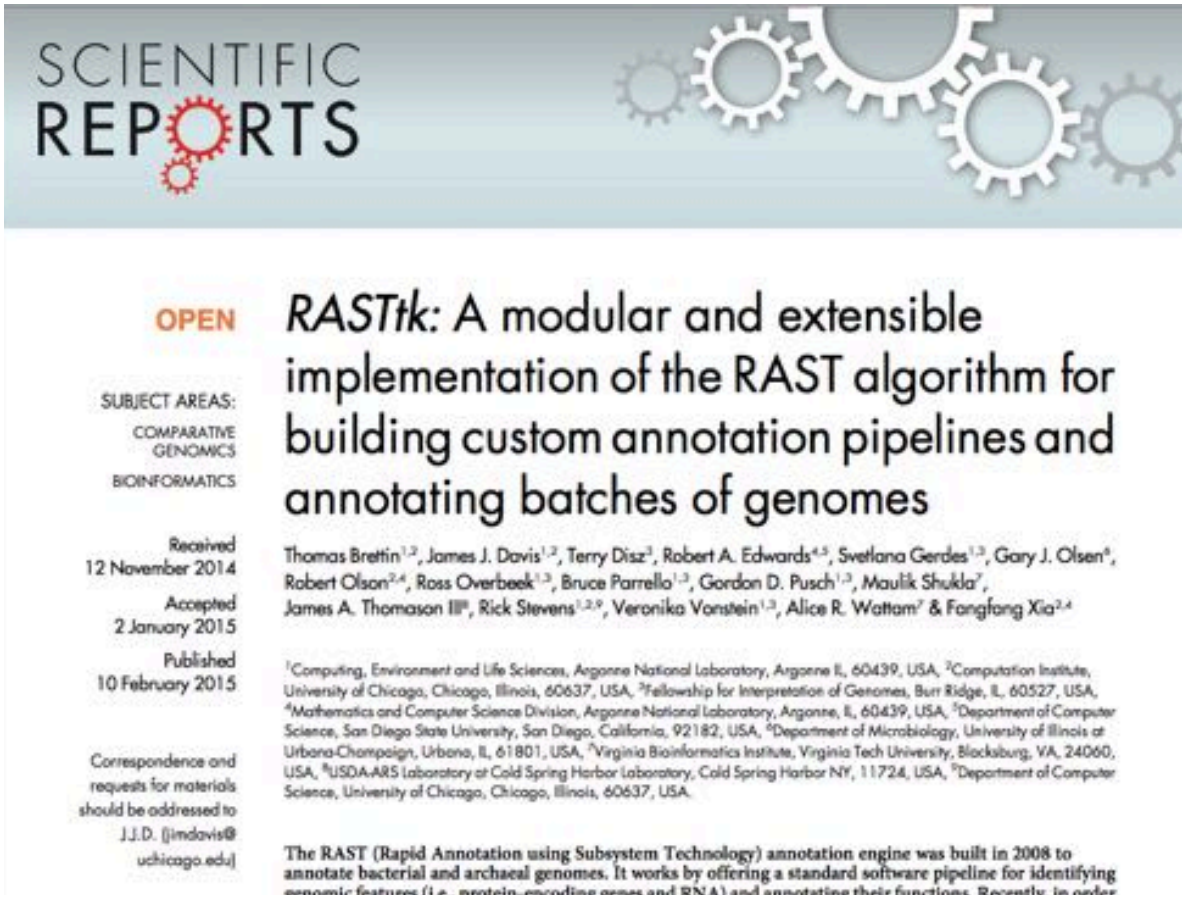
Genome Annotation Pipeline in PATRIC

Marcus Nguyen

What is happening during your annotation job?

RAST tool kit customized for PATRIC



SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
COMPARATIVE GENOMICS
BIOINFORMATICS

Received
12 November 2014

Accepted
2 January 2015

Published
10 February 2015

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RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes

Thomas Brettin[1,2], James J. Davis[1,2], Terry Disz[3], Robert A. Edwards[4,5], Svetlana Gerdes[1,3], Gary J. Olsen[6], Robert Olson[2,4], Ross Overbeek[1,3], Bruce Parrello[1,3], Gordon D. Pusch[1,3], Maulik Shukla[7], James A. Thomason III[8], Rick Stevens[1,2,9], Veronika Vonstein[1,3], Alice R. Wattam[7] & Fangfang Xia[2,4]

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The RAST (Rapid Annotation using Subsystem Technology) annotation engine was built in 2008 to annotate bacterial and archaeal genomes. It works by offering a standard software pipeline for identifying

PATRIC

What is happening during your annotation job?

- Calling rRNAs (16S, 23S, 5S)
- Calling tRNAs with tRNAscanSE
  - (Lowe & Eddy 1997)
- Searching for repeat regions
- Finding special proteins
  - Selenoproteins
  - Pyrrolysylproteins
- Calling CRISPRs
  - clustered regularly interspaced short palindromic repeats

What is happening during your annotation job?

- Calling protein-encoding genes
  - Prodigal (Hyatt et al. 2010)
  - Glimmer3 (Delcher et al. 2007)
- Assigning function
  - First attempt: annotates against CoreSEED
  - Second attempt: annotates against FIGFams
  - Third attempt: BLAST against close relatives
- Overlapping genes are resolved

What is happening during your annotation job?

- Annotates matches to:
  - ARDB (Liu & Pop 2009)
  - CARD (McArthur et al. 2013)
  - VFDB (Chen et al. 2012)
  - Victors (Xiang et al. 2007)
  - PATRIC virulence factors (Mao et al. 2014)
  - DrugBank (Law et al. 2014)
  - TTD (Qin et al. 2014)
  - Human homologs
- Assigns proteins to families
- Finds closest neighbors

AMR Predictions

- SIR prediction based on AdaBoost models
- Only models > 70% accuracy run
- Limits genera that can be predicted
  - Based on available SIR data
  - Lots of resistant genomes
  - Few suseptible

What Genomes Will Have AMR Annotations?

- Acinetobacter baumanni
- Klebsiella pneumoniae
- Mycobacterium tuberculosis
- Peptoclostridium difficile
- Pseudomonas aeruginosa
- Staphylococcus aureus
- Streptococcus pneumoniae

Questions Comments?

- If not, let's look at some annotations

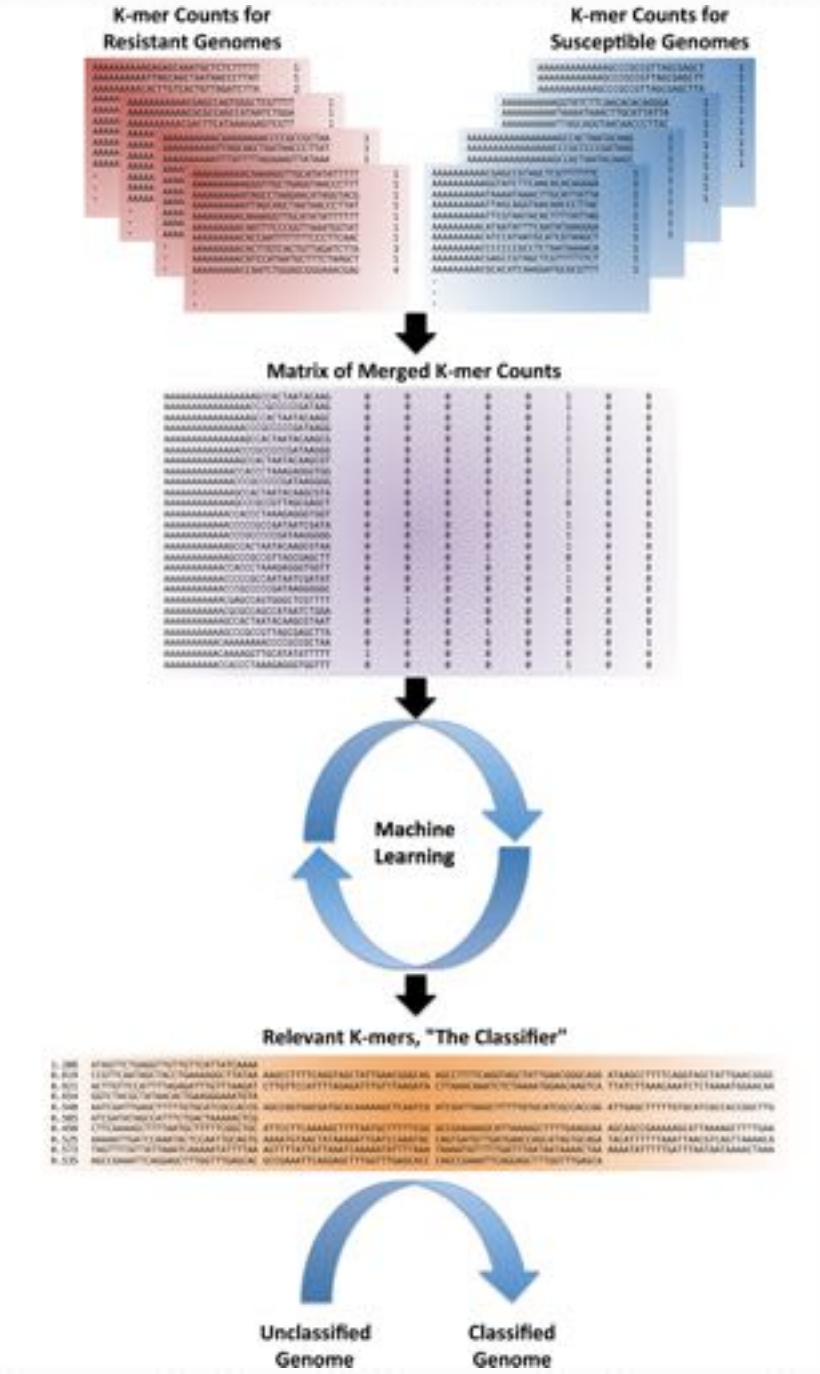
Extra Slides

How Do the Models Work?

- Machine learning
- Give computer
  - Lots of data about genomes
  - And label for genome (S or R)
- Computer finds correlations
  - Between data and label
  - Predict label in unseen genomes

Our Approach

- Give computer
  - Contig 15-mers
  - S or R label
- Computer finds
  - 15-mers related to S or R
  - Uses machine learning technique Adaboost
- Take top 10 15-mers
  - Make S or R prediction



Adaboost

- Stands for *adaptive boosting*
- For each k-mer
  - Sees which k-mer accurately predicts S or R
- Selects best k-mer
- Loop
  - Select best k-mer
    - Predicts well what previous could not

Adaboost Example

15-mer	% S	% R
AATCGACTAA…	0.75	0.25
AATCGCCGTT…	0.05	0.95
ATATGGCATA…	0.45	0.55
ATATATTACG…	0.76	0.24
TTGACAGATA…	0.33	0.67
CGTAGACTAG…	0.11	0.89
TGACATACCA…	0.72	0.28
GTACTACCCA…	0.50	0.50
CGTACCGACT…	0.62	0.38
GATAGATCCG…	0.77	0.23
GATTAAGGCC…	0.20	0.80

15-mer list

Selected 15-mers

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- AATCGCCGTT…

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15-mer list

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- GATAGATCCG…
- ATATATTACG…
- CGTAGACTAG…
- …

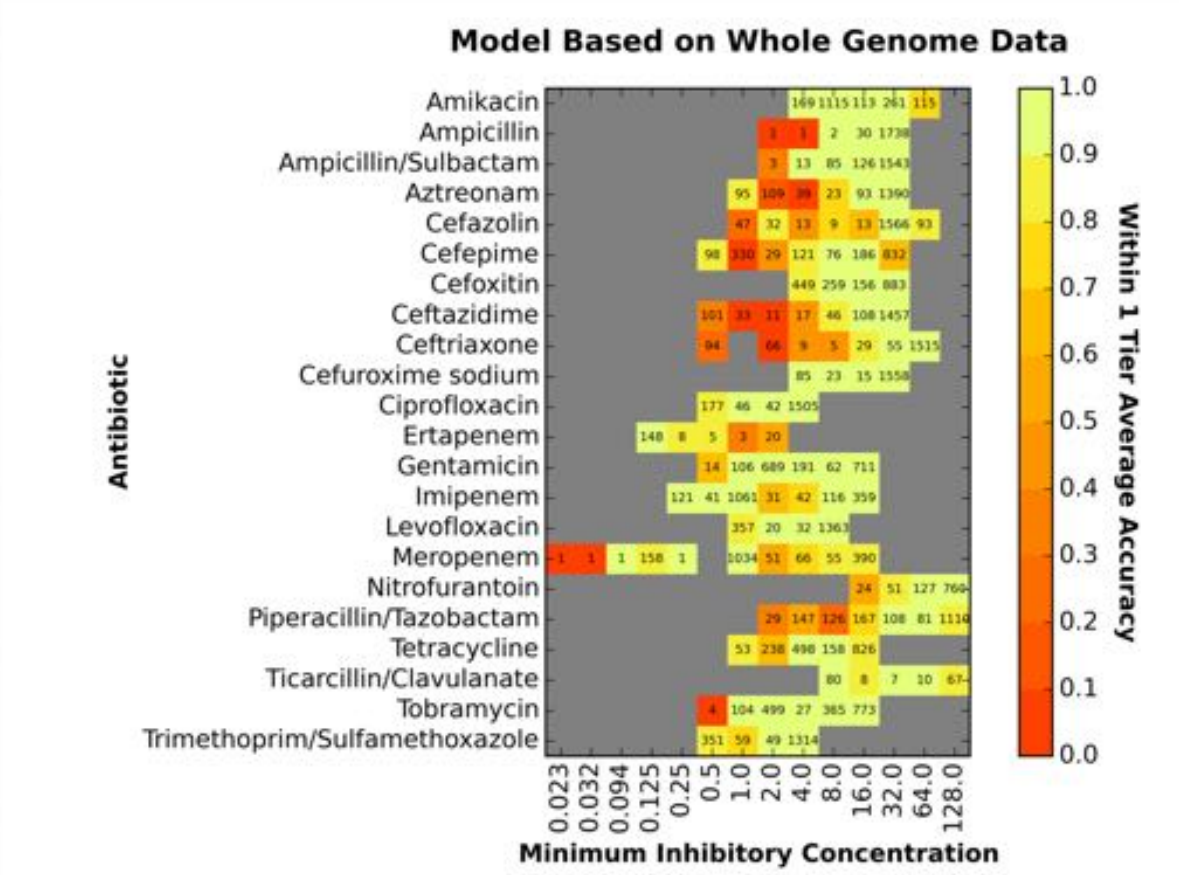
Selected 15-mers

Once 15-mers selected

- Each 15-mer "votes"
  - Susceptible
  - Resistant
- Most "votes" = predicted label
  - If genome has more top-10 resistant k-mers
    - Labeled resistant
  - If genome has more top-10 susceptible k-mers
    - Labeled susceptible

Future Work (predicting MIC)

- Given genome, antibiotic, MIC
  - Train model Using 10-mers
  - Predict MIC
- Building model for *Klebsiella Pneumoniae*
  - Uses gradient boosted trees
  - Overall accuracy (93%)
  - Varies across MIC values and antibiotics



Future Work (predictions with reads)

- Predict AMR using raw reads
  - Susceptibility vs Resistance
  - MIC?
- Clinical setting idea
  - Use MinION
  - Feed reads to model
  - Predict AMR (S, I, R, MIC, etc.)