Detection of Syntenic Regions in Bacterial Genomes Through Statistical Clustering

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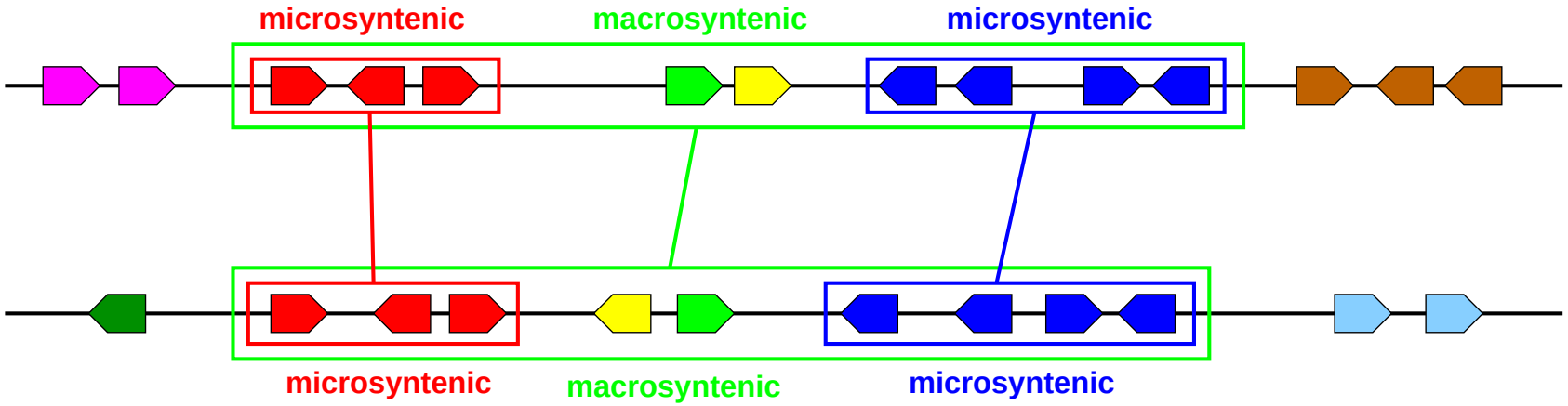
Interface 2007 on Systems Biology, May 24, 2007
Philadelphia, Pennslyvania

Research funded by USDA/ARS

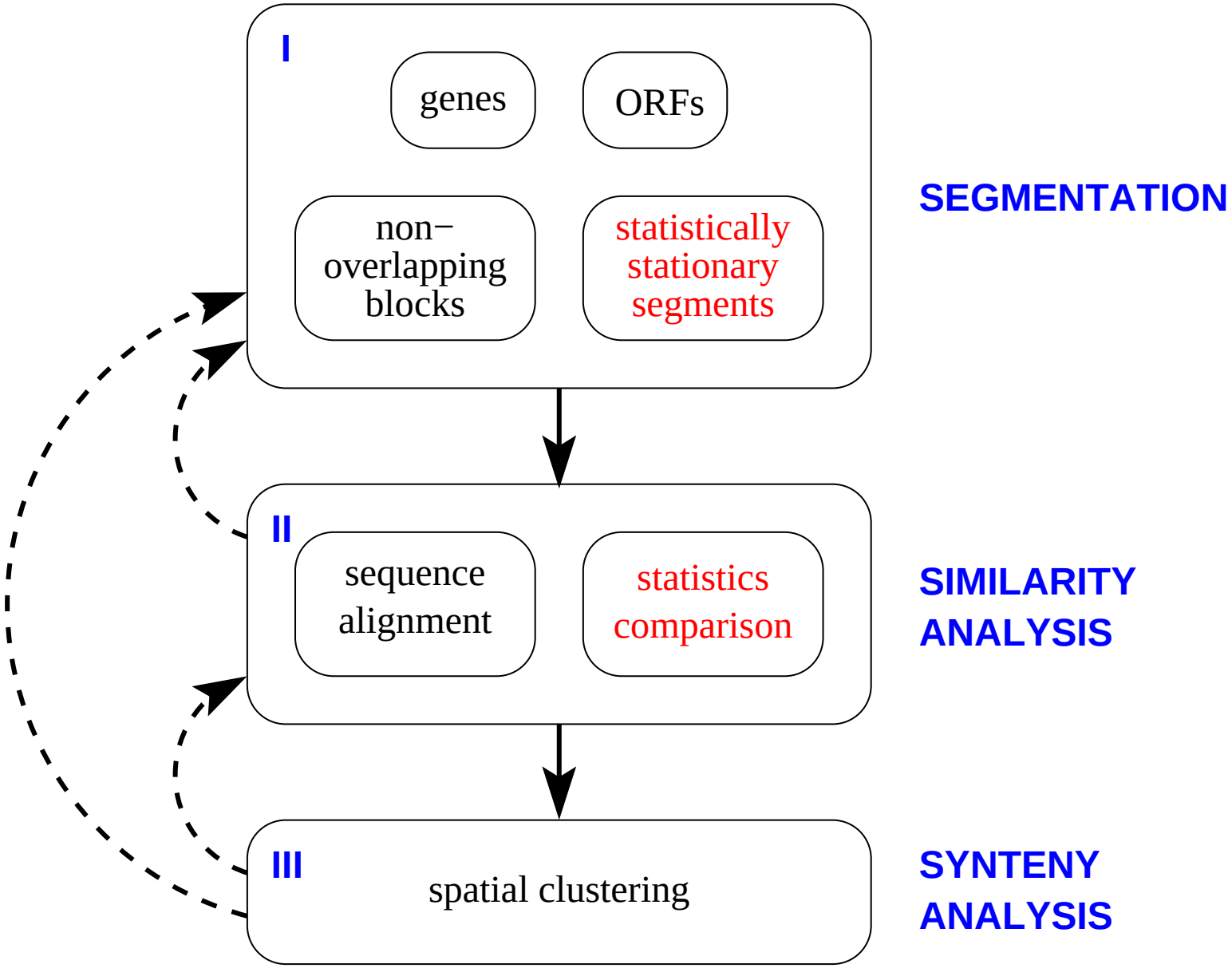
Synteny and Evolution

- The goal of our group to understand the evolutionary roles of horizontal gene transfer and other large-scale rearrangements in shaping organization of bacterial genomes.
- Syntenic regions are homologous multigene regions in two or more genomes in which repertoire of genes are conserved, along with possible conservation of transcription direction and linear gene order.



- Detecting breaks in macrosynteny and microsynteny between closely related bacteria useful tool in unraveling mosaic structures within their genomes.

Computational Framework for Synteny Detection



The Jensen-Shannon Divergence

- Advantage of using Jensen-Shannon divergence [Lin, IEEE Trans. Infor. Theory **37**, 145–151 (1991)] as statistical distance: can be computed for sequences $\mathbf{x}_1$ and $\mathbf{x}_2$ of different lengths.

- For $\mathbf{x}_1$ and $\mathbf{x}_2$ modeled as Markov chains of order K over quaternary alphabet $\mathcal{S} = \{\mathrm{A}, \mathrm{C}, \mathrm{G}, \mathrm{T}\}$ with $S = 4$ letters, Jensen-Shannon divergence given by

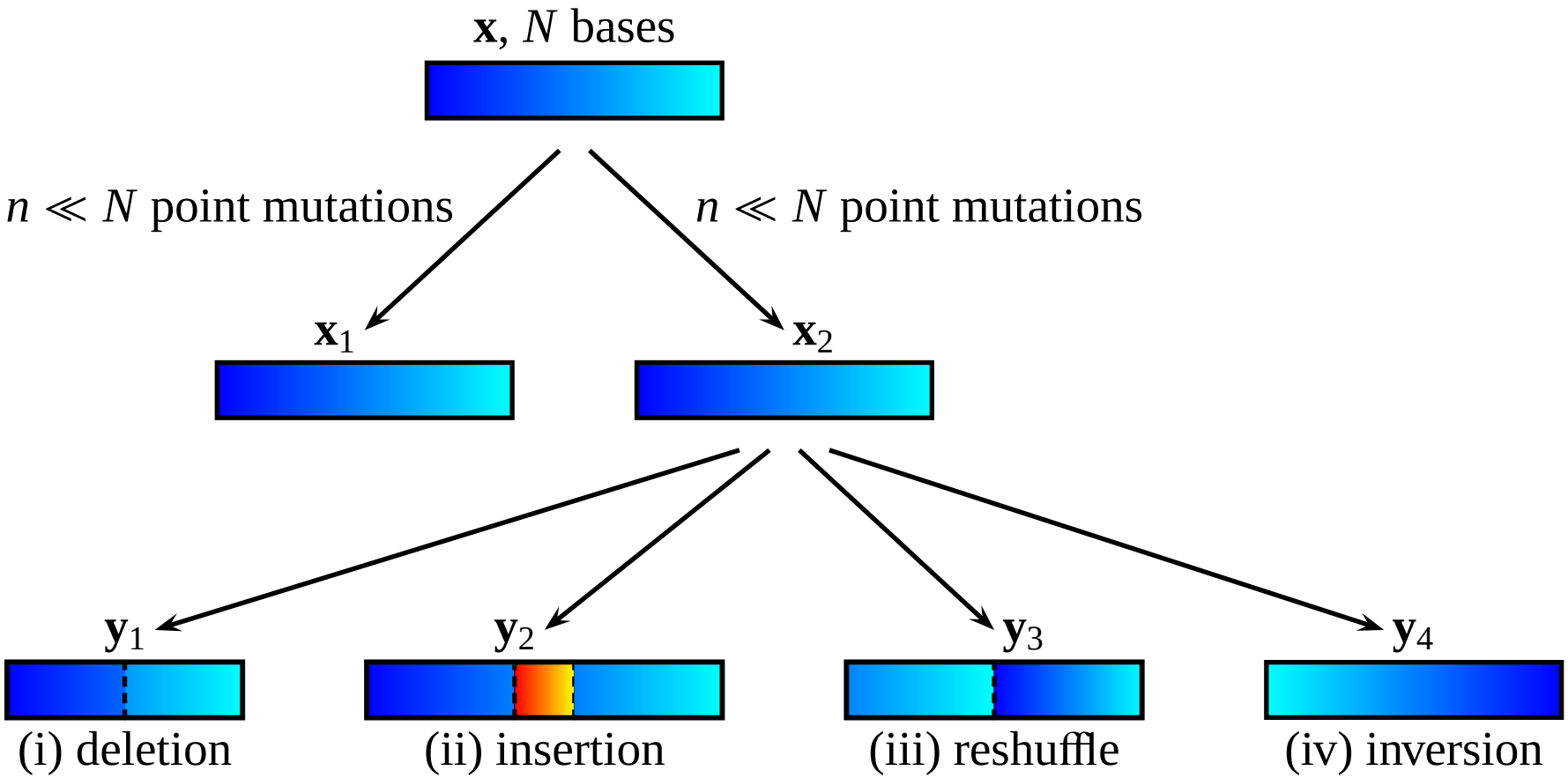
$$\Delta = \sum_{\mathbf{t} \in S^{\otimes K}} \sum_{s=1}^{S} [-f_{\mathbf{t}s} \log \hat{p}_{\mathbf{t}s} + f_{1,\mathbf{t}s} \log \hat{p}_{1,\mathbf{t}s} + f_{2,\mathbf{t}s} \log \hat{p}_{2,\mathbf{t}s}] \geq 0,$$

  where $\mathbf{t} = (t_1, \ldots, t_K) \in \mathcal{S}^K$ is shorthand notation, $f_{1,\mathbf{t}s}$, $f_{2,\mathbf{t}s}$, $f_{\mathbf{t}s} = f_{1,\mathbf{t}s} + f_{2,\mathbf{t}s}$ are transition counts, and

$$\hat{p}_{i,\mathbf{t}s} = \frac{f_{i,\mathbf{t}s}}{\sum_{s'=1}^{S} f_{i,\mathbf{t}s'}}, \quad i = 1, 2; \quad \hat{p}_{\mathbf{t}s} = \frac{f_{\mathbf{t}s}}{\sum_{s'=1}^{S} f_{\mathbf{t}s'}},$$

  are maximum-likelihood transition probabilities.

Mutations and Recombinations



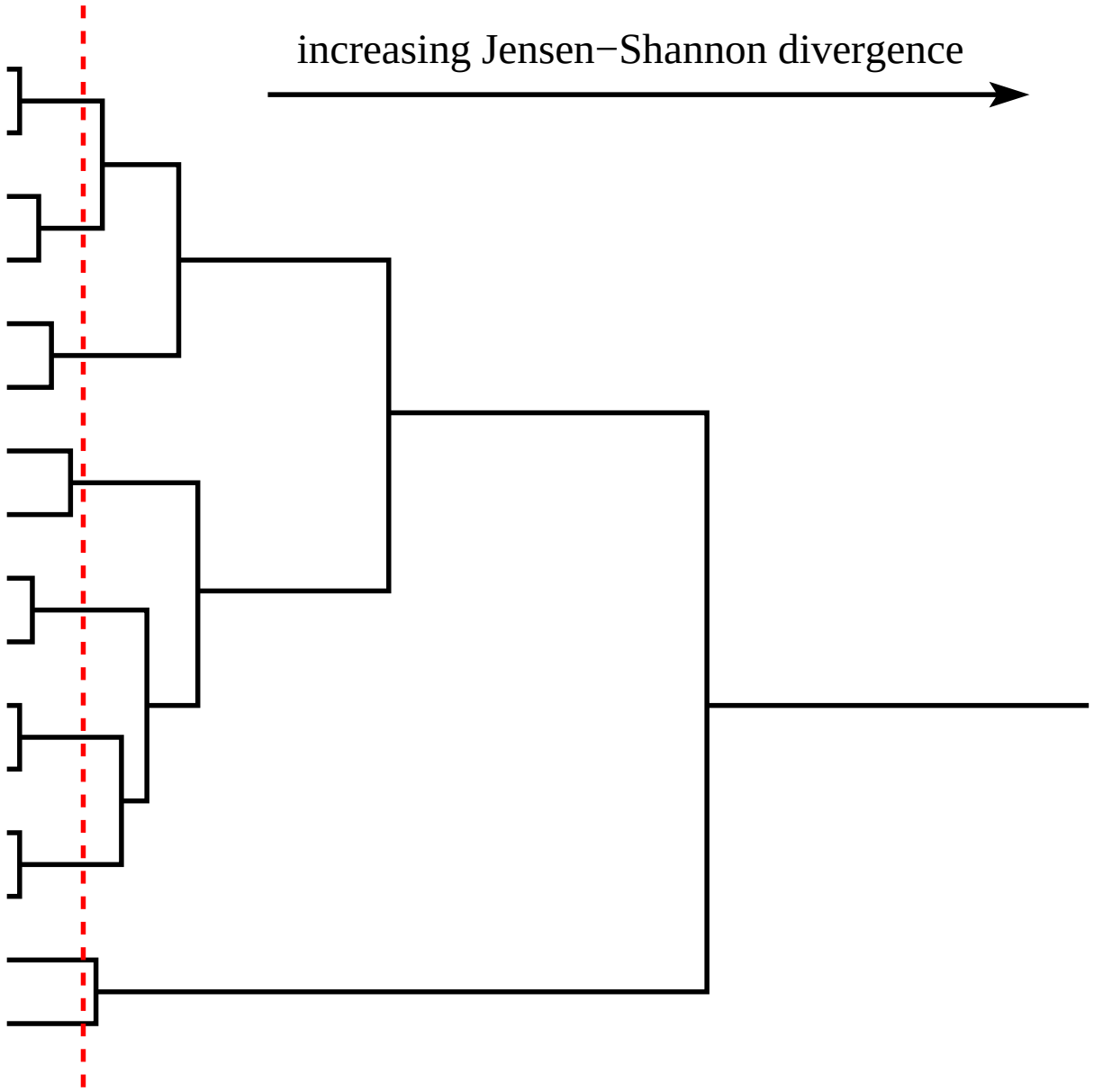
Sequence Alignment and Statistics Comparison

- Without point mutations, $\mathbf{x}_1$ and $\mathbf{x}_2$ perfectly aligned. Also have identical K-mer statistics up to $K = N$.
- With $n \ll N$ point mutations, good alignment between $\mathbf{x}_1$ and $\mathbf{x}_2$. Very similar K-mer statistics up to intermediate K.
- Higher-order statistics strongly constrained by lower-order statistics, therefore, instead of sequence alignment, need only compare K-mer statistics at the few lowest K's to establish homology.
- For recombination cases
  - (i) deletion and (ii) insertion, statistical similarity between $\mathbf{x}_1$ and $\mathbf{y}_1, \mathbf{y}_2$ depends on segment deleted or inserted.
  - (iii) reshuffle, K-mer statistics between $\mathbf{x}_1$ and $\mathbf{y}_3$ similar.
  - (iv) inversion, complementary K-mer statistics of $\mathbf{y}_4$ similar to K-mer statistics of $\mathbf{x}_1$.

Hierarchical Single-Link Clustering

- Use Jensen-Shannon divergence as statistical distance between segments.
- Hierarchical clustering chosen because number and scales of syntenic regions not known beforehand. Hierarchical clustering tree for each K.
- Single-link separation between clusters:
  - clusters of homologous segments diffuse in statistics space, driven by random point mutations;
  - clusters diverging at different evolutionary times have different sizes;
  - two segments close together likely to have evolved from a common ancestor, even if both are far from center of homolog cluster.

Homology Within the Hierarchical Clustering Tree



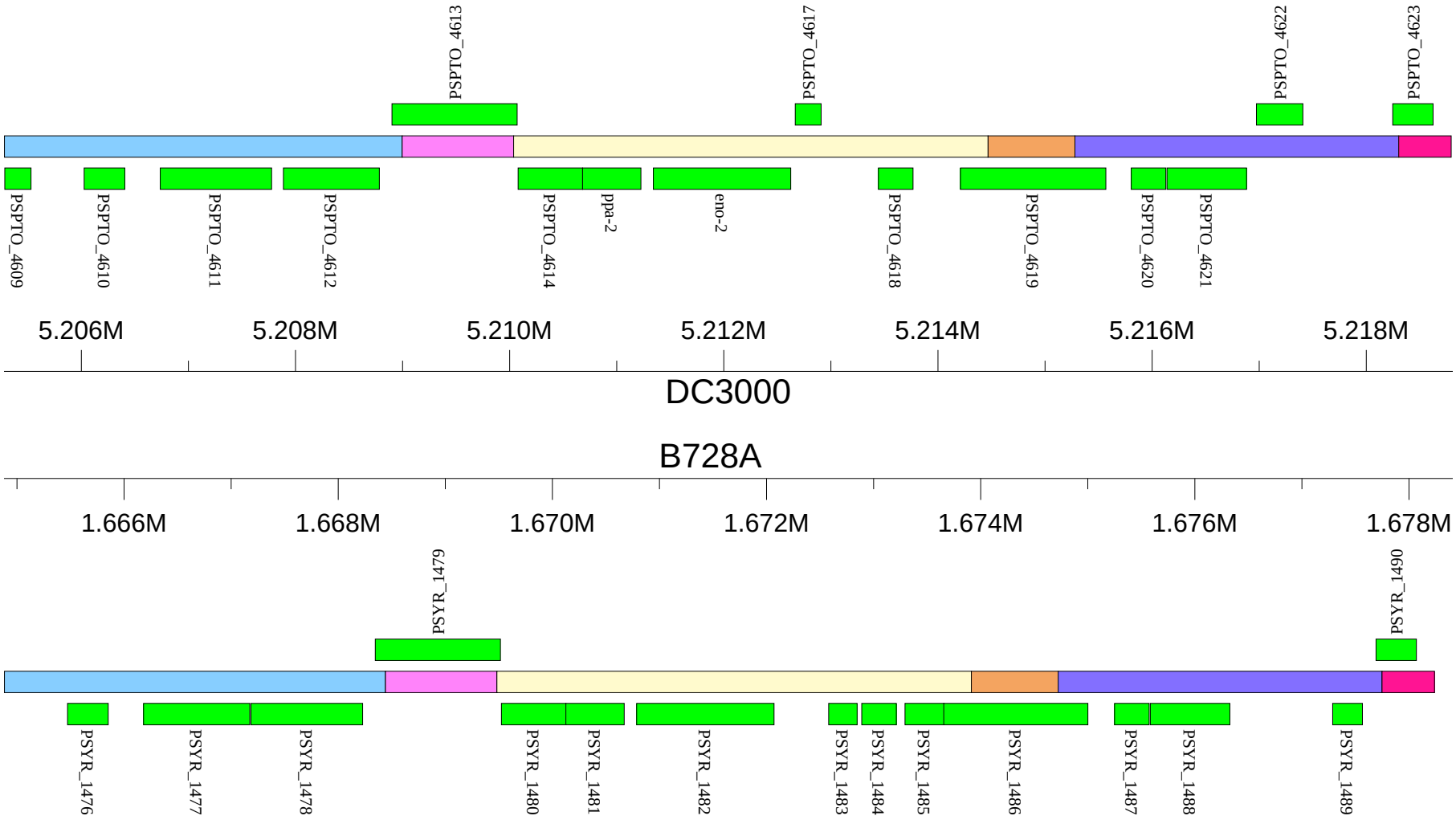
Pilot Study

- Complete genomes of three *Pseudomonas syringae* strains. Plant pathogens.

strain	N (Mbp)
DC3000	6.4
1448A	5.9
B728A	6.1

- Stage I: Segmentations obtained using optimized recursive Jensen-Shannon segmentation scheme [Cheong *et al.*, in preparation].
- Stage II: Cluster at $K = 0, 1, 2, 3$ but not higher, because typical segments are only 5000 bp long.
- Stage III: $K = 0$ hierarchical clustering tree not sufficiently discriminating, but from $1 \leq K \leq 3$ hierarchical clustering trees identified:
  - long syntenic region between DC3000 and B728A; and
  - large number clustering events between paralogous segments containing mobile IS elements.

Syntenic Region Identified



Feedback Between Stages

- Few long syntenic regions identified from hierarchical clustering trees, because syntenic regions segmented differently in different genomes as a result of context sensitivity problem [Cheong *et al.*, in preparation].
- Stage III $\rightarrow$ Stage I: Work with two terminal segmentations per genome: standard (S) and fine (F). (Testing robustness of clustering to segmentation.)
  - cluster S segments against S segments: mask out syntenic regions detected at this level;
  - for remaining segments, cross cluster F segments against S segments: mask out syntenic regions detected at this level; and
  - for remaining segments, cluster F segments against F segments: identify syntenic regions detected at this level.
- Expect cross clustering to detect most, if not all, syntenic regions present.
- Hyperfine segmentation and fine-hyperfine segmentation if necessary.

Conclusions

- Described the three stages in the general framework for synteny detection.
- Stage I: Segmentation. Use statistically stationary segments.
- Stage II: Similarity Analysis.
  - Recasted sequence alignment problem as statistics comparison problem of statistically stationary segments.
  - Devised hierarchical single-link clustering of segments whose pairwise distance is their Jensen-Shannon divergence.
  - Explained how homologous segments cluster at small Jensen-Shannon divergence, and how syntenic blocks emerge as chains of clusters.
- Stage III: Synteny Analysis.
  - Pilot study on three *P. syringae* strains demonstrated feasibility of statistics comparison method for synteny detection.
  - Followup study: cross clustering between standard and fine segmentations of genomes.