus neoformans H	
Cryptococcus gattii WM276	O
Cryptococcus gattii R265	O
Phanerochaete chrysosporiu	
Coprinus cinereus	
Ustilago maydis	Biotroph - maize

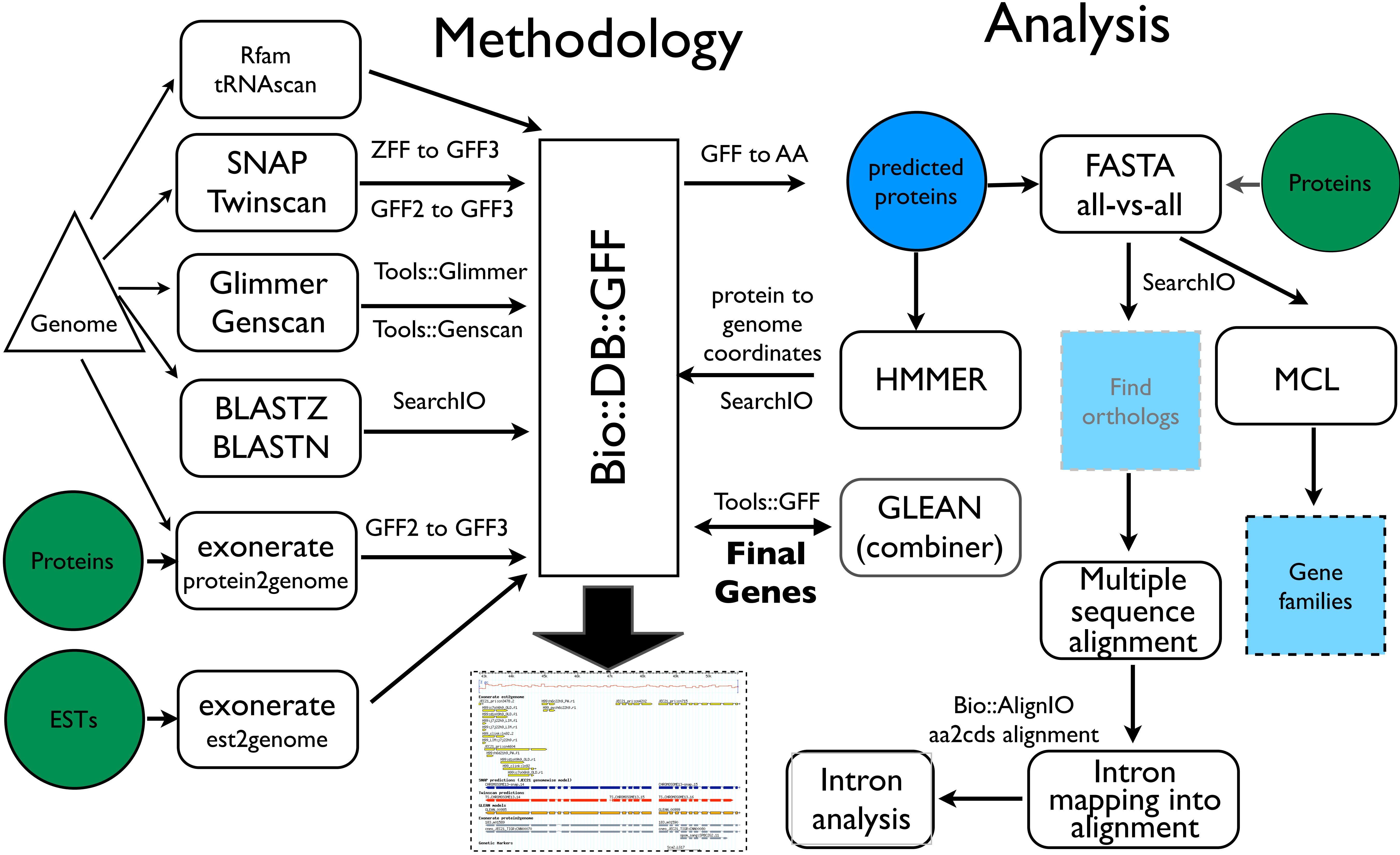






51+ More funded and in progress world-wide

Methodology

Analysis



Rfam
tRNAscan

SNAP
Twinscan

ZFF to GFF3

GFF2 to GFF3

Genome

Glimmer
Genscan

Tools::Glimmer

Tools::Genscan

BLASTZ
BLASTN

SearchIO

Proteins

exonerate
protein2genome

GFF2 to GFF3

ESTs

exonerate
est2genome

Bio::DB::GFF

GFF to AA

predicted proteins

FASTA
all-vs-all

Proteins

SearchIO

protein to genome coordinates

SearchIO

HMMER

Find orthologs

MCL

Tools::GFF

Final Genes

GLEAN
(combiner)

Multiple sequence alignment

Gene families

Bio::AlignIO
aa2cds alignment

Intron analysis

Intron mapping into alignment

http://fungal.genome.duke.edu

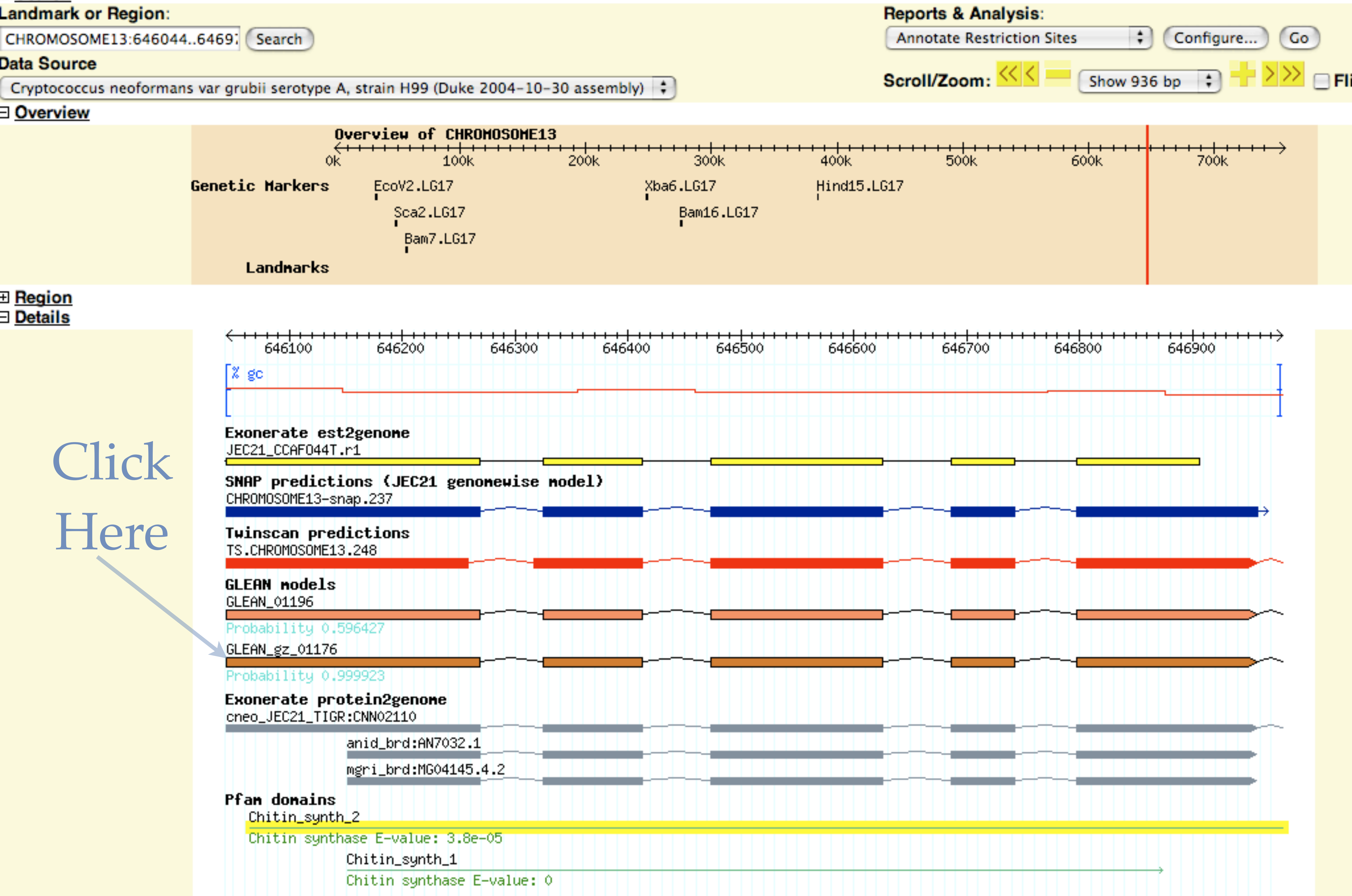
WHAT I NEEDED

- Database for storing and querying genome annotations
  - Bio::DB::GFF (BioPerl & Gbrowse)
- Visualization - Gbrowse
- Analyses
  - Ability to query for a gene's exon-intron structure and sequences
  - Are gene families clustered on chromosome?
  - Are functional classes of genes clustered on chromosome?

GBROWSE

- Visualization of annotation data
- Does not have to be for whole/finished genomes
  - Most projects are unfinished so many contigs (100s - 1000s)
  - BLAST interface with link to Gbrowse view allows user to start with query sequence and get to the genomic location

GBROWSE VIEW



Landmark or Region: CHROMOSOME13:646044..6469

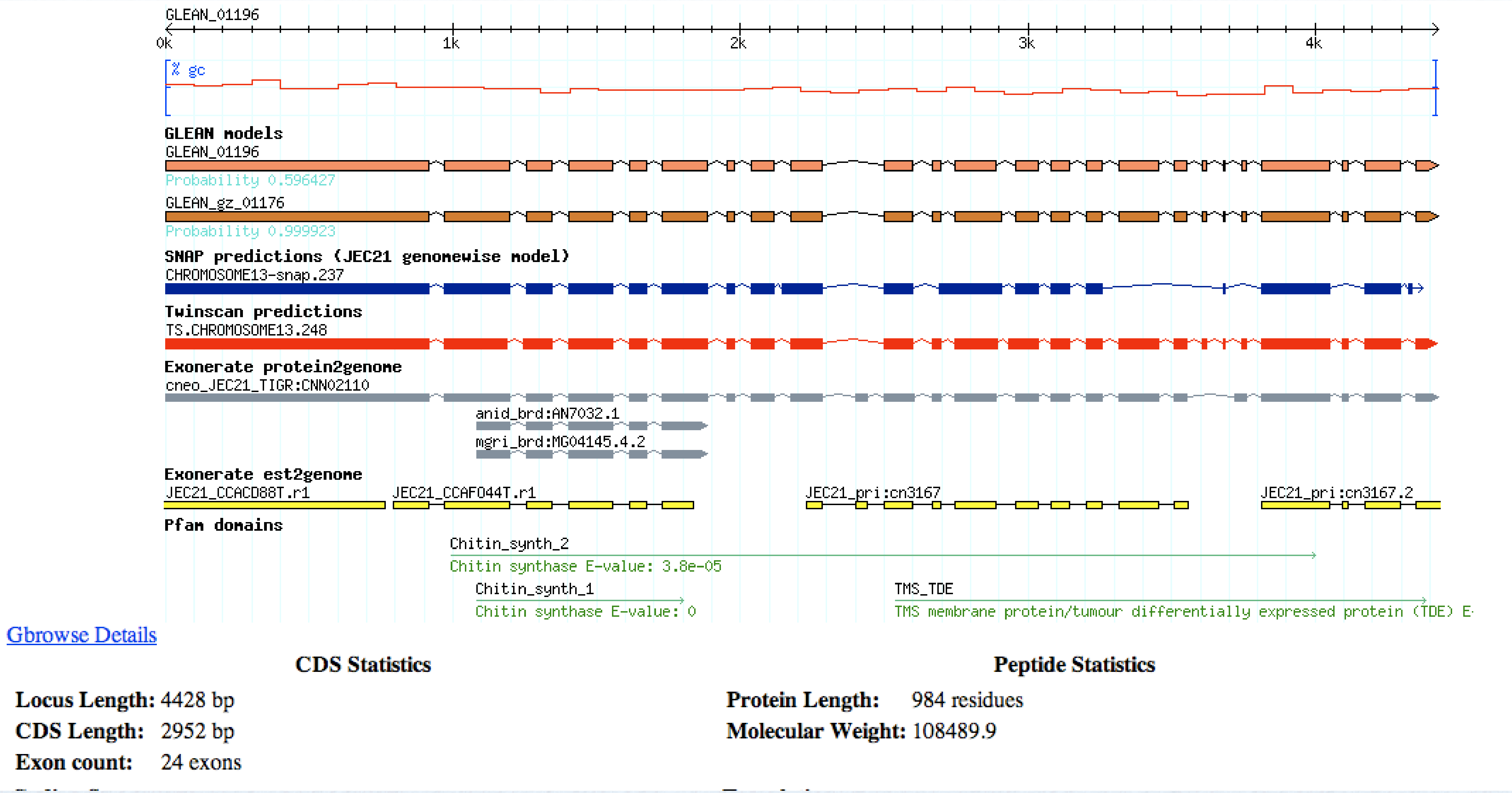
Data Source: Cryptococcus neoformans var grubii serotype A, strain H99 (Duke 2004-10-30 assembly)

Reports & Analysis: Annotate Restriction Sites

Scroll/Zoom: Show 936 bp

Click Here

GENE PAGE (1)



Gbrowse Details

CDS Statistics		Peptide Statistics	
Locus Length:	4428 bp	**Protein Length:**	984 residues
CDS Length:	2952 bp	**Molecular Weight:**	108489.9
Exon count:	24 exons		

GENE PAGE (2)

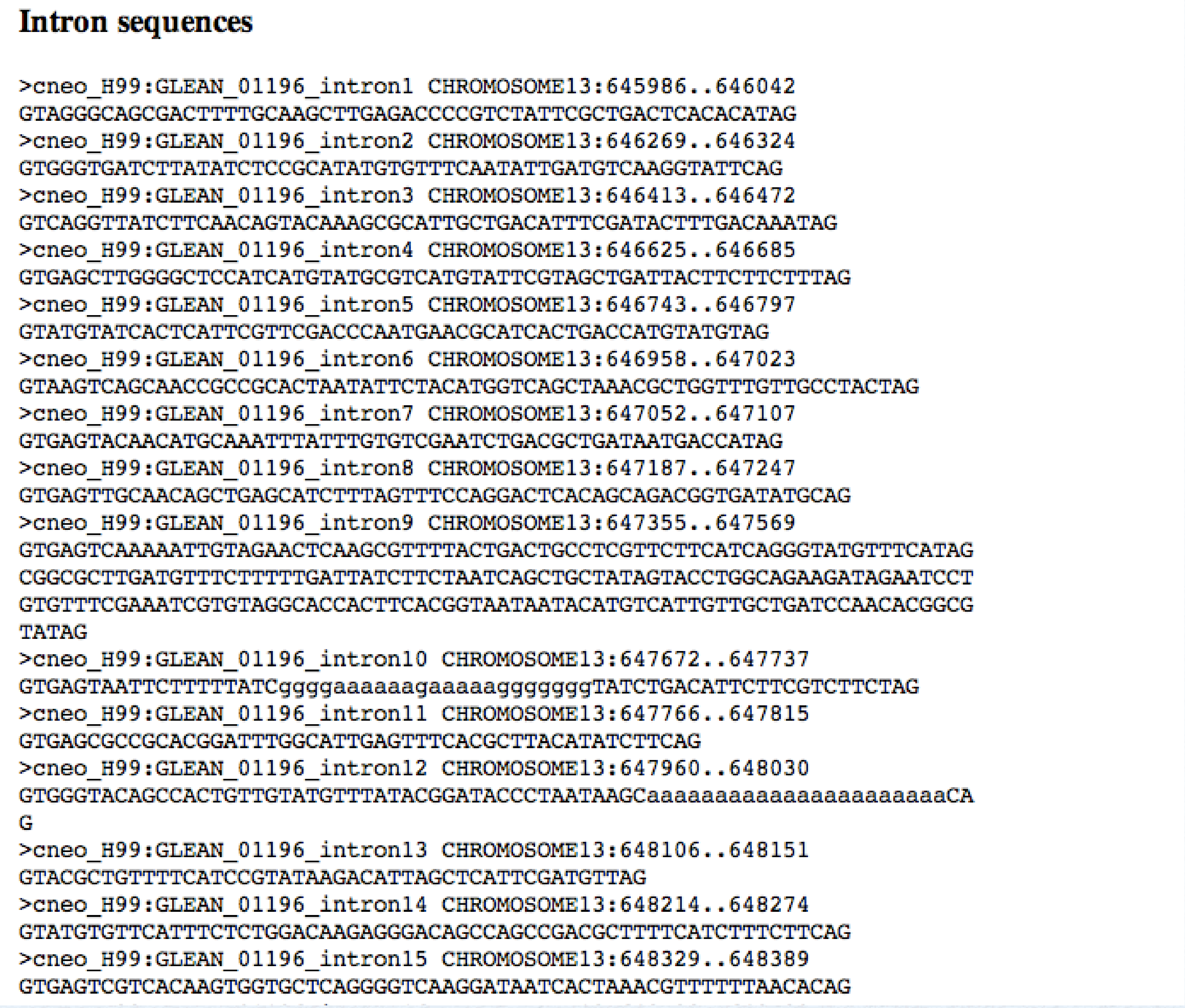
Coding Sequence

```
>cneo_H99:GLEAN_01196
ATGCCAAACATATCACGCAAACCTCCTCCGCGcttctactctccttctcactccccttcgccttcacttt
ATGCTCCCATACAATCACCCCCGGCCCCCTCTTATGACTACCACGCCAACCCCAGGACGTTGAATCCATT
CTCCGACGCACGTGAAGTCGGTGGATATGCTCAACTCCAAGGGGAAGATCAAATGACTGGCGCACCTTTA
TACCAGCCTCCGTATGCTCCTCAACTACTCGTTGCTCAACCAActcctgtttcttcccgcctcccgtttt
tcGAAGCTGCGCTTGCCCGCGCGCGCGGTATCCAAACGCCCAGCTTACCAGAAGCCCCGACTCCAGCTTA
TGcccaacctttaccttcctacctcccgcctcccGACCCCAATCACCCTGATCTATCTGTTGGTTTGACT
CAGGCCAATACAGTTCGCTACGCCATCAATCCAAGATCTCAGTTGAAGGAAGGATCCCGTTCGCCTTCAC
CGTTCATGGATGACAGTTTCGTTTACAATGATGCTGCTCACCTCTATAACGTTGAGCCGGACGTCGAAAA
AGCTTTGCTCGGAAGTGGACTGGGTTATGAATCGGAGAAGCGTGTCGAATCTTCGATGGGCTTCAATGAC
AATGATGGCGACCTCTCAGTCCCTCAGTCGTTTGGTGGCCGACCTCCTTCATGGGAGCCGAGCGGCATAT
TGGATGAAAAAGGGGAAATGTCGACTACAAAGCATTTTGGGCCTGCACCTGCGGGTCGAGTCGGTCGGCG
AGCGCACAACGCTGCAGGGTACCGCAGGATCAAACAATCAGCGACCCTCGATGAGAATGGTTTCTTTGCT
ATCGAGATGAACATCCCCACCCGACTGGCGCAATTTCTACCCATCAAGGGAGTTGAAGAGCAAAAGACTA
CAAGGTATACTGCGATTACCACCGACCCAGATGATGTCCCAGCAGCTGGCTTCCGTCTTCGCCAGAACAT
GACTTCTCCGCCCCGACAGACTGAACTTTTTATCGTGATCACTATGTACAATGAGAACGCCGAGCTCTTT
TGTCGAACACTTTATGGTGTCATGAAGAATATAGCCCACCTATGTGGGCGTAAGAACTCAAGGGTCTGGG
GCAAGGATGGTTGGCAAAAGGTTGTCGTTTGCATTGTCGCGGACGGACGTAAGGCGGTTAACCCCCGCGT
CCTCGATTGTTTAGCAGCTCTTGGAGTTTACCAAGAAGGCGCAATGACGAACACAGTAAAGGATCGACCG
GTCACAGCGCATGTTTTCGAATACACGACCAGCTTTGCTCTTGACGGTGATTTACACTTCAAATATCCAG
ACAAAGGCATTGTCCCCTGCCAGATTATCTTCTGCATGAAAGAGAAAAATGCCAAAAAGATCAACTCCCA
TCGATGGTTTTTCAACGCCTTCGCGCCCTTGCTATCACCAAATGTCTGCATTCTTCTTGATGTGGGAACC
CAGCCAGCTCCGAAATCCATCTATCATCTTTGGAAAGCATTTGATGTCAATTCTAATGTTGGTGGTGCCT
GTGGAGAAATTGCGACCTTCAAGGGCAAAACTTGGAGGAGTTTATTGAACCCCCTTGTCGCGGCCCAAGC
CTTTGAGTACAAGATGTCCAACATCCTCGACAAACCTTTGGAGAGTCTCTTCGGATACTGCACTGTGTTG
CCTGGTGCCTTCTCGGCTTACAGGTGGATCGCTTTGCAAAACAATGGGGATGGGAGAACGGGACCTTTGG
CGAGTTATTTTGCTGGTGAACAGCTCAATACTGGAAAGGCAGACACATTCACTGGTAATATGGCCAAACC
CAAGGCCAACTGGGTGCTGAAATTCGTTAAGGCTGCTGTTGGAGAAACAGATTGCCCTGATACCATCCCA
GAGTTTATTGCTCAAAGAAGAAGATGGCTTAACGGTTCCTTCTTTGCAGCTGTCTATGCGTTGATGCACA
CGAACCAAATTTGGCGATCCGACCATTCGTTCGCGAGAAAGTCAGCCCTGATGTTGGAATCAGTGTACAA
CTTTCTGAACCTGATATTCTCGTGGTTCGCTTTGGCAAACTTTTACATTTTCTTTGTCATCCTTACGAGC
GCTTTGGAGGGCAGCGCTTTCAATGTCCCTCATATCGATGTGCTCAATACTATTGCACGATATGGTTACC
ttggtgctttggttggttgtttCATCTTCGCAATGGGAAACAGGCCACAAGGTTCGCCTTGGAAGTATAA
AGCAGCAATCTACTTTTTCGCCCTTTTGACTACCTATATGCTGGTCGCAGCAGTGCTTTGTACGGTACAG
GCAATCAAAAATATAAACAGCCCAATTTTTGCCAAGATGGTAGTATCACTCATATCAACCTATGGTATTT
ATGTGATTTCCAGTTTCTTGGCCCTTGACCCTTGGCACATCTTTACTTGCTTTATTCAATATGTTCTCTT
CTCACCTACTTATATCAATGTTCttaatgttTATGCCTATTCCAACCTTCACGACTTGTCATGGGGTACA
AAAGGCTCTGATGCAACCCAGGCGTCGGATTTGGGTGCTGTTTCCGGAGTGGGAAAGCACGTCGAAGTGG
AACTTGTAACTGCCCAGCAAGACATTGATATTGCCTATCAGGATGCTTTGGACAATATTAGATTAAGAGG
ATCAAAAGTTGACTCTGCTGAATCTGAGCCCAAAAAGGAGCAATCTGAACAAGCCCAGAAGGATACTTAT
GCCAACTTTCGTACCAATTTACTTTTGGTCTGGTCGCTGTCAAACGCCCTTCTCGCAAGTGTTATCCTTA
CAGGCAACAATTCTGGAGCGTTTGACGAGGGTTCCGGCAGTTCAAAAGCCACAATATACATGCTTGTGAT
```

Translation

```
>cneo_H99:GLEAN_01196
MPNISRKPPPRFYSPSHSPSPSLYAPIQSPPAPSYDYHANPRTLNPFSDAREVGGYAQLQGEDQMTGAPL
YQPPYAPQLLVAQPTPVSSRLPFFEAALARARGIQTPSLPEAPTPAYAQPLPSYLPPPDPNHPDLSVGLT
QANTVRYAINPRSQLKEGSRSPSPFMDDSFVYNDAAHLYNVEPDVEKALLGSGLGYESEKRVESSMGFND
NDGDLSVPQSFGGRPPSWEPSGILDEKGEMSTTKHFGPAPAGRVGRRAHNAAGYRRIKQSATLDENGFFA
IEMNIPTRLAQFLPIKGVEEQKTTRYTAITTDPDDVPAAGFRLRQNMTSPPRQTELFIVITMYNENAELF
CRTLYGVMKNIAHLCGRKNSRVWGKDGWQKVVVCIVADGRKAVNPRVLDCLAALGVYQEGAMTNTVKDRP
VTAHVFEYTTSFALDGDLHFKYPDKGIVPCQIIFCMKEKNAKKINSHRWFFNAFAPLLSPNVCILLDVGT
QPAPKSIYHLWKAFDVNSNVGGACGEIATFKGKTWRSLLNPLVAAQAFEYKMSNILDKPLESLFGYCTVL
PGAFSAYRWIALQNNGDGRTGPLASYFAGEQLNTGKADTFTGNMAKPKANWVLKFVKAAVGETDCPDTIP
EFIAQRRRWLNGSFFAAVYALMHTNQIWRSDHSFARKSALMLESVYNFLNLIFSWFALANFYIFFVILTS
ALEGSAFNVPHIDVLNTIARYGYLGALVGCFIFAMGNRPQGSPWKYKAAIYFFALLTTYMLVAAVLCTVQ
AIKNINSPIFAKMVVSLISTYGIYVISSFLALDPWHIFTCFIQYVLFSPTYINVLNVYAYSNLHDLSWGT
KGSDATQASDLGAVSGVGKHVEVELVTAQQDIDIAYQDALDNIRLRGSKVDSAESEPKKEQSEQAQKDTY
ANFRTNLLLVWSLSNALLASVILTGNNSGAFDEGSGSSKATIYMLVILIFVAGMSIFRFICSTLYLVISL
FTG*
```

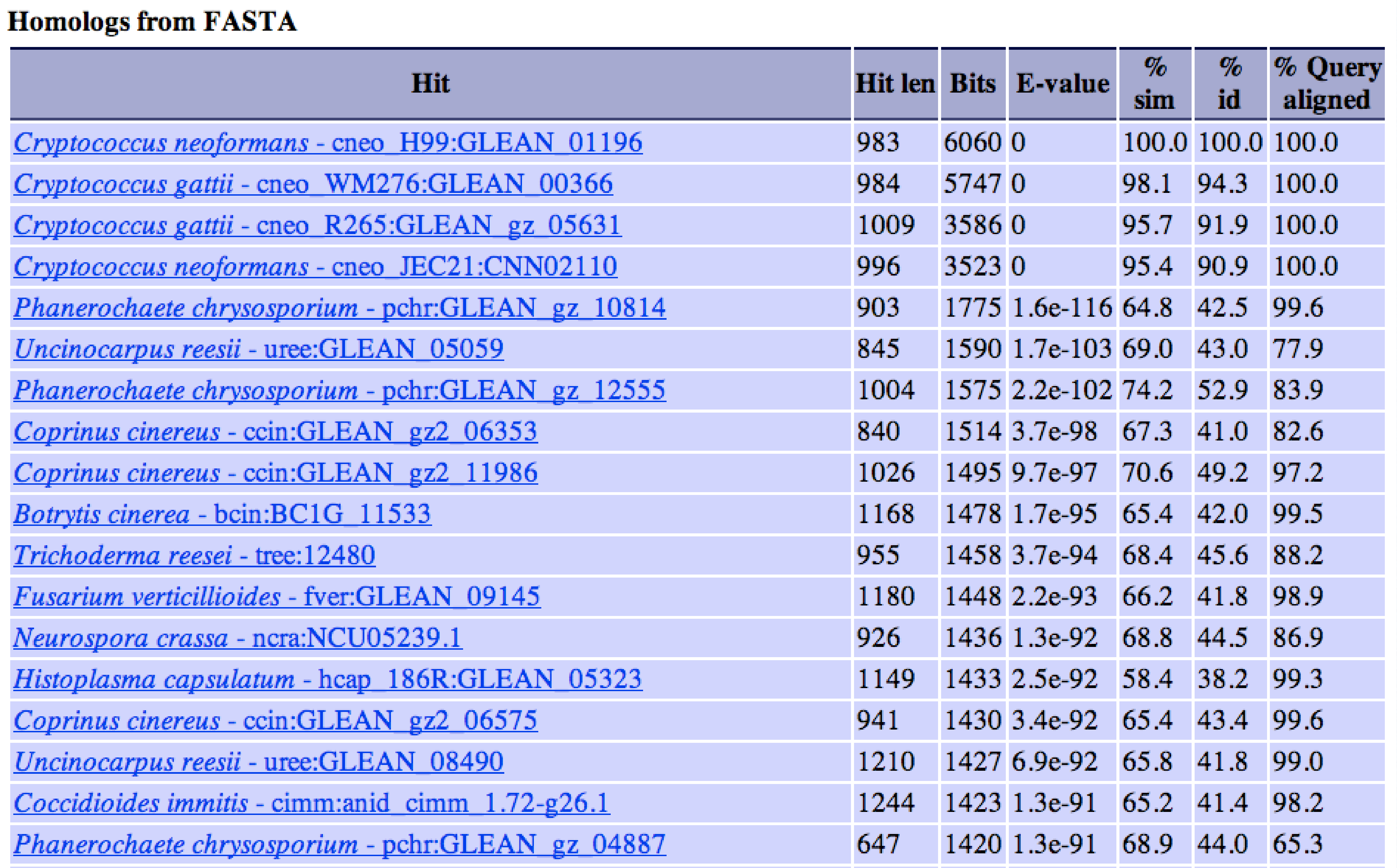
GENE PAGE (3)



Intron sequences

```
>cneo_H99:GLEAN_01196_intron1 CHROMOSOME13:645986..646042
GTAGGGCAGCGACTTTTGCAAGCTTGAGACCCCGTCTATTCGCTGACTCACACATAG
>cneo_H99:GLEAN_01196_intron2 CHROMOSOME13:646269..646324
GTGGGTGATCTTATATCTCCGCATATGTGTTTCAATATTGATGTCAAGGTATTCAG
>cneo_H99:GLEAN_01196_intron3 CHROMOSOME13:646413..646472
GTCAGGTTATCTTCAACAGTACAAAGCGCATTGCTGACATTTCGATACTTTGACAAATAG
>cneo_H99:GLEAN_01196_intron4 CHROMOSOME13:646625..646685
GTGAGCTTGGGGCTCCATCATGTATGCGTCATGTATTCGTAGCTGATTACTTCTTCTTTAG
>cneo_H99:GLEAN_01196_intron5 CHROMOSOME13:646743..646797
GTATGTATCACTCATTCGTTCGACCCAATGAACGCATCACTGACCATGTATGTAG
>cneo_H99:GLEAN_01196_intron6 CHROMOSOME13:646958..647023
GTAAGTCAGCAACCGCCGCACTAATATTCTACATGGTCAGCTAAACGCTGGTTTGTTGCCTACTAG
>cneo_H99:GLEAN_01196_intron7 CHROMOSOME13:647052..647107
GTGAGTACAACATGCAAATTTATTTGTGTCGAATCTGACGCTGATAATGACCATAG
>cneo_H99:GLEAN_01196_intron8 CHROMOSOME13:647187..647247
GTGAGTTGCAACAGCTGAGCATCTTTAGTTTCCAGGACTCACAGCAGACGGTGATATGCAG
>cneo_H99:GLEAN_01196_intron9 CHROMOSOME13:647355..647569
GTGAGTCAAAAATTGTAGAACTCAAGCGTTTTACTGACTGCCTCGTTCTTCATCAGGGTATGTTTCATAG
CGGCGCTTGATGTTTCTTTTTGATTATCTTCTAATCAGCTGCTATAGTACCTGGCAGAAGATAGAATCCT
GTGTTTCGAAATCGTGTAGGCACCACTTCACGGTAATAATACATGTCATTGTTGCTGATCCAACACGGCG
TATAG
>cneo_H99:GLEAN_01196_intron10 CHROMOSOME13:647672..647737
GTGAGTAATTCTTTTTATCggggaaaaaagaaaaagggggggTATCTGACATTCTTCGTCTTCTAG
>cneo_H99:GLEAN_01196_intron11 CHROMOSOME13:647766..647815
GTGAGCGCCGCACGGATTTGGCATTGAGTTTCACGCTTACATATCTTCAG
>cneo_H99:GLEAN_01196_intron12 CHROMOSOME13:647960..648030
GTGGGTACAGCCACTGTTGTATGTTTATACGGATACCCTAATAAGCaaaaaaaaaaaaaaaaaaaaaaCA
G
>cneo_H99:GLEAN_01196_intron13 CHROMOSOME13:648106..648151
GTACGCTGTTTTCATCCGTATAAGACATTAGCTCATTCGATGTTAG
>cneo_H99:GLEAN_01196_intron14 CHROMOSOME13:648214..648274
GTATGTGTTCATTTCTCTGGACAAGAGGGACAGCCAGCCGACGCTTTTCATCTTTCTTCAG
>cneo_H99:GLEAN_01196_intron15 CHROMOSOME13:648329..648389
GTGAGTCGTCACAAGTGGTGCTCAGGGGTCAAGGATAATCACTAAACGTTTTTAACACAG
```

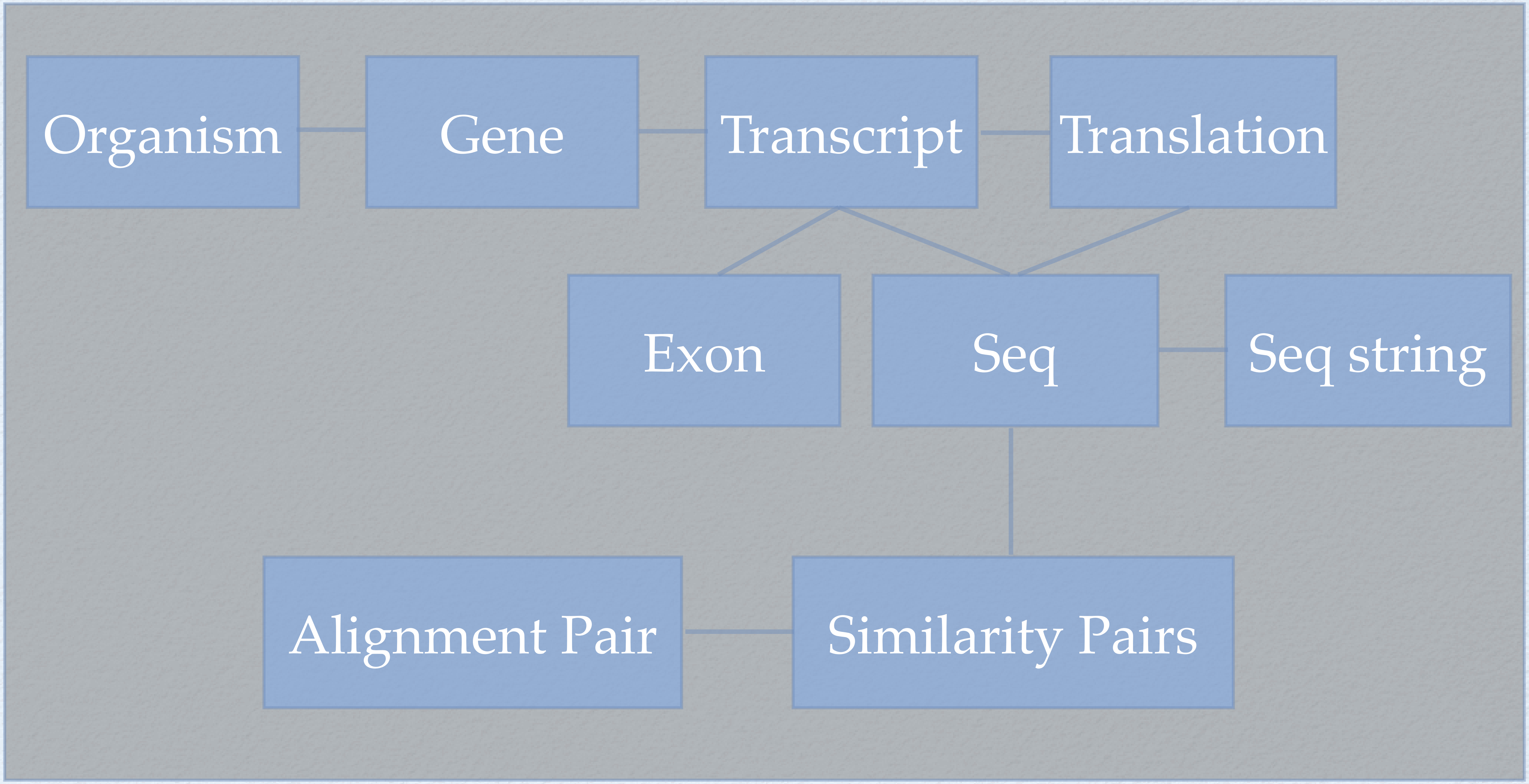
GENE PAGE (4)



Homologs from FASTA

Hit	Hit len	Bits	E-value	% sim	% id	% Query aligned
Cryptococcus neoformans - cneo_H99:GLEAN_01196	983	6060	0	100.0	100.0	100.0
Cryptococcus gattii - cneo_WM276:GLEAN_00366	984	5747	0	98.1	94.3	100.0
Cryptococcus gattii - cneo_R265:GLEAN_gz_05631	1009	3586	0	95.7	91.9	100.0
Cryptococcus neoformans - cneo_JEC21:CNN02110	996	3523	0	95.4	90.9	100.0
Phanerochaete chrysosporium - pchr:GLEAN_gz_10814	903	1775	1.6e-116	64.8	42.5	99.6
Uncinocarpus reesii - uree:GLEAN_05059	845	1590	1.7e-103	69.0	43.0	77.9
Phanerochaete chrysosporium - pchr:GLEAN_gz_12555	1004	1575	2.2e-102	74.2	52.9	83.9
Coprinus cinereus - ccin:GLEAN_gz2_06353	840	1514	3.7e-98	67.3	41.0	82.6
Coprinus cinereus - ccin:GLEAN_gz2_11986	1026	1495	9.7e-97	70.6	49.2	97.2
Botrytis cinerea - bcin:BC1G_11533	1168	1478	1.7e-95	65.4	42.0	99.5
Trichoderma reesei - tree:12480	955	1458	3.7e-94	68.4	45.6	88.2
Fusarium verticillioides - fver:GLEAN_09145	1180	1448	2.2e-93	66.2	41.8	98.9
Neurospora crassa - ncra:NCU05239.1	926	1436	1.3e-92	68.8	44.5	86.9
Histoplasma capsulatum - hcap_186R:GLEAN_05323	1149	1433	2.5e-92	58.4	38.2	99.3
Coprinus cinereus - ccin:GLEAN_gz2_06575	941	1430	3.4e-92	65.4	43.4	99.6
Uncinocarpus reesii - uree:GLEAN_08490	1210	1427	6.9e-92	65.8	41.8	99.0
Coccidioides immitis - cimm:anid_cimm_1.72-g26.1	1244	1423	1.3e-91	65.2	41.4	98.2
Phanerochaete chrysosporium - pchr:GLEAN_gz_04887	647	1420	1.3e-91	68.9	44.0	65.3

SIMILARITY DATABASE



Organism

Gene

Transcript

Translation

Exon

Seq

Seq string

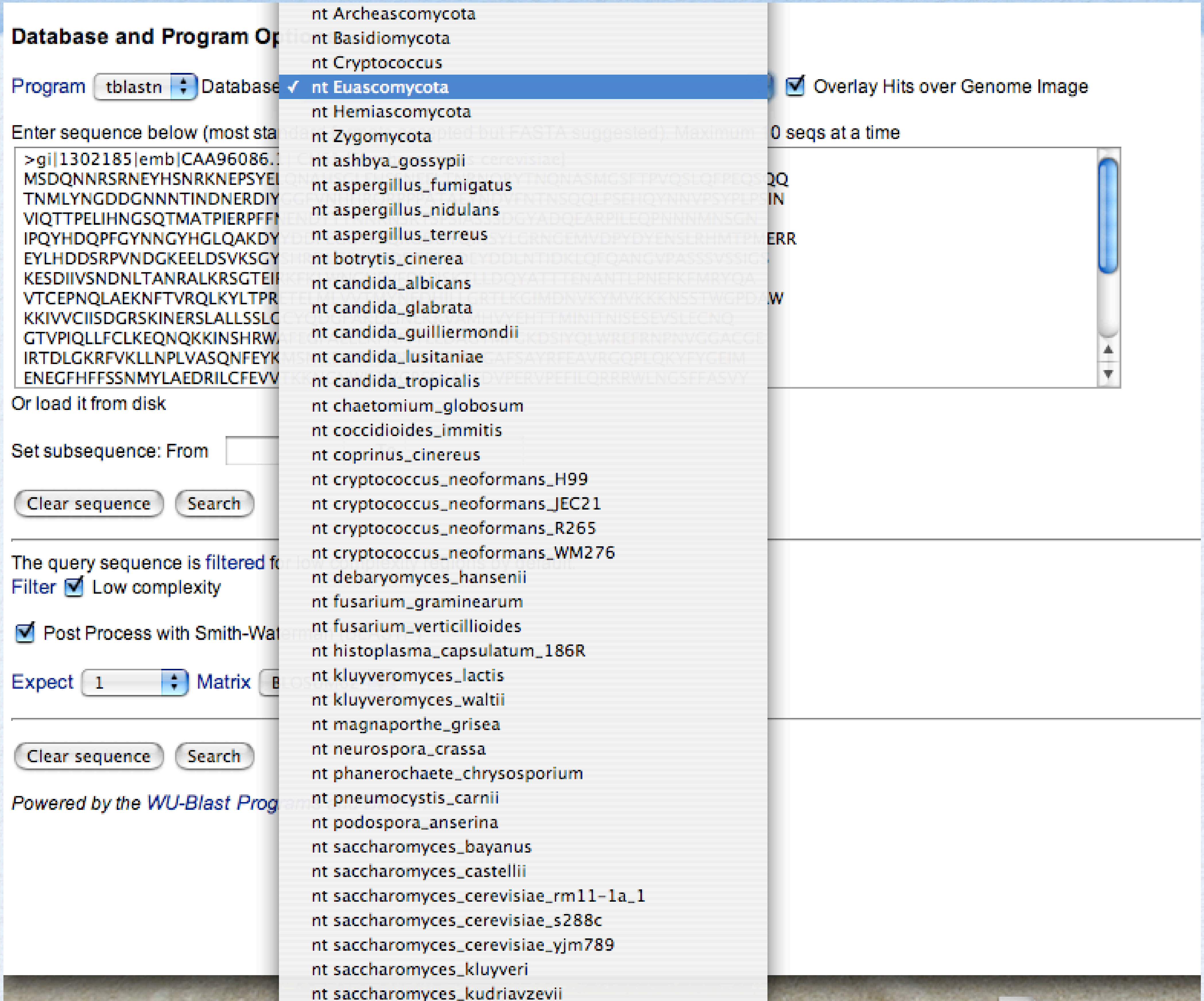
Alignment Pair

Similarity Pairs

OTHER TOOLS

- BLAST interface
  - Search your sequence and get marked up results with links to Gbrowse
  - "Yeast protein to genomic visualization of locus in your organism of interest"

BLAST TOOL



Database and Program Op

Program tblastn Database nt Euascomycota ☑ Overlay Hits over Genome Image

Enter sequence below (most sta … 0 seqs at a time

```
>gi|1302185|emb|CAA96086.
MSDQNNRSRNEYHSNRKNEPSYEI                                         QQ
TNMLYNGDDGNNNTINDNERDIY                                          IN
VIQTTPELIHNGSQTMATPIERPFFN
IPQYHDQPFGYNNGYHGLQAKDY                                          ERR
EYLHDDSRPVNDGKEELDSVKSGY
KESDIIVSNDNLTANRALKRSGTEIF
VTCEPNQLAEKNFTVRQLKYLTPR                                         W
KKIVVCIISDGRSKINERSLALLSSLC
GTVPIQLLFCLKEQNQKKINSHRW
IRTDLGKRFVKLLNPLVASQNFEYK
ENEGFHFFSSNMYLAEDRILCFEVV
```

Or load it from disk

Set subsequence: From

The query sequence is filtered f

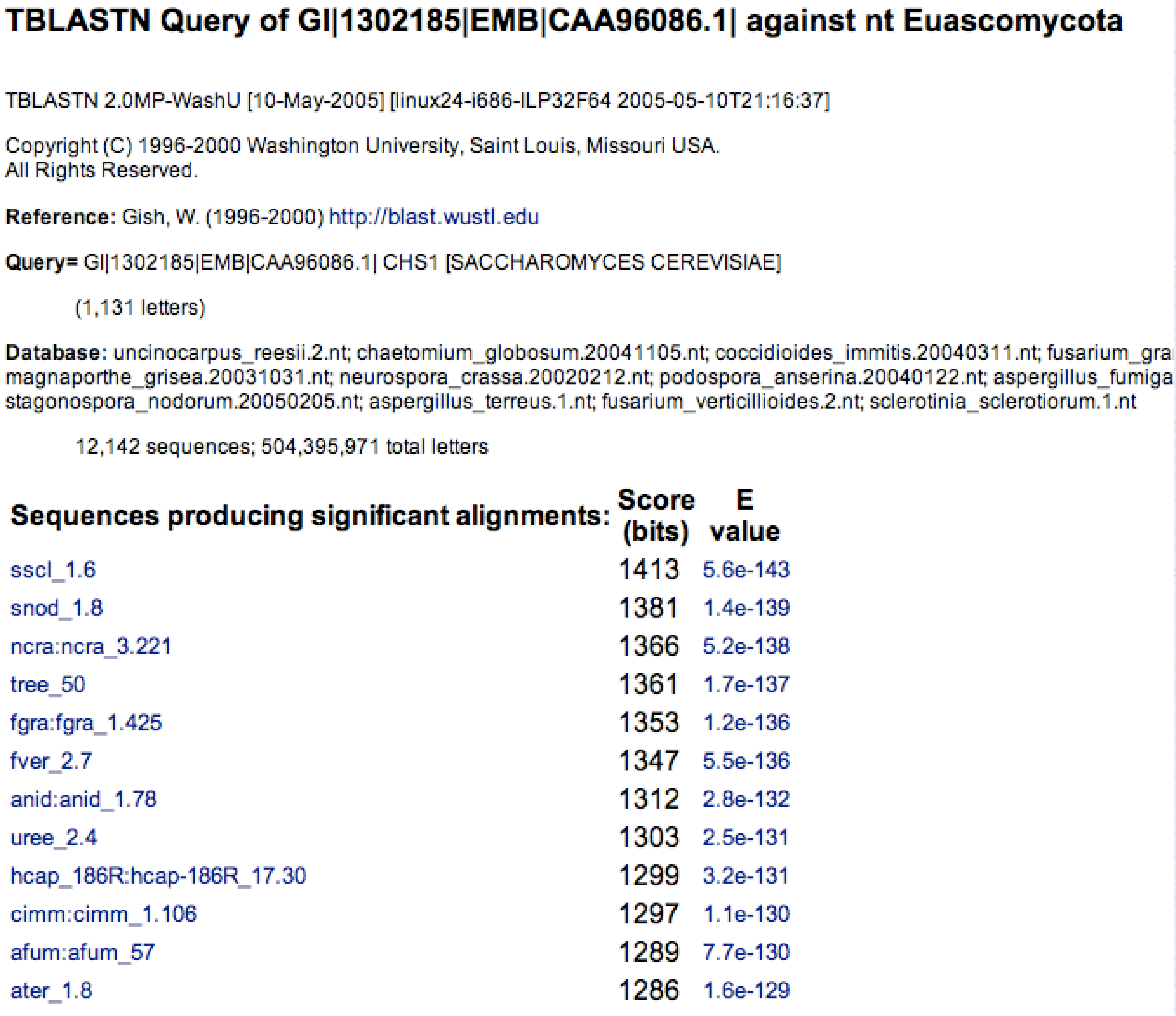
Filter ☑ Low complexity

☑ Post Process with Smith-Wat

Expect 1 Matrix B

Powered by the *WU-Blast Prog*

RE-FORMATTED BLAST



TBLASTN Query of GI|1302185|EMB|CAA96086.1| against nt Euascomycota

TBLASTN 2.0MP-WashU [10-May-2005] [linux24-i686-ILP32F64 2005-05-10T21:16:37]

Copyright (C) 1996-2000 Washington University, Saint Louis, Missouri USA.
All Rights Reserved.

Reference: Gish, W. (1996-2000) http://blast.wustl.edu

Query= GI|1302185|EMB|CAA96086.1| CHS1 [SACCHAROMYCES CEREVISIAE]

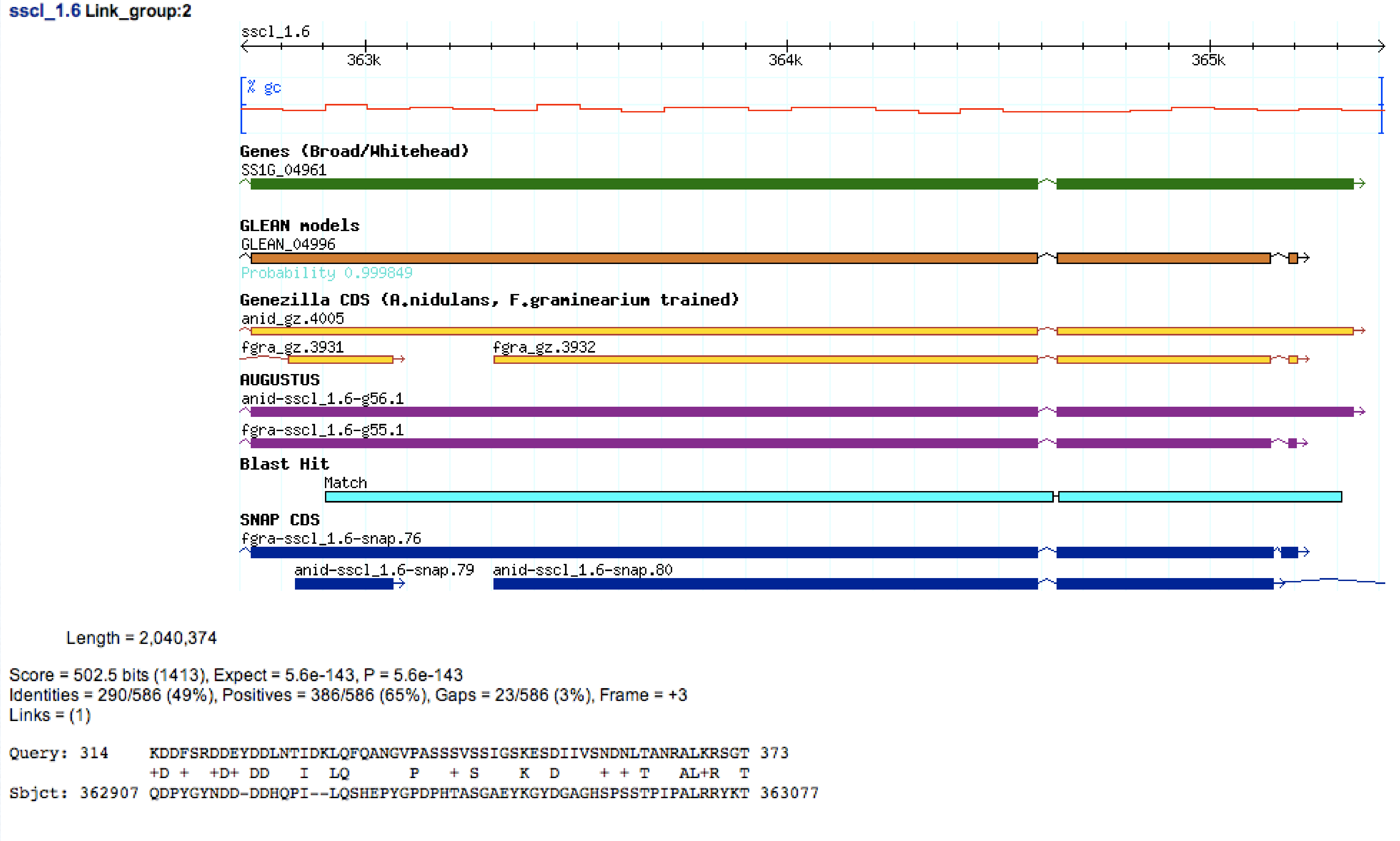
(1,131 letters)

Database: uncinocarpus_reesii.2.nt; chaetomium_globosum.20041105.nt; coccidioides_immitis.20040311.nt; fusarium_gra
magnaporthe_grisea.20031031.nt; neurospora_crassa.20020212.nt; podospora_anserina.20040122.nt; aspergillus_fumiga
stagonospora_nodorum.20050205.nt; aspergillus_terreus.1.nt; fusarium_verticillioides.2.nt; sclerotinia_sclerotiorum.1.nt

12,142 sequences; 504,395,971 total letters

Sequences producing significant alignments:	**Score (bits)**	**E value**
sscl_1.6	1413	5.6e-143
snod_1.8	1381	1.4e-139
ncra:ncra_3.221	1366	5.2e-138
tree_50	1361	1.7e-137
fgra:fgra_1.425	1353	1.2e-136
fver_2.7	1347	5.5e-136
anid:anid_1.78	1312	2.8e-132
uree_2.4	1303	2.5e-131
hcap_186R:hcap-186R_17.30	1299	3.2e-131
cimm:cimm_1.106	1297	1.1e-130
afum:afum_57	1289	7.7e-130
ater_1.8	1286	1.6e-129

WITH LINKS



```
        Length = 2,040,374

Score = 502.5 bits (1413), Expect = 5.6e-143, P = 5.6e-143
Identities = 290/586 (49%), Positives = 386/586 (65%), Gaps = 23/586 (3%), Frame = +3
Links = (1)

Query: 314    KDDFSRDDEYDDLNTIDKLQFQANGVPASSSVSSIGSKESDIIVSNDNLTANRALKRSGT 373
              +D +  +D+ DD   I  LQ      P   + S    K  D    + + T   AL+R  T
Sbjct: 362907 QDPYGYNDD-DDHQPI--LQSHEPYGPDPHTASGAEYKGYDGAGHSPSSTPIPALRRYKT 363077
```

ADDITIONAL DATA TO INTEGRATE

- Curated life-history information about sequenced fungi (with Anne Pringe, Harvard)
- Expression data...
- Mart-enabled?

WHAT'S MISSING

- Homolog/Ortholog/Paralog capturing
  - Pairwise focused summary stastistics
  - Multiway ortholog summaries
  - Ensembl Compara --> GMOD Compara?
  - Linking to gene trees

QUERIES TO ADDRESS

- "All the genes in closely related pathogenic fungi not present in non-pathogenic outgroup"
  - Species-tree defined unique genes,etc
- "Rapidly evolving cell-surface associated genes"
- Gene family size change (paralogous expansions)