Cool New Stuff!

NCBI Resources for Phylogenetically-Defined Next Generation Analysis in and out of the Cloud

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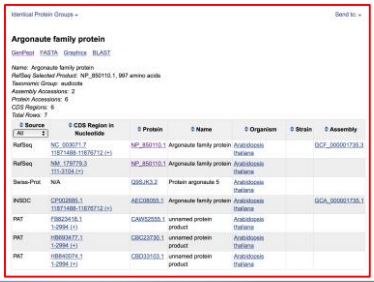


Tom Madden's BLAST_v5 Talk (slightly modified)

If you'd like to see the original slides from Tom, or this slide deck, check out https://www.slideshare.net/benbusby

Search the Argonaute protein family with BLAST!

- Against the nr protein database
- Limit the search to Schizosaccharomyces pombe

BLASTDBv5: a better BLAST database!

- Limit search by taxonomy with a command-line parameter

  -taxids

- Improved performance when limiting BLAST search with accessions.
  - **Faster!**
- Retrieve sequences by taxonomy from the BLAST database.
  - Extract data!


```
Database: All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF
excluding environmental samples from WGS projects
           148,264,992 sequences; 54,322,627,498 total letters


Query= NP_850110.1 Argonaute family protein [Arabidopsis thaliana]

Length=997
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

NP_587782.1  argonaute [Schizosaccharomyces pombe]                    407     2e-128


>NP_587782.1 argonaute [Schizosaccharomyces pombe]
 CAA19275.1 argonaute [Schizosaccharomyces pombe]
Length=834

 Score = 407 bits (1047),  Expect = 2e-128, Method: Compositional matrix adjust.
 Identities = 284/892 (32%), Positives = 449/892 (50%), Gaps = 91/892 (10%)

Query  136  SLPPASSKAVTFPVRPGRGTLGKKVMVRANHF-LVQVADRDLYHYDVSINPEVISKTVNR  194
            S  P+S  A+     RPG G LGK++ ++AN F ++ + +  +  Y V +       V R
Sbjct  2    SYKPSSEIAL----RPGYGGLGKQITLKANFFQIISLPNETINQYHVIVGD----GSRVPR  54

Query  195  NVMKLLVKNVKDSHLGGKS----PAYDGRKSLYTAGPLPFDSKEFVVNLAEKRADGSSGKD  251
            +L+  + +     G S     YDGR   ++ G +    + VN+      GS
Sbjct  55   KQSQLIWNSKEVKQYFGSSWMNSVYDGRSMCWSKGDIADGTIK---VNI------GSESHP  106
```

BLAST command-line parameters

```
blastp
  -db nr
    -query NP_850110.fsa
      -outfmt "7 qaccver saccver pident length evalue sscinames"  # tabular output
        -out NP_850110.4896.tab
          -task blastp-fast                    # runs faster!
            -num_threads 8                     # use 8 CPUs
              -evalue 0.05                     # limit to more significant matches
                -taxids 4896                   # limit search to Schizosaccharomyces pombe
```


Need taxids for a whole Phylum?

```
bash-4.2$ get_species_taxids.sh -n Ascomycota

Taxid: 4890
 rank: phylum
 division: ascomycetes
 scientific name: Ascomycota
 common name: ascomycetes

1 match(es) found.

bash-4.2$ get_species_taxids.sh -t 4890 > Ascomycota.taxidlist
bash-4.2$ head Ascomycota.taxidlist
4754
4896
4897
4899
4903
4909
4911
4914
```


A script to find taxid

Distributed with the BLAST package!

```
bash-4.2$ get_species_taxids.sh -n "Schizosaccharomyces pombe"

Taxid: 4896
 rank: species
 division: ascomycetes
 scientific name: Schizosaccharomyces pombe
 common name: fission yeast

1 match(es) found.
```


BLAST against Ascomycota sequences!

```
blastp
  -db nr
    -query NP_850110.fsa
      -outfmt "7 qaccver saccver pident length evalue sscinames"  # tabular output
        -out NP_850110.Ascomycota.tab
          -task blastp-fast                    # runs faster!
            -num_threads 8                     # use 8 CPUs
              -evalue 0.05                     # limit to more significant matches
                -taxidlist Ascomycota.taxids   # limit search to Ascomycota
```


Tabular output

```
# BLASTP 2.8.0+
# Query: NP_850110.1 Argonaute family protein [Arabidopsis thaliana]
# Database: nr
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, evalue, subject sci name
# 1 hits found
NP_850110.1     NP_587782.1     31.839  892     1.57e-128       Schizosaccharomyces pombe
# BLAST processed 1 queries
```


Ascomycota results

```
# BLASTP 2.8.0+
# Query: NP_850110.1 Argonaute family protein [Arabidopsis thaliana]
# Database: nr
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, evalue, subject sci name
# 501 hits found
NP_850110.1     GAO47878.1      36.139  891     1.93e-164       Saitoella complicata NRRL Y-17804
NP_850110.1     XP_019027272.1  36.550  829     1.39e-156       Saitoella complicata NRRL Y-17804
NP_850110.1     ODQ75150.1      34.220  865     1.03e-146       Lipomyces starkeyi NRRL Y-11557
NP_850110.1     CUS09702.1      31.767  894     6.68e-134       Tuber aestivum
NP_850110.1     XP_002839547.1  30.624  898     3.85e-130       Tuber melanosporum Mel28
NP_850110.1     XP_013025347.1  32.540  882     1.01e-127       Schizosaccharomyces cryophilus OY26
NP_850110.1     ORYB1937.1      32.993  882     2.93e-127       Protomyces lactucaedebilis
NP_850110.1     NP_587782.1     31.839  892     1.45e-125       Schizosaccharomyces pombe
NP_850110.1     XP_013019123.1  33.333  852     5.49e-125       Schizosaccharomyces octosporus yFS286
NP_850110.1     EPS44580.1      30.710  902     7.32e-125       Dactylellina haptotyla CBS 200.50
NP_850110.1     CUS14138.1      33.684  855     1.87e-124       Tuber aestivum
NP_850110.1     XP_011120241.1  31.180  901     2.47e-124       Arthrobotrys oligospora ATCC 24927
NP_850110.1     EWC46593.1      31.823  905     1.41e-122       Drechslerella stenobrocha 248
NP_850110.1     EWC45585.1      31.042  931     3.45e-120       Drechslerella stenobrocha 248
NP_850110.1     KFZ00212.1      30.444  969     1.22e-116       Pseudogymnoascus pannorum VKM F-4518 (FW-2643)
NP_850110.1     XP_002173826.1  31.818  858     4.95e-116       Schizosaccharomyces japonicus yFS275
NP_850110.1     XP_011120792.1  31.973  882     8.11e-116       Arthrobotrys oligospora ATCC 24927
NP_850110.1     KFZ13012.1      30.341  969     9.18e-116       Pseudogymnoascus pannorum VKM F-4520 (FW-2644)
NP_850110.1     XP_011119032.1  31.585  877     2.56e-114       Arthrobotrys oligospora ATCC 24927
NP_850110.1     KFY31959.1      30.714  967     1.34e-112       Pseudogymnoascus pannorum VKM F-4281 (FW-2241)
NP_850110.1     EPS43673.1      29.814  966     1.75e-111       Dactylellina haptotyla CBS 200.50
```

BLAST excluding Viridiplantae

```
blastp
   -db nr
      -query NP_850110.fsa
         -outfmt "7 qaccver saccver pident length evalue sscinamе"
            -out NP_850110.noGreenPlants.tab
               -task blastp-fast
                  -num_threads 8
                     -evalue 0.05
                        -negative_taxidlist Viridiplantae.taxids          # exclude green plants
```


Everything

```
# BLASTP 2.8.0+
# Query: NP_850110.1 Argonaute family protein [Arabidopsis thaliana]
# Database: nr
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, evalue, subject sci name
# 521 hits found
NP_850110.1     NP_850110.1     100.000 997     0.0     Arabidopsis thaliana
NP_850110.1     OAP08078.1      99.900  997     0.0     Arabidopsis thaliana
NP_850110.1     AAD21514.1      99.900  997     0.0     Arabidopsis thaliana
NP_850110.1     XP_002880962.1  89.634  984     0.0     Arabidopsis lyrata subsp. lyrata
NP_850110.1     XP_010473185.1  82.513  995     0.0     Camelina sativa
NP_850110.1     XP_010417939.1  81.590  994     0.0     Camelina sativa
NP_850110.1     XP_019101467.1  81.894  961     0.0     Camelina sativa
NP_850110.1     XP_010510717.1  81.894  961     0.0     Camelina sativa
NP_850110.1     XP_006296291.1  77.756  1016    0.0     Capsella rubella
NP_850110.1     XP_010435959.1  74.926  1009    0.0     Camelina sativa
NP_850110.1     XP_013692158.1  75.540  973     0.0     Brassica napus
NP_850110.1     XP_013636559.1  75.540  973     0.0     Brassica oleracea var. oleracea
NP_850110.1     XP_009103628.1  74.795  976     0.0     Brassica rapa
NP_850110.1     KFK44936.1      73.953  979     0.0     Arabis alpina
NP_850110.1     XP_013647627.1  74.693  976     0.0     Brassica napus
NP_850110.1     XP_018440495.1  79.402  869     0.0     Raphanus sativus
NP_850110.1     XP_006409803.1  72.700  989     0.0     Eutrema salsugineum
NP_850110.1     XP_010490899.1  72.819  986     0.0     Camelina sativa
NP_850110.1     XP_010521702.1  67.306  991     0.0     Cleome hassleriana
NP_850110.1     XP_021610669.1  65.119  926     0.0     Manihot esculenta
NP_850110.1     PNT55850.1      67.086  875     0.0     Populus trichocarpa
```


No Viridiplantae

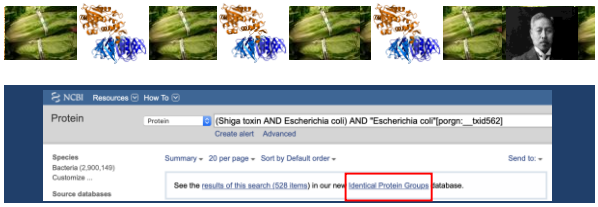
```
# BLASTP 2.8.0+
# Query: NP_850110.1 Argonaute family protein [Arabidopsis thaliana]
# Database: nr
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, evalue, subject sci name
# 500 hits found
NP_850110.1     XP_021356153.1  42.081  884     0.0     Mizuhopecten yessoensis
NP_850110.1     OWF49186.1      41.968  884     0.0     Mizuhopecten yessoensis
NP_850110.1     XP_021356154.1  41.968  884     0.0     Mizuhopecten yessoensis
NP_850110.1     XP_021356147.1  41.742  884     0.0     Mizuhopecten yessoensis
NP_850110.1     XP_021965366.1  41.619  877     0.0     Folsomia candida
NP_850110.1     ODN00002.1      41.686  878     0.0     Orchesella cincta
NP_850110.1     OXA40521.1      41.733  877     0.0     Folsomia candida
NP_850110.1     XP_014666293.1  42.640  856     0.0     Priapulus caudatus
NP_850110.1     XP_002413188.1  42.272  854     0.0     Ixodes scapularis
NP_850110.1     AMN88363.1      40.765  915     0.0     Mytilus galloprovincialis
NP_850110.1     XP_014666292.1  42.740  854     0.0     Priapulus caudatus
NP_850110.1     XP_021965365.1  41.563  883     0.0     Folsomia candida
NP_850110.1     XP_021965367.1  41.563  883     0.0     Folsomia candida
NP_850110.1     XP_002045323.1  40.761  893     0.0     Drosophila sechellia
NP_850110.1     ELT99790.1      41.344  878     0.0     Capitella teleta
NP_850110.1     XP_012170889.1  40.252  954     0.0     Bombus terrestris
NP_850110.1     XP_021356148.1  41.290  884     0.0     Mizuhopecten yessoensis
NP_850110.1     XP_021356151.1  41.516  884     0.0     Mizuhopecten yessoensis
NP_850110.1     KRF99116.1      40.400  901     0.0     Drosophila willistoni
NP_850110.1     XP_015033121.1  40.400  901     0.0     Drosophila willistoni
NP_850110.1     XP_002068762.1  40.400  901     0.0     Drosophila willistoni
```


Timings

Search set	Run time (seconds)
Schizosaccharomyces pombe	8.7
Ascomycota	11.7
No Viridiplantae	101
Everything	91

8 CPUs, best of three runs using time command.


```
                              Tax BLAST report

Query= NP_850110.1 Argonaute family protein [Arabidopsis thaliana]

Length=997

                              Organism Report

Accession                       Description                                        Score E-value

                 Mizuhopecten yessoensis (Yesso scallop) [bivalves]
 XP_021356153 protein argonaute-2-like isoform X6 [Mizuhopecten yessoensis]         668   0.0
 OWF49186     Protein argonaute-2 [Mizuhopecten yessoensis]                         666   0.0
 XP_021356154 protein argonaute-2-like isoform X7 [Mizuhopecten yessoensis]         665   0.0
 XP_021356147 protein argonaute-2-like isoform X2 [Mizuhopecten yessoensis]         665   0.0
 XP_021356148 protein argonaute-2-like isoform X3 [Mizuhopecten yessoensis]         654   0.0
 XP_021356151 protein argonaute-2-like isoform X5 [Mizuhopecten yessoensis]         654   0.0
 XP_021356158 protein argonaute-2-like isoform X11 [Mizuhopecten yessoensis]        652   0.0
 XP_021356150 protein argonaute-2-like isoform X4 [Mizuhopecten yessoensis]         652   0.0
 XP_021356157 protein argonaute-2-like isoform X10 [Mizuhopecten yessoensis]        652   0.0
 XP_021356156 protein argonaute-2-like isoform X9 [Mizuhopecten yessoensis]         652   0.0
 XP_021356155 protein argonaute-2-like isoform X8 [Mizuhopecten yessoensis]         652   0.0
 XP_021356146 protein argonaute-2-like isoform X1 [Mizuhopecten yessoensis]         652   0.0
 XP_021356149 protein argonaute-2-like isoform X1 [Mizuhopecten yessoensis]         652   0.0

                       Folsomia candida  [springtails]
 XP_021965366 protein argonaute-2-like isoform X2 [Folsomia candida]                661   0.0
 OXA40521     Protein argonaute-2 [Folsomia candida]                                660   0.0
 XP_021965365 protein argonaute-2-like isoform X1 [Folsomia candida]                657   0.0
 XP_021965367 protein argonaute-2-like isoform X3 [Folsomia candida]                657   0.0

                      Orchesella cincta  [springtails]
 ODN00002     Protein argonaute-2 [Orchesella cincta]                               660   0.0

                      Priapulus caudatus  [priapulids]
 XP_014666293 PREDICTED: protein argonaute-2-like isoform X6 [Priapulus caudatus]   659   0.0
 XP_014666292 PREDICTED: protein argonaute-2-like isoform X5 [Priapulus caudatus]   658   0.0
```

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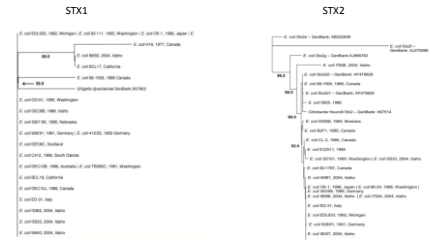
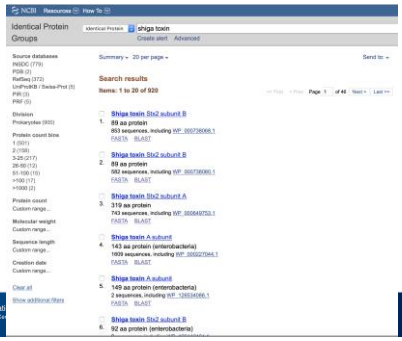
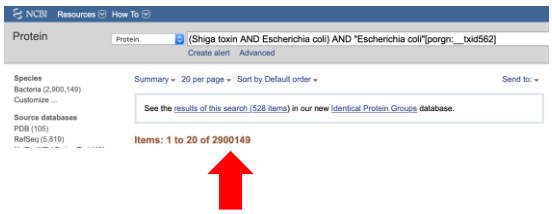




Search the Shiga toxin family protein with BLAST

- Against the nr protein database
- Limit the search to E coli

Why Use Identical Protein Groups?



Search the Shiga toxin family protein with BLAST

- Against the nr pr…base
- Limit the searc…to E coli

But *which* Shiga toxin?!

Stx1 and Stx2 Example Sequences

But what did I need to do to make my demo?

Grab sequences!

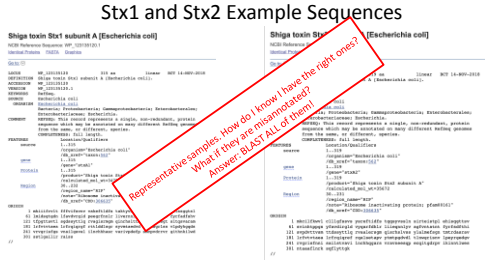
```
ben.busby@known-blast-workbench-sbx-camacho:~$ esearch -db protein -query WP_123135120 | efetch -format fasta
>WP_123135120.1 Shiga toxin Stx1 subunit A [Escherichia coli]
MKIIIFRVLTFFFVIFSVNVVAKEFTLDFSTAKTYVDSLNVIRSAIGTPLQTISSGGTSLLMIDSGTGDN
LFAVDVRGIDPEEGRFNNLRLIVERNNLYVTGFVNRTNNVFYRFADFSHVTFPGTTAVTLSGDSSYTTLQ
RVAGISRMGMQINRHSLTTSYLDLMSHSGTSLTQSVARAMLRFVTVTAEALRFRQIQRGFRTTLDDLSGR
SYVMTAEDVDLTLNWGRLSSVLPDYHGQDSVRVGRISFGSVNAILGSVALILNCHHHASRVARIVPDEFP
SMCPVDRRVRGITHNKILWDSSTLGAILIRRAISS
```

Do I need to get all of nr?!

update_blast.pl -- decompress works

But theres a better way!

Stx1 and Stx2 Example Sequences



CloudBLAST and Docker Images to the Rescue!

- http://ncbi.github.io/blast-cloud/
- https://github.com/ncbi/makeblastdb4cloud
- Also, check out magicBLAST!

BLAST command-line parameters

```
blastp
   -db refseq-protein_v5
      -query  WP_123135120_123130813.fasta
         -outfmt "7 qaccver saccver pident length evalue sscinname"   # tabular output
            -out Shiga_Ecoli.tab
               -task blastp-fast                                      # runs faster!
                  -num_threads 32                                     # use 8 CPUs
                     -evalue 0.00005                                  # limit to more significant matches
                        -taxids   562                                 # limit search to Escherichia coli
```


But how do I use the BLAST Docker(s)?!

https://github.com/ncbi/docker/blob/master/blast/README.md

But how do I use the BLAST Docker(s)?!

https://github.com/ncbi/docker/blob/master/blast/README.md

Interactive BLAST

One can login to the container and run commands inside the container if multiple BLAST runs will be executed interactively.

```
docker run --rm -it \
  -v $BLASTDB_DIR:/blast/blastdb:ro -v $HOME/blastdb_custom:/blast/blastdb_custom:ro \
  -v $HOME/queries:/blast/queries:ro \
  -v $HOME/results:/blast/results:rw \
  ncbi/blast \
  /bin/bash
```

This will open a login shell in the container and one can run BLAST+ as if it was locally installed.

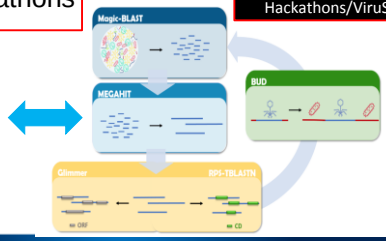
Scripted BLAST

One approach to deal with this situation is to start the blast container in detached mode and execute commands on it.

```
# Start a container named "blast" in detached mode
docker run --rm -dit --name blast \
  -v $BLASTDB_DIR:/blast/blastdb:ro -v $HOME/blastdb_custom:/blast/blastdb_custom:ro \
  -v $HOME/queries:/blast/queries:ro \
  -v $HOME/results:/blast/results:rw \
  ncbi/blast \
  sleep infinity
```


Hackathons

https://github.com/NCBI-Hackathons/ViruSpy



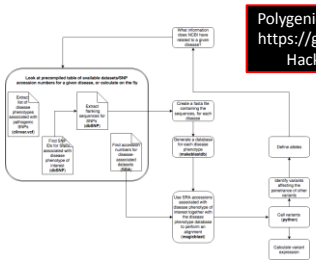
But how do I use the BLAST Docker(s)?!

https://github.com/ncbi/docker/blob/master/blast/README.md

sudo docker run --rm -v $BLASTDB_DIR:/blast/blastdb:ro -v $HOME/queries:/blast/queries:rw -w /blast/blastdb ncbi/blast blastp -db nr_v5 -query /blast/queries/WP_123135120_123130813.fasta -out /blast/queries/Shiga_Ecoli.tab -task blastp-fast -num_threads 32 -evalue .00005 -taxids 562

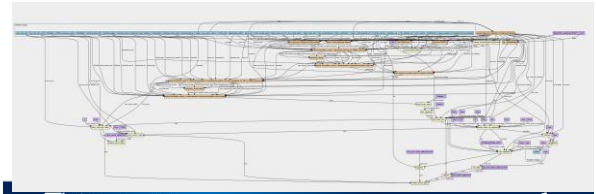
Variant calling on the fly!

Polygenic SNP Search Tool
https://github.com/NCBI-Hackathons/PSST



Bonus: Prokaryotic Genome Annotation Pipeline Wrapped with Docker and CWL

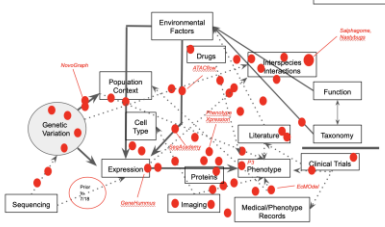
https://github.com/ncbi/pgap



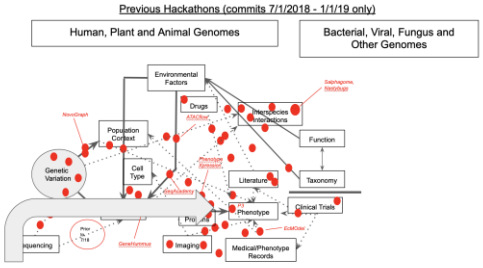
Previous Hackathons (commits 7/1/2018 - 1/1/19 only)

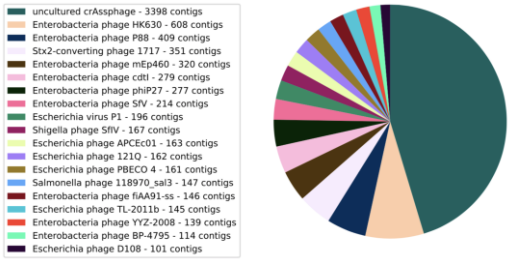
Human, Plant and Animal Genomes

Bacterial, Viral, Fungus and Other Genomes

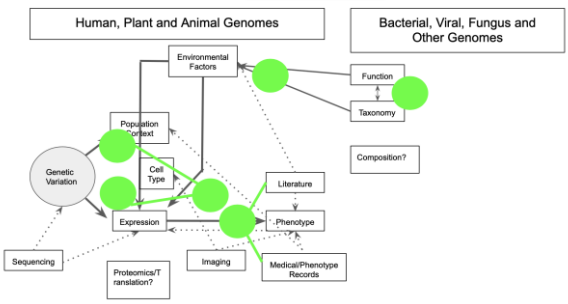


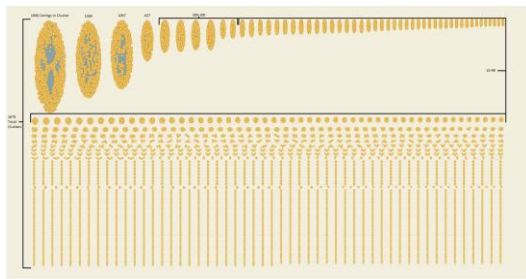
Previous Hackathons (commits 7/1/2018 - 1/1/19 only)

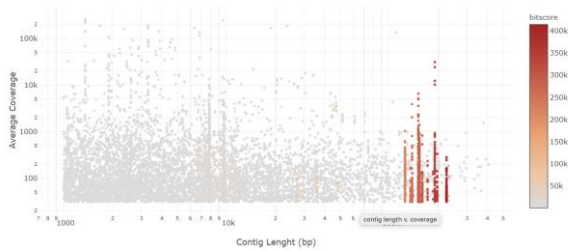




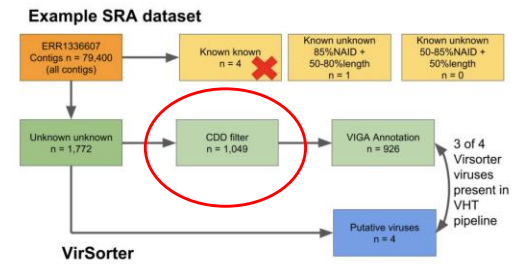
"STRIDES" Hackathons!

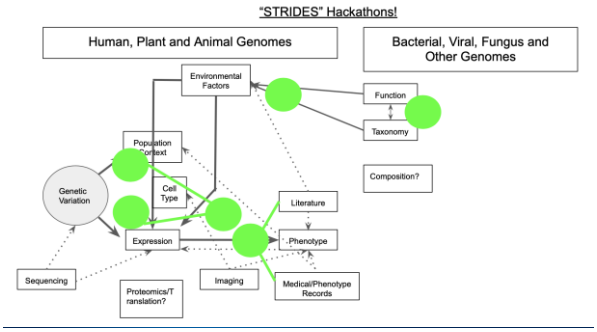


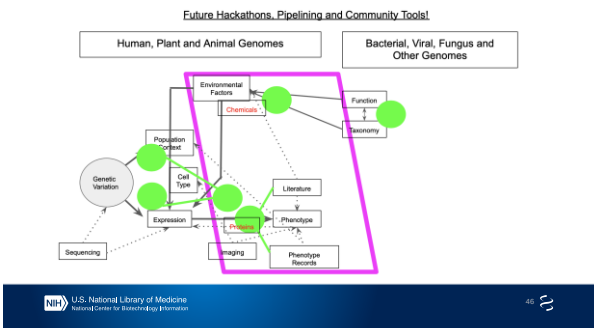




Example SRA dataset







44

Data Analysis Hackathons!

NCBI to assist in Virus Hunting Data Science Hackathon January 9-11, 2019

Planned Events

RNAseq – UNC, March 11-13 2019; Palo Alto, June 2019
Smaller events at USF and Pittsburgh

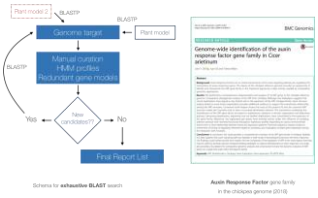
Human PanGenomics – UCSC, 25-27 March 2019;
Haplotype Annotation, Baltimore, June 2019

Variant Synthetic Dataset Generation – Boston, April 15-16, 2019

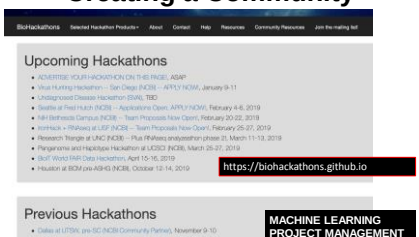
AMR and Prokaryotic Genome Annotate-athon
NIH Campus, August 2019

GeneHummus

Automated Gene Family Discovery!



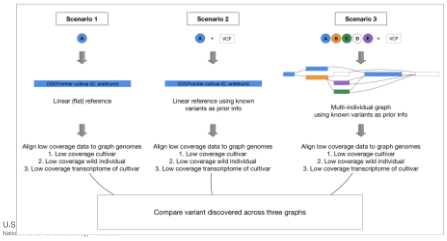
Creating a Community



MACHINE LEARNING
PROJECT MANAGEMENT
PRODUCT MANAGEMENT

HummusGraph

Because graph genomes aren't just for humans!



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Jan Buchmann
Chaochih Liu
Tom Madden
Moamen Elmassry
Kim LeBlanc
Allissa Dillman
Olaitan Awe
Anjana Raina
Peter Meric
Francoise Thibaud-Nissen
Douglas Slotta
Michael Crusoe

Hackathon Participants!

And visiting bioinformaticians!

Eric Cox
Vamsi Kodali
Brian Smith-White
Bart Trawick
Kim Pruitt

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National Center for Biotechnology Information

Watch NCBI News for updates!

http://www.ncbi.nlm.nih.gov/news/
https://www.youtube.com/user/NCBINLM

49

NCBI Genome Resources Workshop

Time	Topic
12:50 – 1:10	Submission of Genomes to GenBank *Karen Clark*
1:10 – 1:30	GEO Submissions and Usage *Steve Wilhite*
1:30 – 1:55	From Annotation to Visualization: Exploring Genes and Genomes with NCBI Tools *Eric Cox*
1:55 – 2:15	Programmatic Access to Genomic Data: E-Utilities and FTP *Vamsi K. Kodali*
2:15 – 2:35	NCBI Resources for Phyletically-Defined Next Generation Analysis in and out of the Cloud (a.k.a. Cool New Stuff!) *Ben Busby*
2:35 – 3:00	Q & A session

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