

# Comparison of bacteriophage annotation methods



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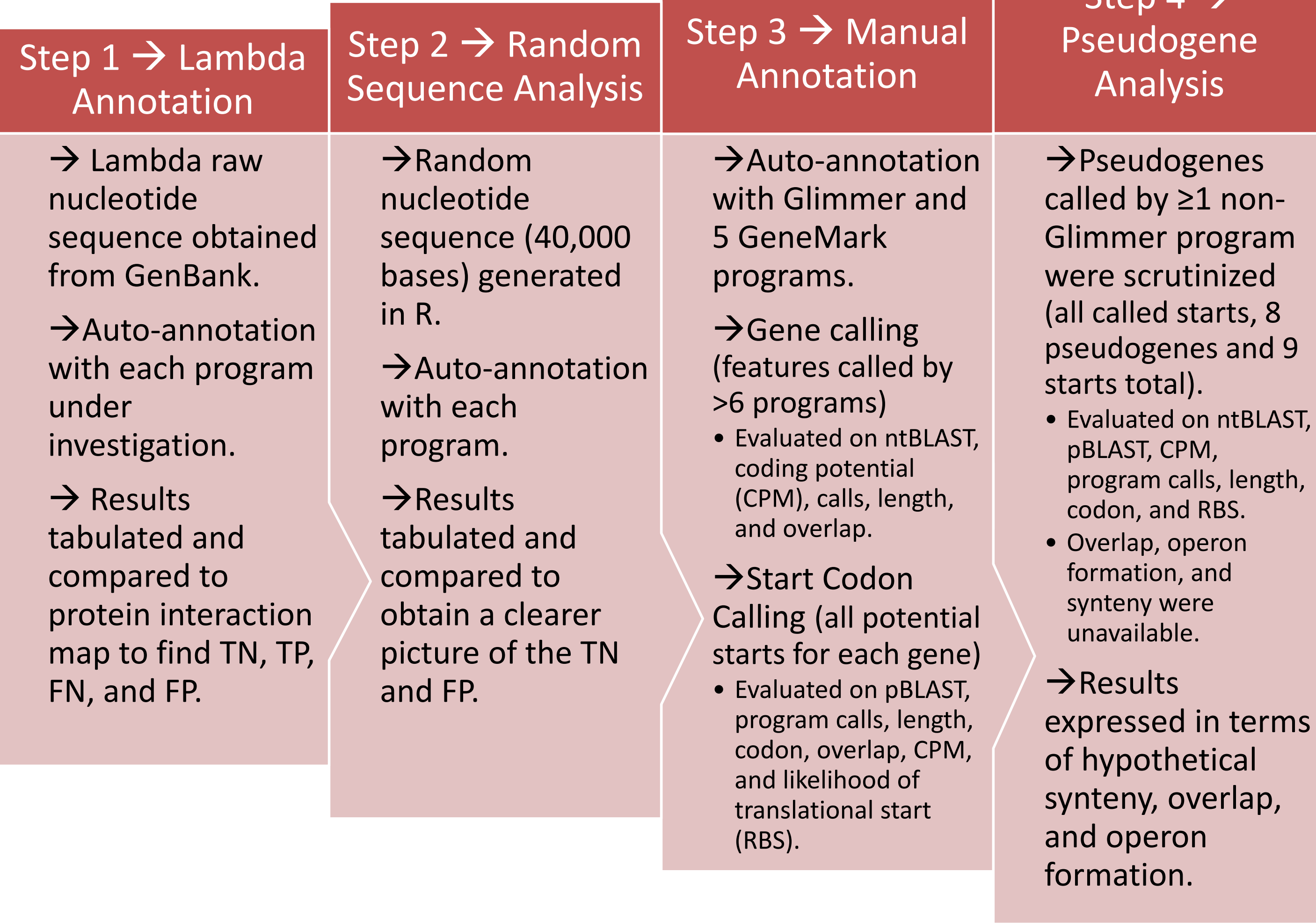
## Abstract:

The rise of antibiotic-resistant bacteria has increased interest in bacteriophages (viruses that kill bacteria) in recent years. Due to the decreasing cost of genome sequencing, the number of sequenced phage genomes is growing at a geometric rate. Sequencing is followed by annotation, in which genes, start codons, and putative protein functions are identified. Most phage genomes are auto-annotated with programs designed for prokaryotes. Accuracy metrics for these programs with regard to phage genomes are not available. The genome of *Escherichia coli* phage Lambda was used to benchmark the accuracy of several genome annotation methods and programs. Discovered in 1951, Lambda is the most well studied phage, with nearly all gene functions and start sites demonstrated experimentally. Eight programs were used to annotate the Lambda genome: Glimmer, BASys, RAST, GeneMark, GeneMark.hmm, GeneMarkS, GeneMarkS2, and GeneMark with Heuristic models. Calls were compared to the reference genome from the literature.

**Goal:** To determine the accuracy of the eight selected programs in regard to bacteriophage genome annotation.

**Hypothesis:** Manual curation and compilation of auto-annotation results obtained from several programs will yield more accurate gene feature and start codon prediction than auto-annotation alone.

## Methods:



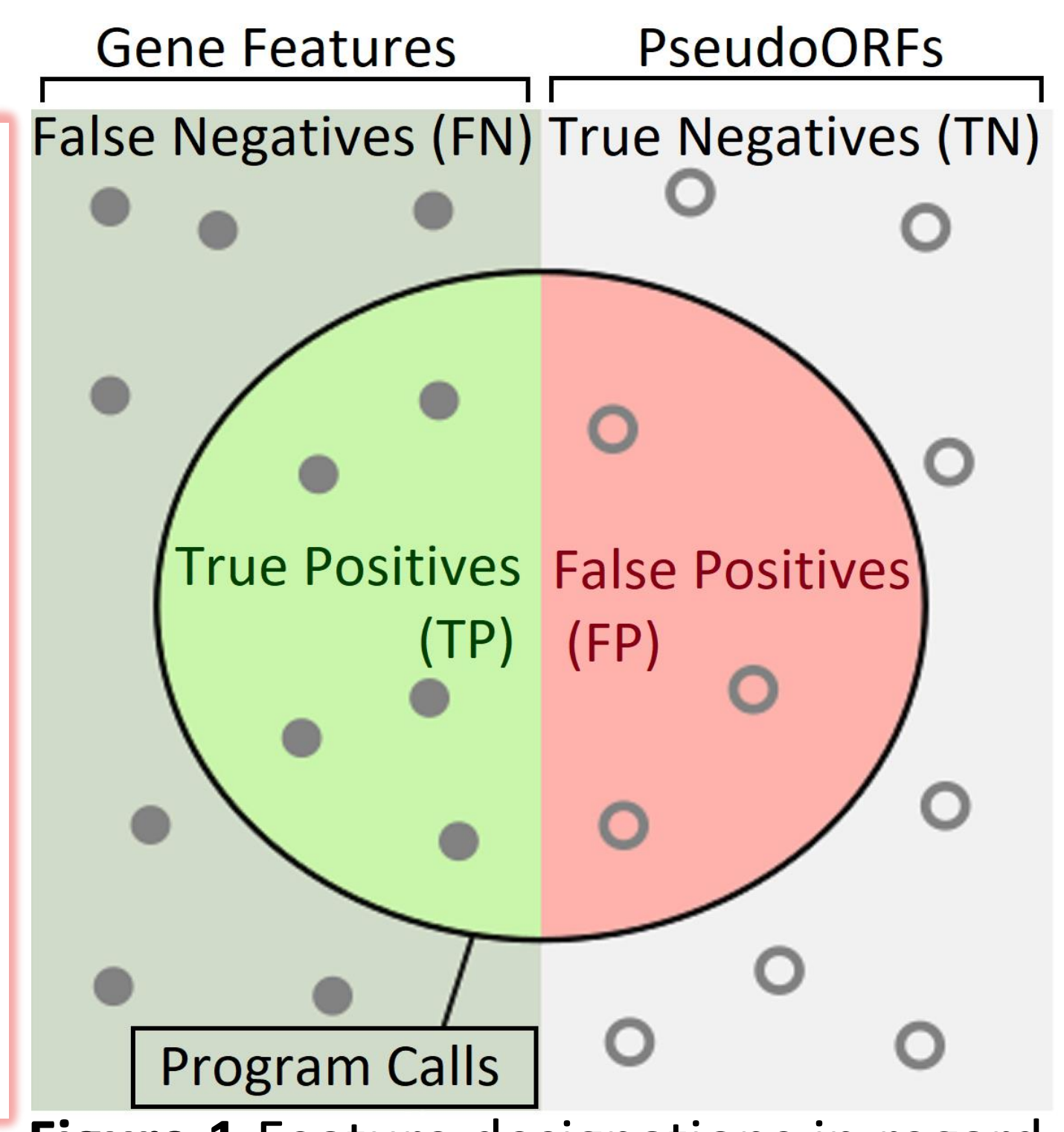
## Calculations:

**Sensitivity**  
 True Positive Rate (TPR)  
 Describes the proportion of genes called correctly

$$TPR = \frac{TP}{TP+FN}$$

**Precision**  
 Positive Predictive Value (PPV)  
 Describes the probability that a call is a gene

$$PPV = \frac{TP}{TP+FP}$$



**Figure 1** Feature designations in regard to phage genomics. A pseudoORF is a continuous reading frame ≤75bp long.

## Data:

**Table 1** Calling programs, coordinates, length, CPM for all PseudoORFs generated by non-Glimmer programs in the randomly generated sequence.

Feature	1	2	3	4	5	6	7	8
Direction	R	F	R	F	R	F	F	R
Stop	6259	7068	7403	22324	27950	31520	35911	39017
Start	6384	6904	7570	22130	28174	31299	35636	39196
GeneMark		called	called	called	called	called		called
Glimmer							called	called
GeneMark.hmm			called		called		called	
GeneMarkS		called	called	called	called	called		called
Heuristic		called	called	called	called	called		called
GeneMark								called
RAST	called			called		called		called
Length	126	165	168	195	225	222	276	201
Coding Potential								

**Table 2** Positives and negatives. 73 genes, 545 true negatives in the reference.

Gene Calls	GeneMark	.hmm	S	Heuristic	S2	RAST	BASys	Glimmer	Manual
False Positives	3	4	3	3	2	2	4	4	4
True Positives	58	62	58	58	58	62	62	63	65
True Negatives	542	541	542	542	543	543	541	541	541
False Negatives	16	11	15	15	15	11	11	10	9
<b>Sensitivity (TPR)</b>	0.784	0.849	0.795	0.795	0.795	0.863	0.849	0.849	0.878
<b>Precision (PPV)</b>	0.951	0.939	0.951	0.951	0.967	0.940	0.969	0.939	0.942

**Table 3** Starts called long or short relative to the reference genome.

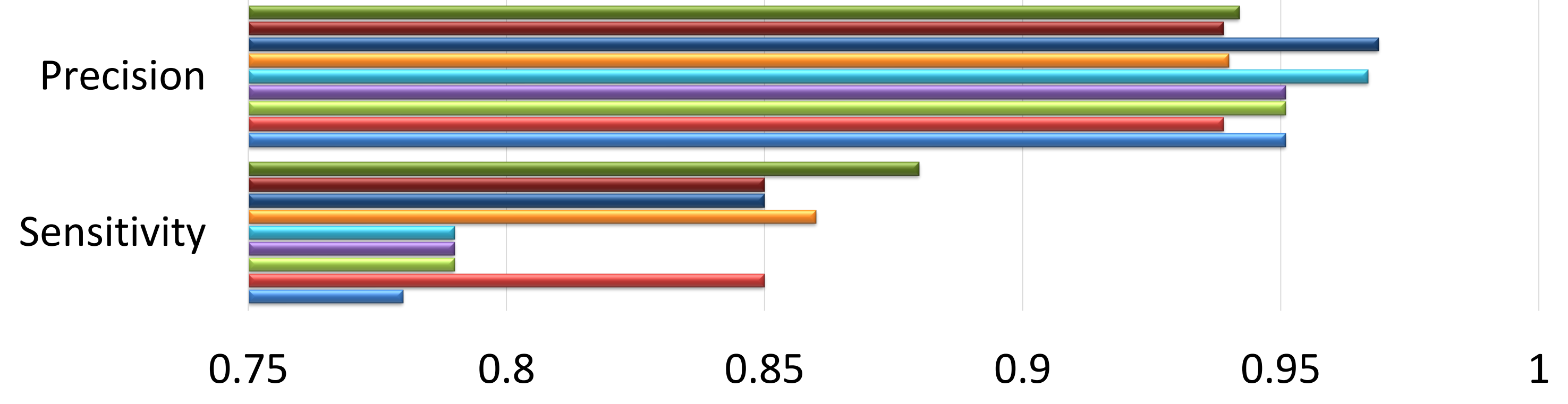
Start Calls	GeneMark	.hmm	S	Heuristic	S2	RAST	BASys	Glimmer	Manual
Called Long	6	1	6	6	3	2	1	4	1
Called Short	5	7	5	5	7	7	5	7	4
Genes Called	58	62	58	58	58	62	62	63	64
<b>% Accurate</b>	81.03%	87.10%	81.03%	81.03%	82.76%	85.48%	90.32%	82.54%	92.19%

**Table 4** PseudoORFs generated for the randomly generated sequence, in which 628 true negatives exist. GeneMarkS2 and BASys returned no result.

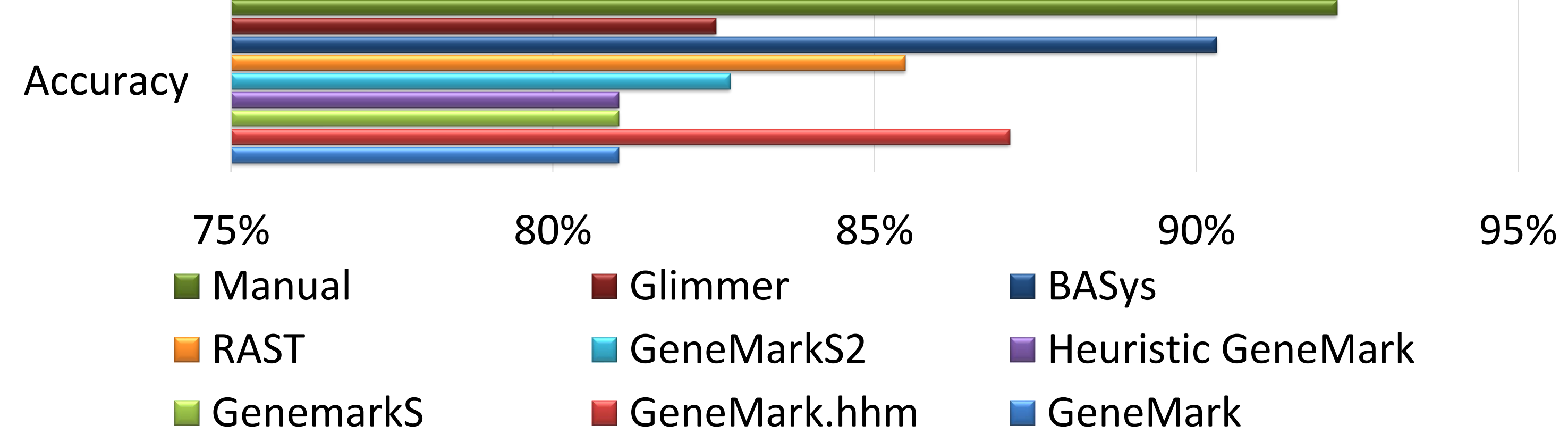
Generated	GeneMark	Glimmer	.hmm	S	Heuristic	RAST
False Positives	6	47	3	6	6	4
True Negatives	622	581	625	622	622	624

## Results:

### Gene Calling



### Start Codon Calling



## Conclusions:

- Manual annotation is slightly more accurate, particularly in start calling.
- No gene called by all programs was a false positive.
- Some genes were not detected by any annotation method.
- Glimmer is disproportionately prone to nonsense FN.
- Pseudogenes called by any program other than Glimmer represent borderline features, which, during manual annotation, would be:
  - Deleted in the absence of any additional evidence.
  - Kept if they satisfied one of the following conditions:
    1. Filled a gap completely without generating overlap or direction change.
    2. Created 4bp overlap on one or both ends, suggesting an operon.
- Pseudogenes called in the random sequence have:
  1. No plateaus of coding potential.
  2. No significant ntBLAST result.
  3. No significant pBLAST results.
  4. Coding potential does not align well with start and STOP coordinates.

## Future Research:

- Uncalled genes will be scrutinized and compared to pseudogenes called in the random sequence.

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