TADA: phylogenetic augmentation of microbiome samples enhances phenotype classification

Erfan Sayyari[1], Ban Kawas [2], Siavash Mirarab[1]

[1] University of California, San Diego,
[2] IBM, Research—Almaden

Erfan Sayyari

Ban Kawas

Many studies report successful application of supervised learning to microbiome data

Gut microbiome development along the colorectal adenoma–carcinoma sequence

Qiang Feng, Suisha Liang […] Jun Wang

Nature Communications **6**, Article number: 6528 (2015) | Download Citation

Gastrointestinal Microbiome Signatures of Pediatric Patients With Irritable Bowel Syndrome

Delphine M. Saulnier[*,†,§], Kevin Riehle[‡,¶], Toni–Ann Mistretta[*,‡], Maria–Alejandra Diaz[*,‡], Debasmita Mandal[#], Sabeen Raza[*,‡], Erica M. Weidler[**,‡‡], Xiang Qin[§§], Cristian Coarfa[‡,¶], Aleksandar Milosavljevic[‡,¶], Joseph F. Petrosino[§§,ıı,¶¶], Sarah Highlander[§§,ıı], Richard Gibbs[§§], Susan V. Lynch[#], Robert J. Shulman[**,‡‡], James Versalovic[*,†,‡,**,ıı,*]

A Metagenomic Approach to Characterization of the Vaginal Microbiome Signature in Pregnancy

Kjersti Aagaard, Kevin Riehle, Jun Ma, Nicola Segata, Toni-Ann Mistretta, Cristian Coarfa, Sabeen Raza, Sean Rosenbaum, Ignatia Van den Veyver, Aleksandar Milosavljevic, Dirk Gevers, Curtis Huttenhower, Joseph Petrosino, James Versalovic

Published: June 13, 2012 • https://doi.org/10.1371/journal.pone.0036466

Machine Learning Techniques Accurately Classify Microbial Communities by Bacterial Vaginosis Characteristics

Daniel Beck, James A. Foster

Published: February 3, 2014 • https://doi.org/10.1371/journal.pone.0087830

Article | OPEN | Published: 10 October 2017

The gut microbiome in atherosclerotic cardiovascular disease

Zhuye Jie, Huihua Xia, […] Karsten Kristiansen

Nature Communications **8**, Article number: 845 (2017) | Download Citation

Article

Prediction of Early Childhood Caries via Spatial-Temporal Variations of Oral Microbiota

Fei Teng [1, 2, 5], Fang Yang [3, 5], Shi Huang [2, 5], Cunpei Bo [2], Zhenjiang Zech Xu [4], Amnon Amir [4], Rob Knight [4], Junqi Ling [1], Jian Xu [2]

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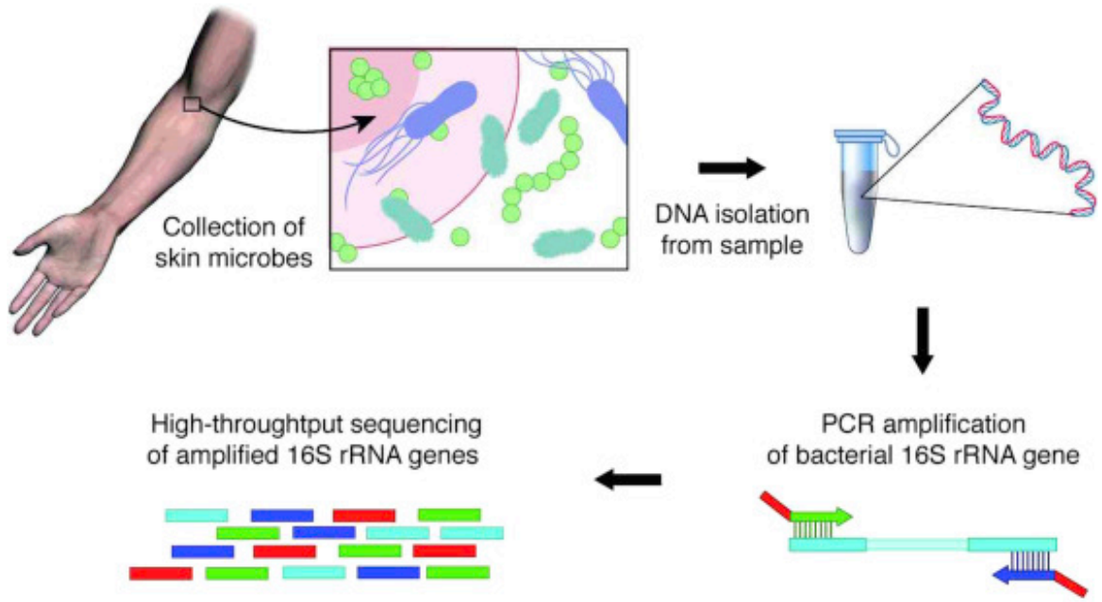
https://doi.org/10.1016/j.chom.2015.08.005

Sequencing microbiome (16S)



Jo et al., Journal of Investigative Dermatology, 2016

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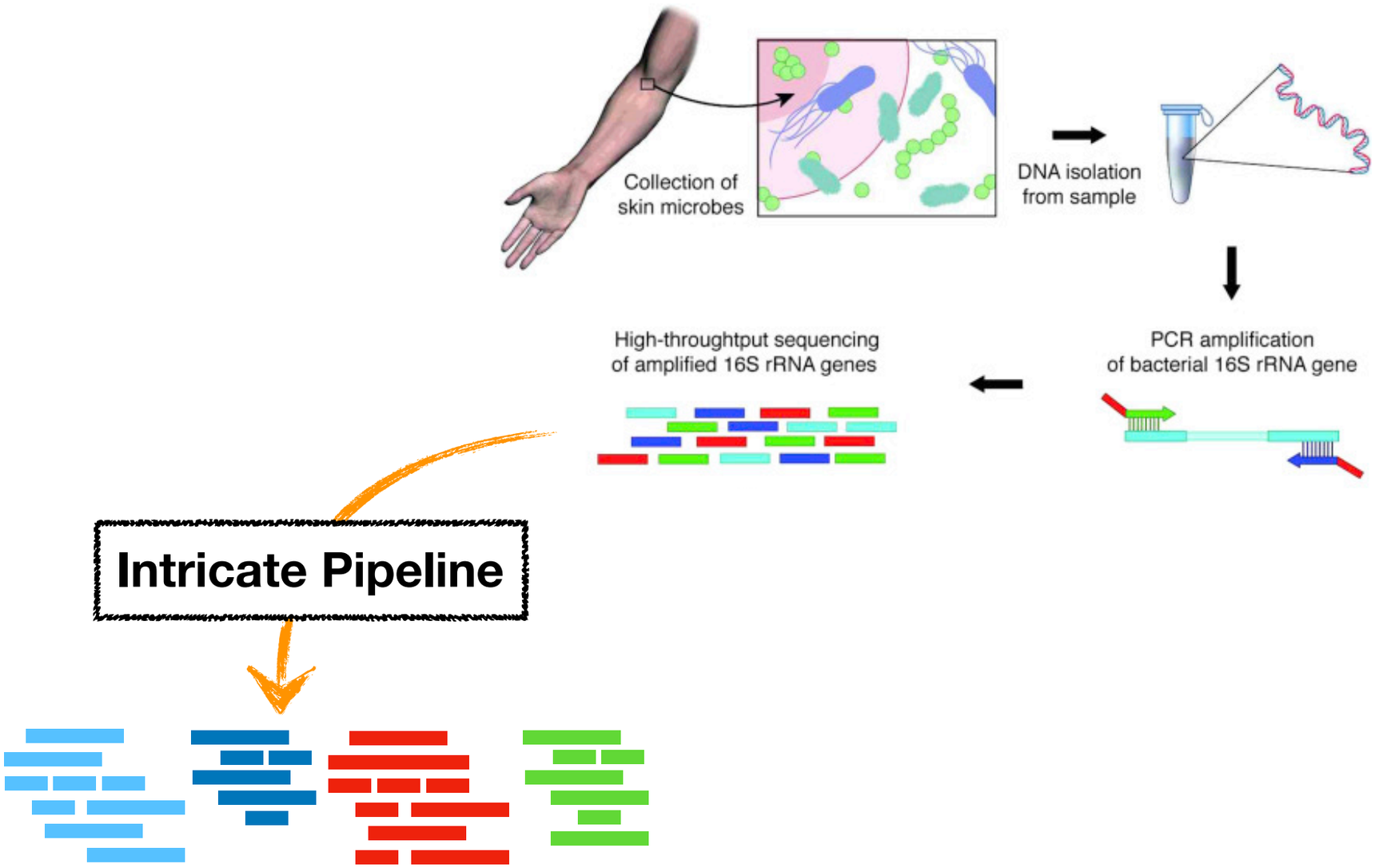

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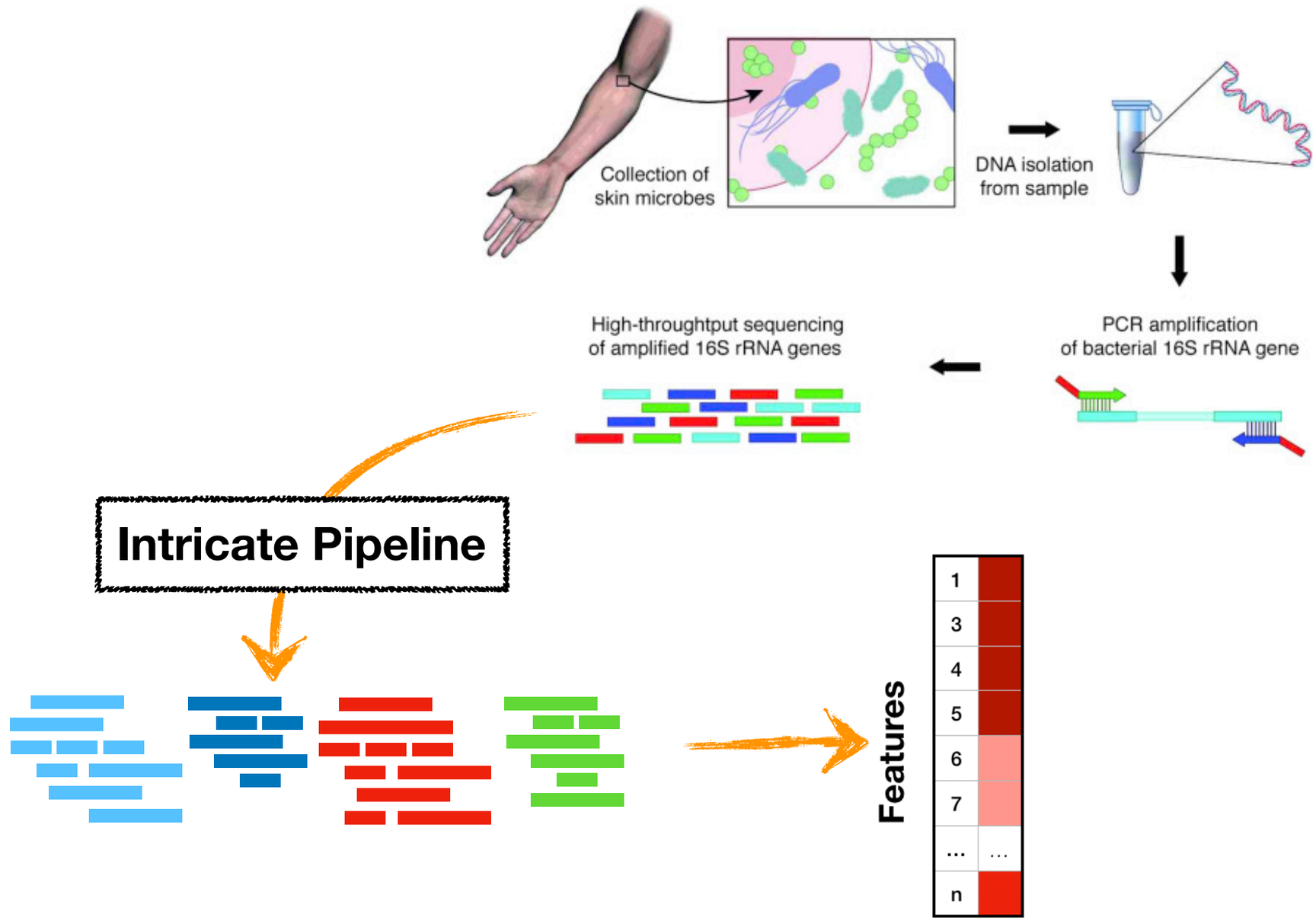

Jo et al., Journal of Investigative Dermatology, 2016

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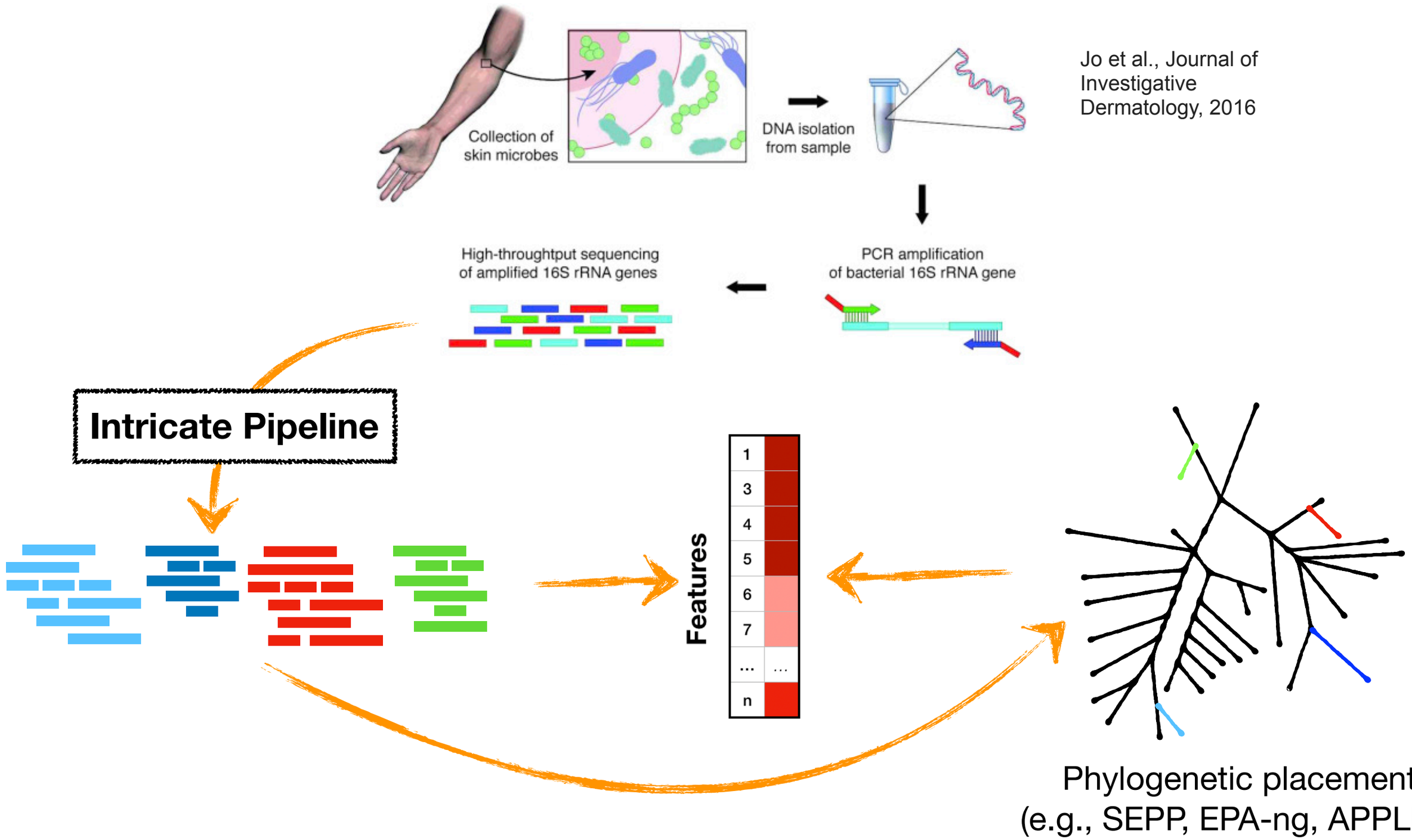
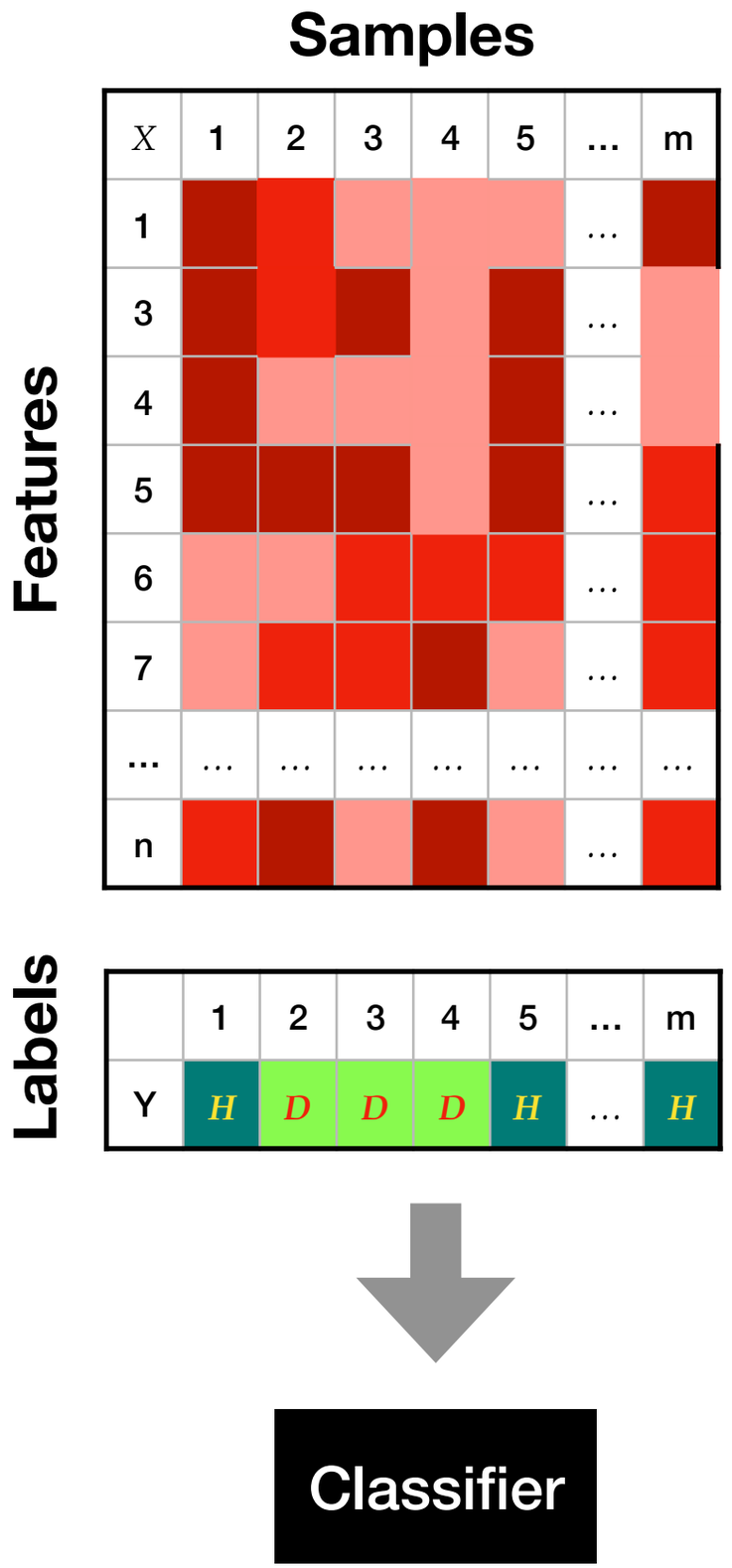


Jo et al., Journal of Investigative Dermatology, 2016

Supervised learning from microbiome data

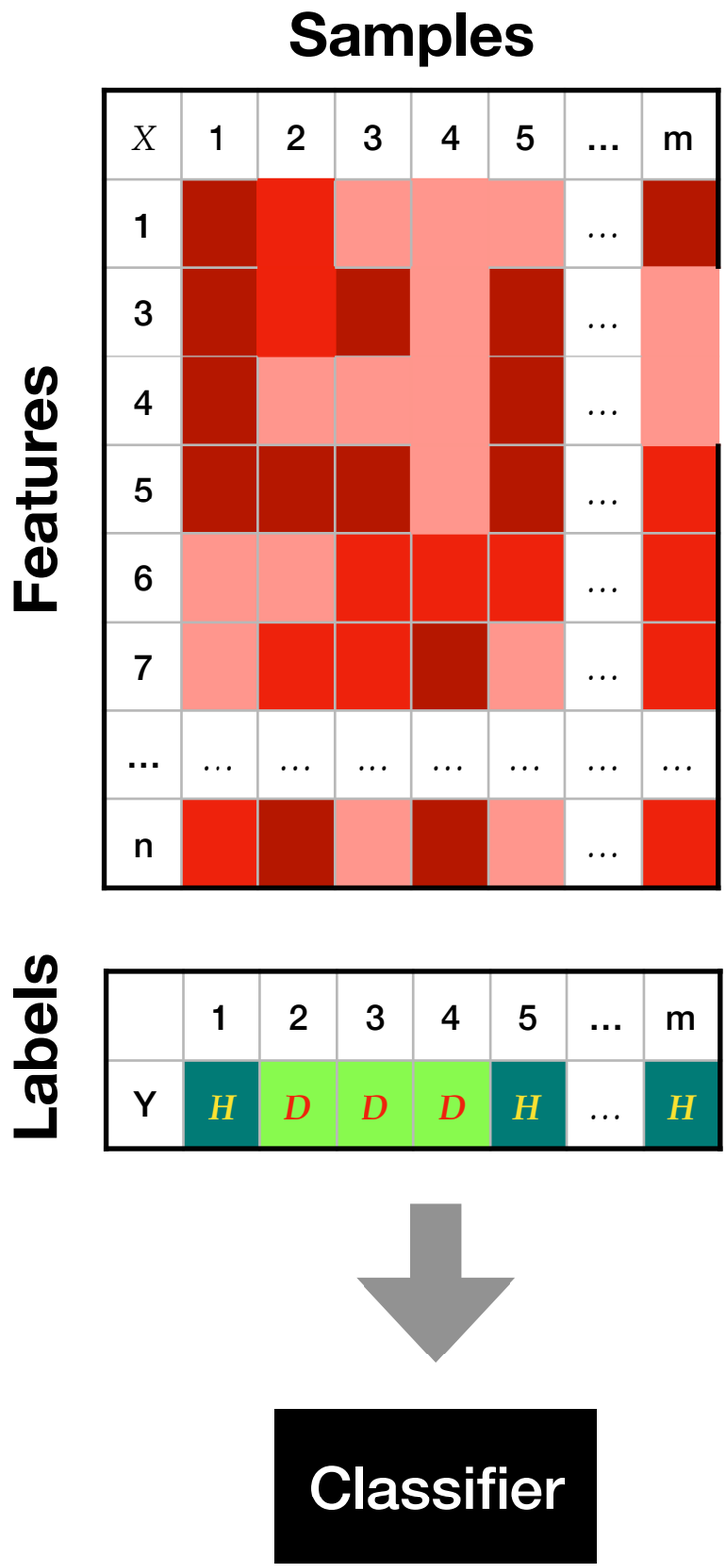
- **Goal**: train a classifier to predict the labels (**phenotype**) **from** the feature matrix (**genotype**)



Supervised learning from microbiome data

- **Goal**: train a classifier to predict the labels (**phenotype**) **from** the feature matrix (**genotype**)

- Random Forest has outperformed other methods, including Neural Networks
  [Statnikov et al., Microbiome, 2013]



Overfitting seems prevalent

Sze and Schloss, Looking for a Signal in the Noise: Revisiting Obesity and the Microbiome, mBio, 2016

Dave et. al., The human gut microbiome: current Knowledge, challenges, and future directions, Translational Research, 2012

- Large number of features, but small number of samples
- High (biological) variation (confounders)
- Highly noisy and incomplete data

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- Large number of features, but small number of samples
- High (biological) variation (confounders)
- Highly noisy and incomplete data
- **Unbalanced and biased representation** of labels
  — Example: control group often is underrepresented

Benchmarking dataset

- Inflammatory Bowel Disease (IBD) study [Gevers *et al., 2014*]
- 1,359 samples, reduced after filtering to
  - 647 diseased and 243 healthy samples
- Around 10,000 features
  - Roughly: denoised unique sequences (defined using deblur)

[Amir et al., 2017]

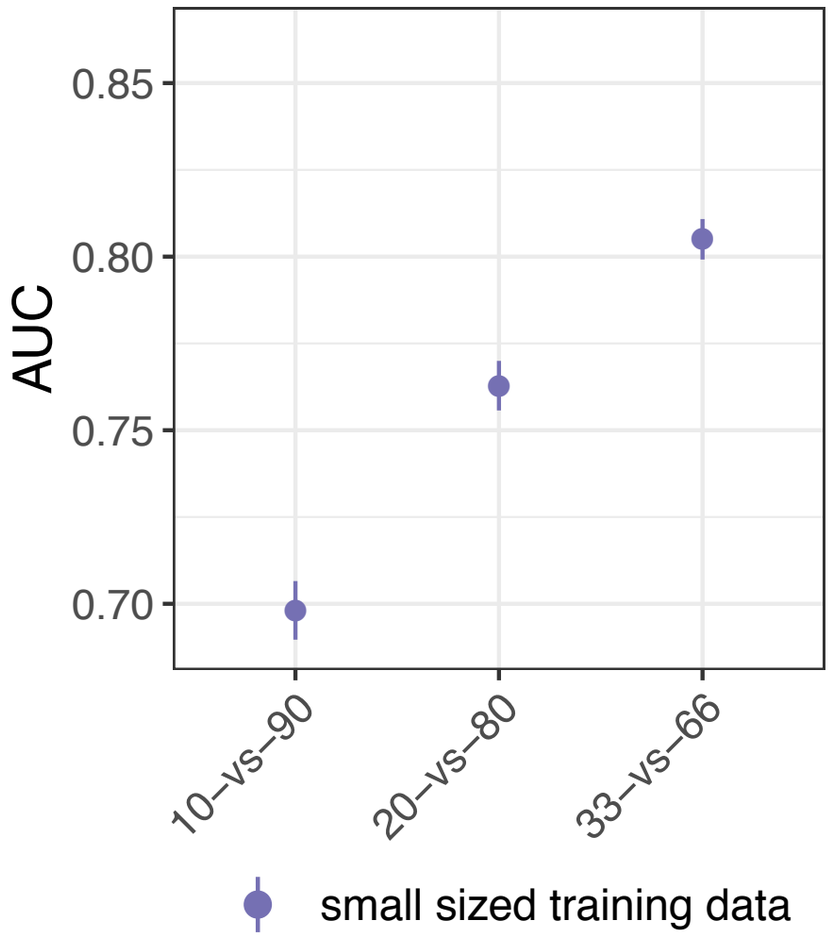
Cell Host & Microbe
Resource

The Treatment-Naive Microbiome in New-Onset Crohn's Disease

Dirk Gevers,[1] Subra Kugathasan,[4,24] Lee A. Denson,[5,24] Yoshiki Vázquez-Baeza,[6] Will Van Treuren,[7] Boyu Ren,[8] Emma Schwager,[8] Dan Knights,[9,10] Se Jin Song,[7] Moran Yassour,[1] Xochitl C. Morgan,[8] Aleksandar D. Kostic,[1] Chengwei Luo,[1] Antonio González,[7] Daniel McDonald,[7] Yael Haberman,[5] Thomas Walters,[11] Susan Baker,[12] Joel Rosh,[13] Michael Stephens,[14] Melvin Heyman,[15] James Markowitz,[16] Robert Baldassano,[17] Anne Griffiths,[18] Francisco Sylvester,[19] David Mack,[20] Sandra Kim,[21] Wallace Crandall,[21] Jeffrey Hyams,[19] Curtis Huttenhower,[1,8] Rob Knight,[7,22,23] and Ramnik J. Xavier[1,2,3,*]

Are **unbalanced classes** problematic?

- Fixed the size of the training dataset (243 samples in total)
- Control the proportion of healthy-vs-diseased



Data augmentation

- Downsample the overrepresented class

Data augmentation

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- Upsample: generate synthetic data to augment the underrepresented class until classes have similar sizes
  - Synthetic data should resemble real data (could have been but are not seen)
  - SMOTE [Chawla *et al*., 2002]
    ADASYN [He *et al*.,2008]
    (kNN+linear combination)

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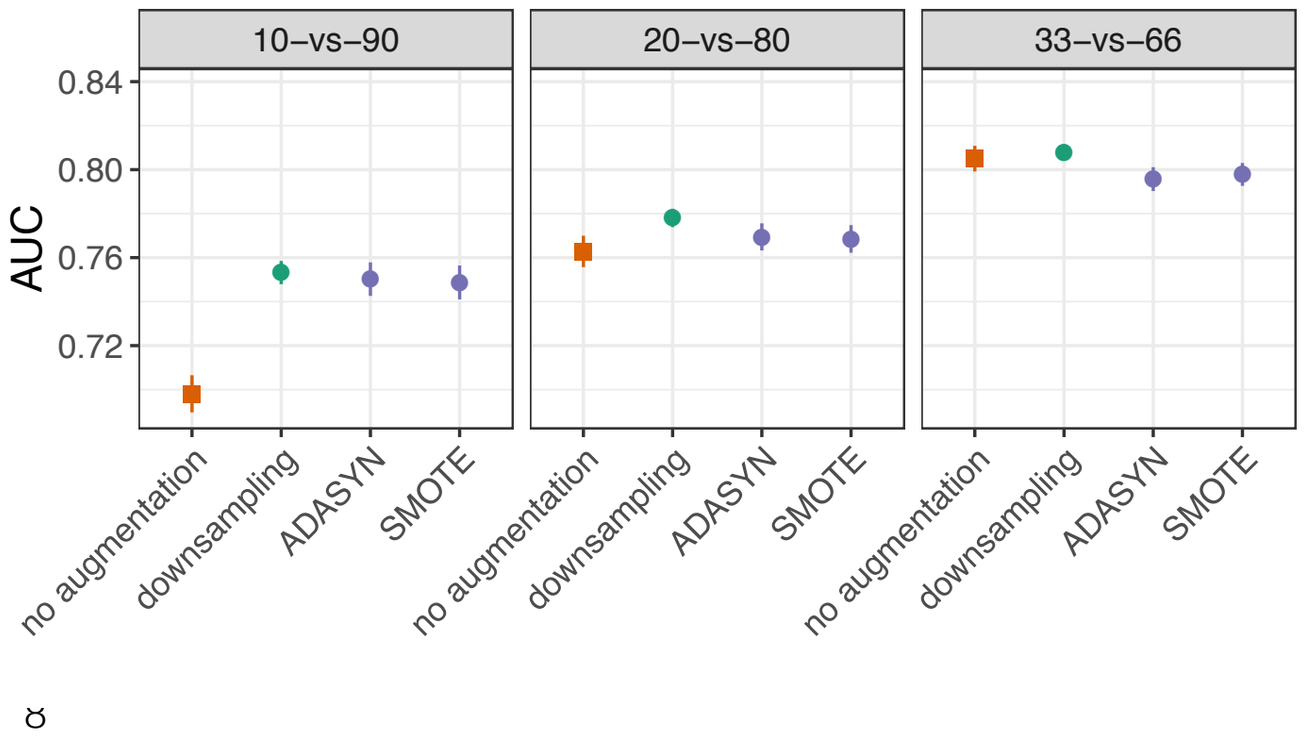

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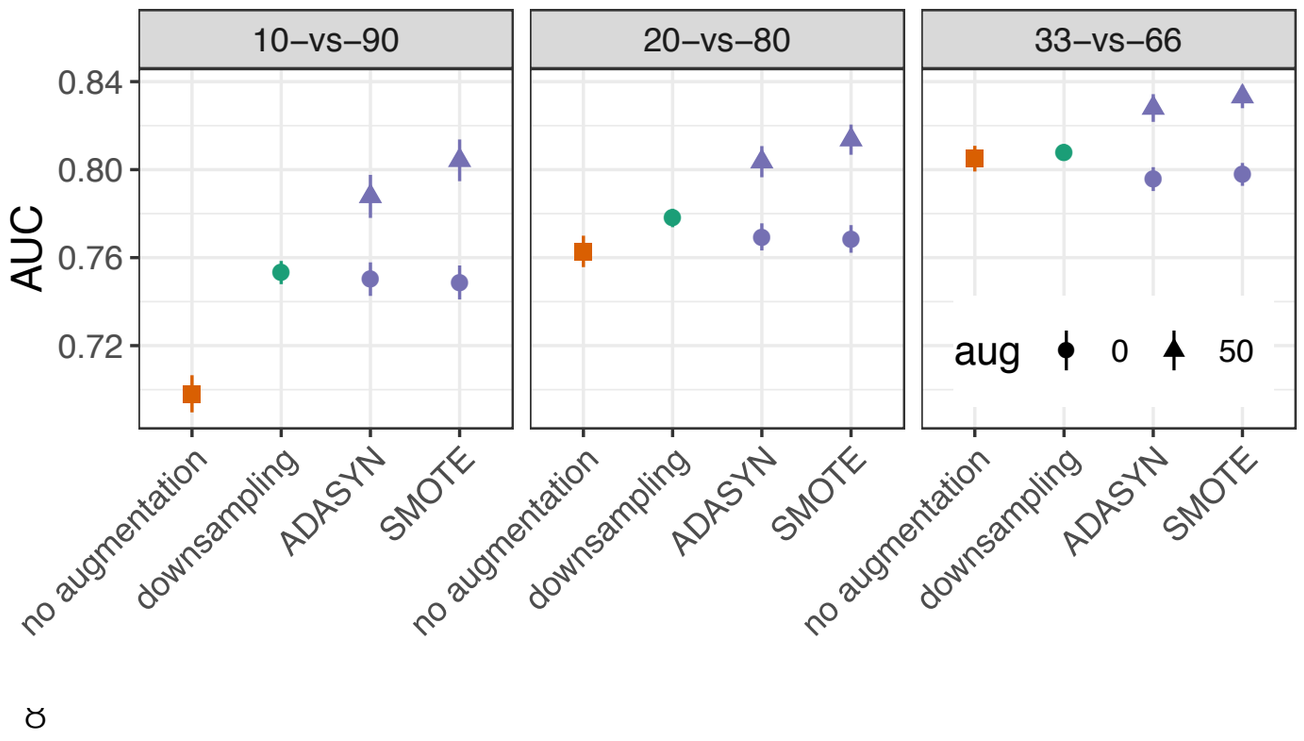
    (kNN+linear combination)
- Keep augmenting (50X) *after* balancing labels (▲)



Combining domain knowledge with data augmentation

- Microbes are related through a phylogeny

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- **Hypothesis:** Variations in the feature vectors across samples are governed by the phylogeny

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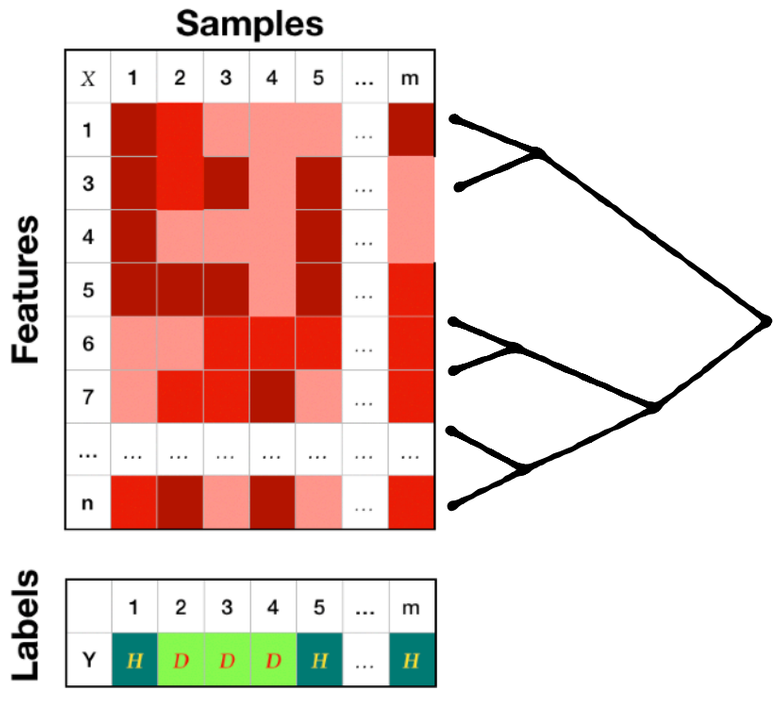
- Microbes are related through a phylogeny

- **Hypothesis:** Variations in the feature vectors across samples are governed by the phylogeny

- Thus, data augmentation should take the phylogeny into account



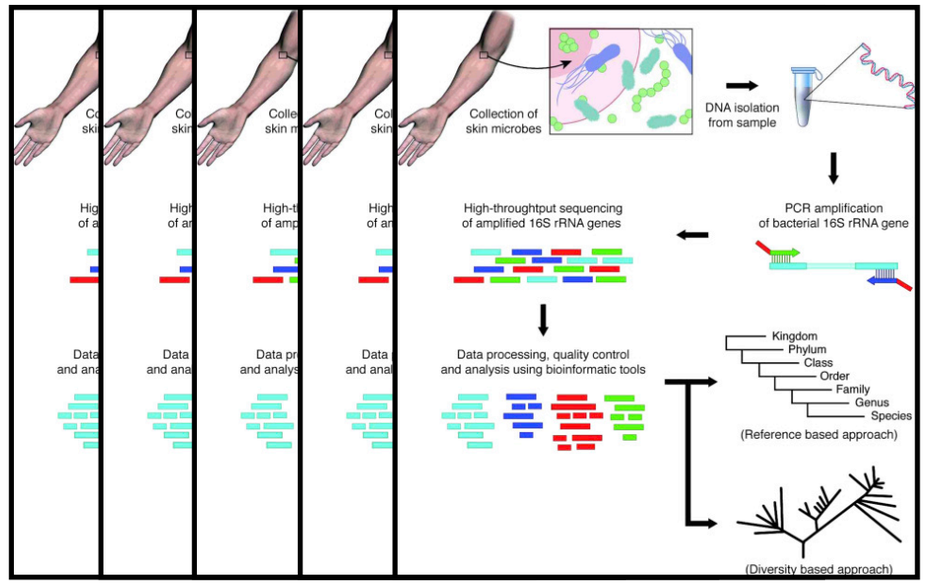
TADA: Tree-based Associative Data Augmentation

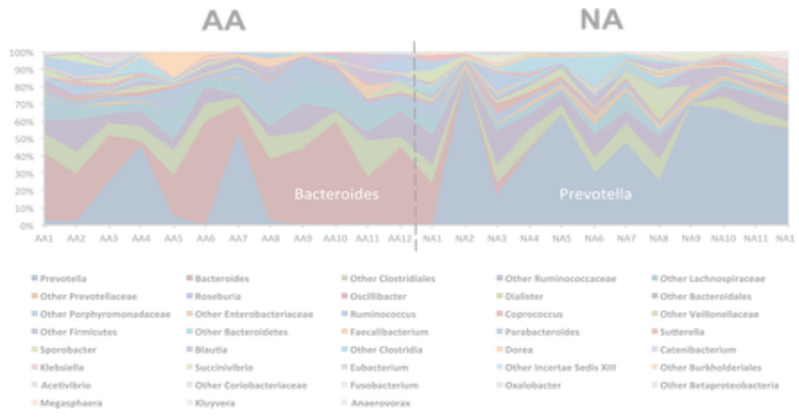
- Design a phylogeny-aware generative model with parameters that can be learnt from (subsets of) training data

- Use the generative model to generate synthetic samples and add them to the training data

Sources of variation

- **Sampling (sequencing) variation**
- Biological variation
  - Confounding factors: ethnicity, age, gender, diet, lifestyle
- Temporal variations
- Not fully understood

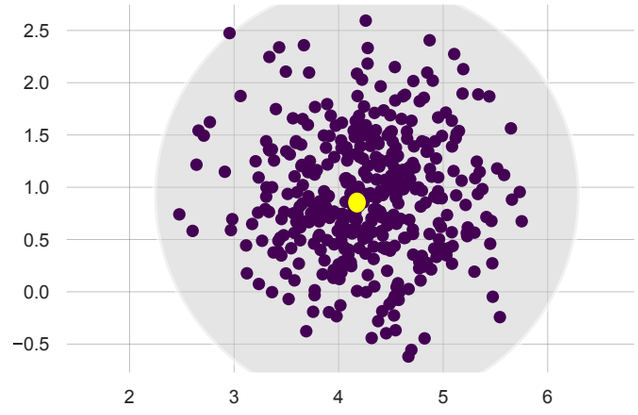




Ou et al, Am J Clin Nutr 98:111, 2013

Sampling variation (SV)

- If another round of sequencing is performed, what could we observe?



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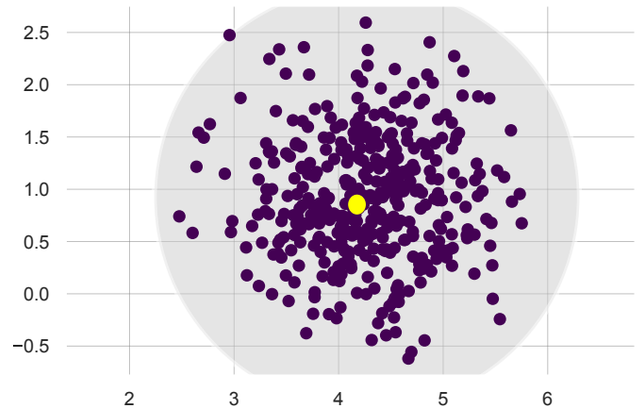
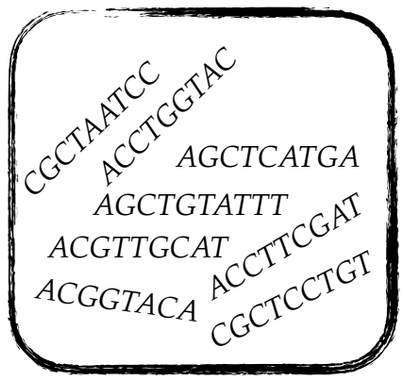
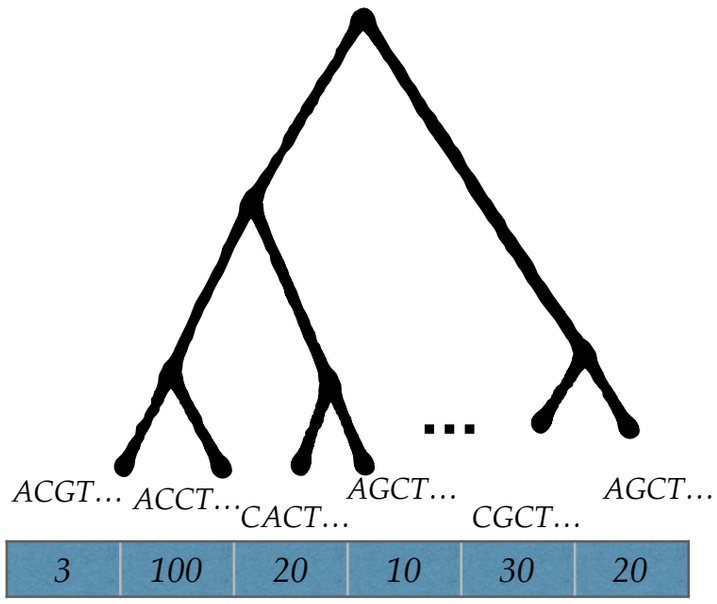




ACGT…	3
ACCT…	100
CACT…	20
AGCT…	10
…	…
CGCT…	30
AGCT…	20



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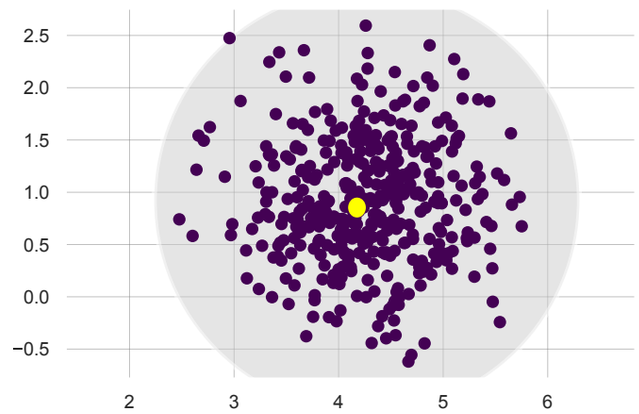
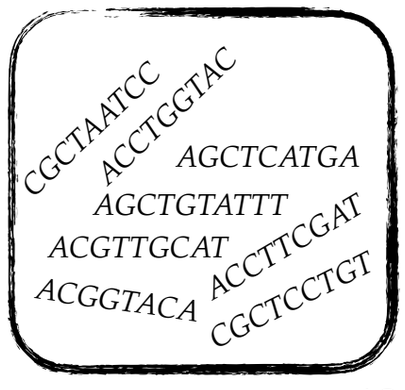


- Map normalized abundances to nodes



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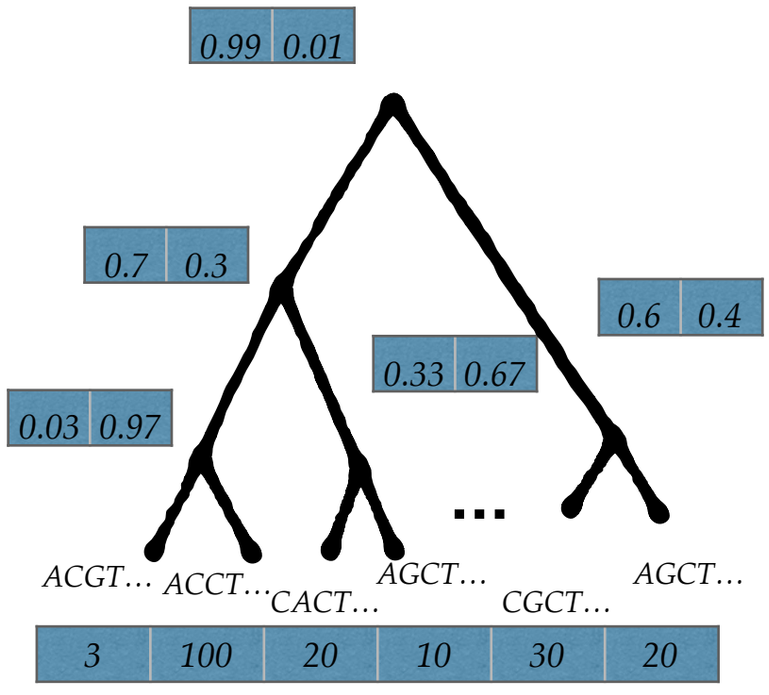

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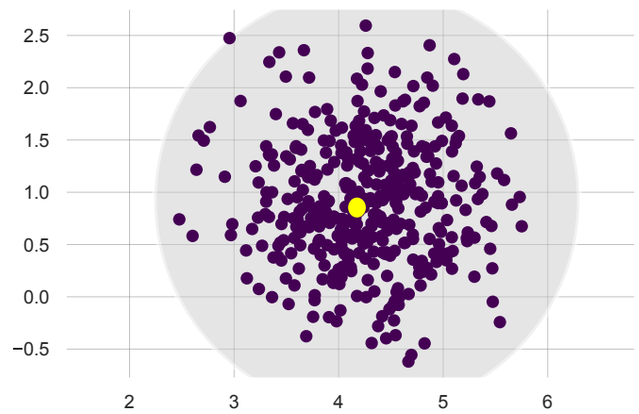
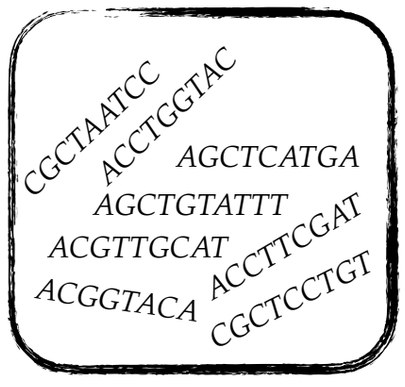


- Map normalized abundances to nodes

- Gives a hierarchy of binomial distributions



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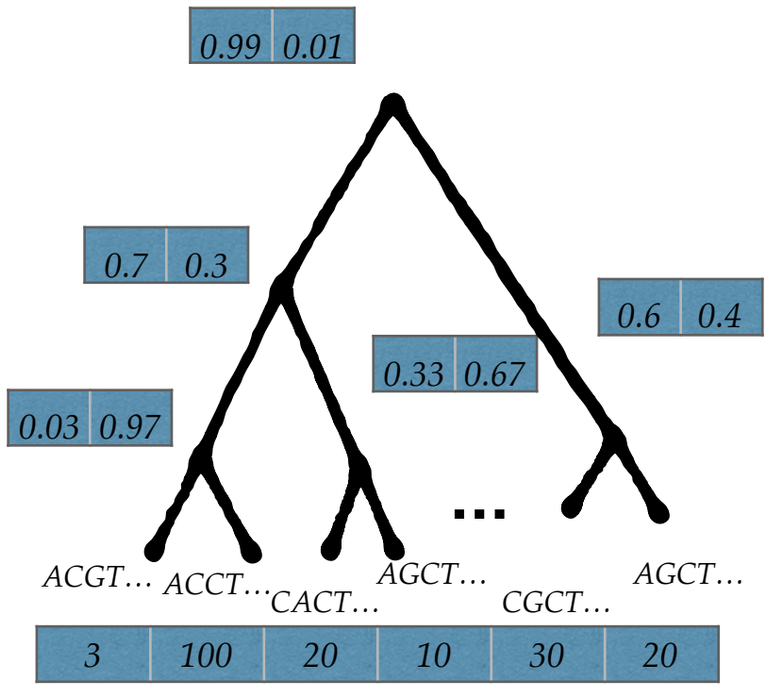
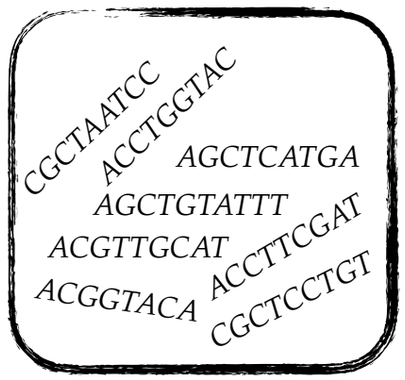


TADA-SV

Repeat:



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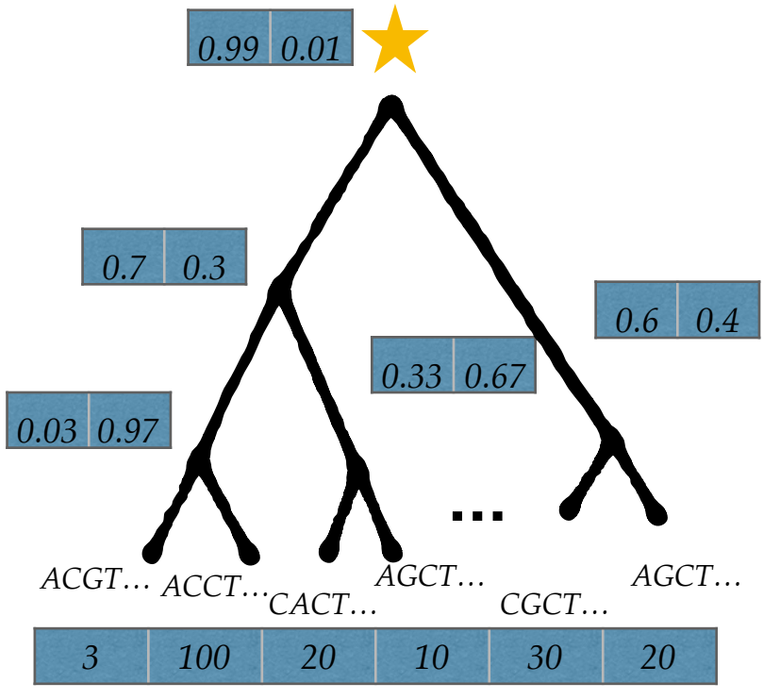
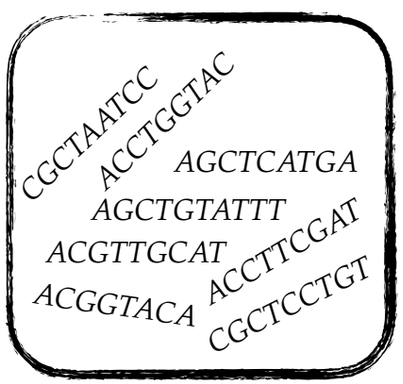


TADA-SV

Repeat:

- Start from the root
    - Assume some total counts over the root



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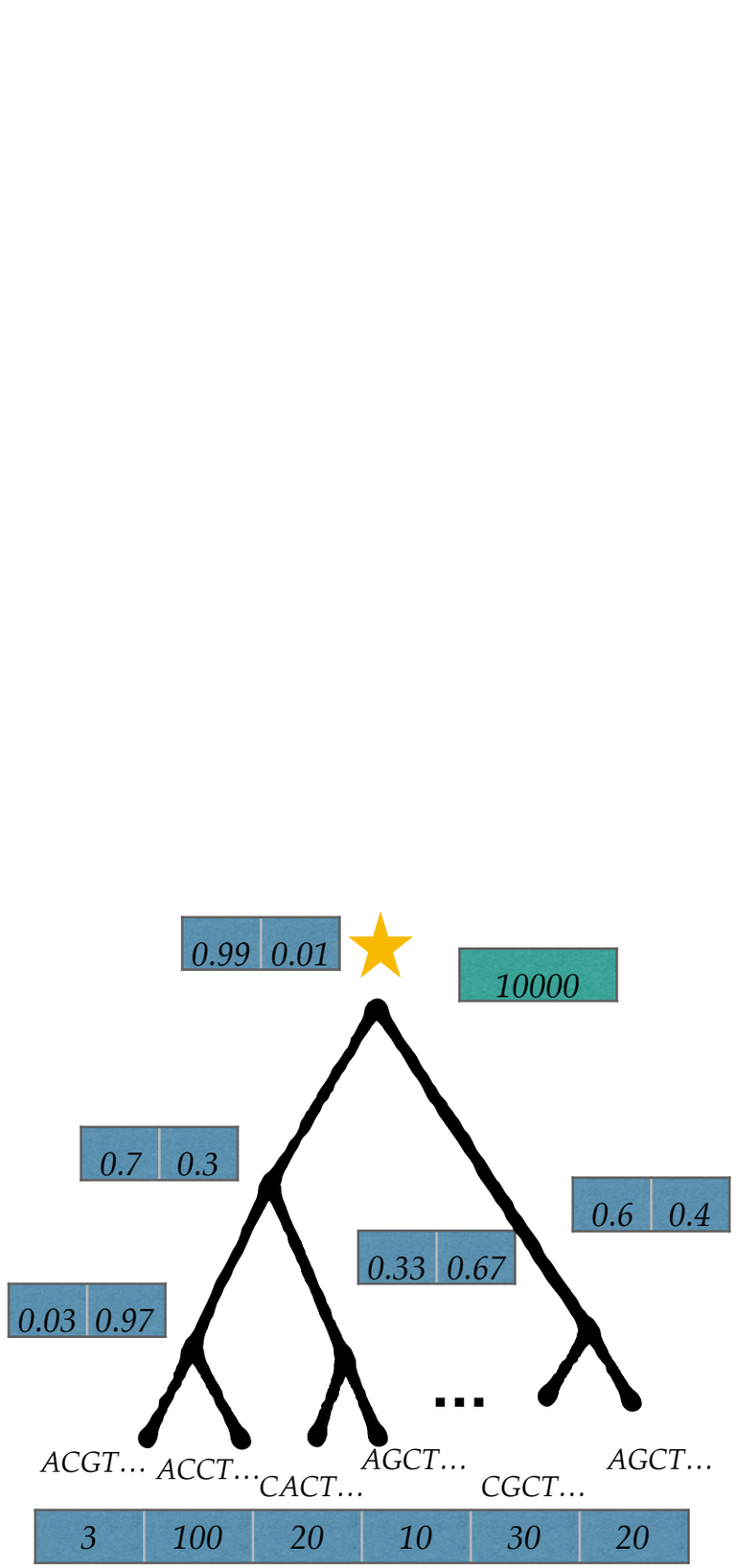
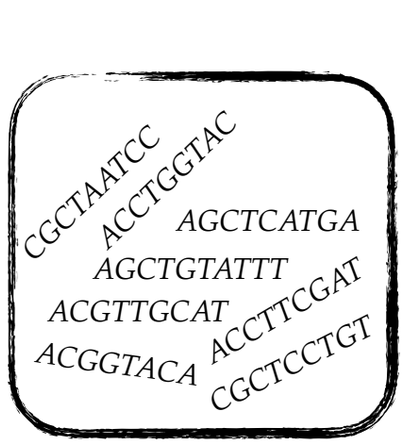


TADA-SV

Repeat:

- Start from the root
    - Assume some total counts over the root
- Draw a number from Binomial distribution



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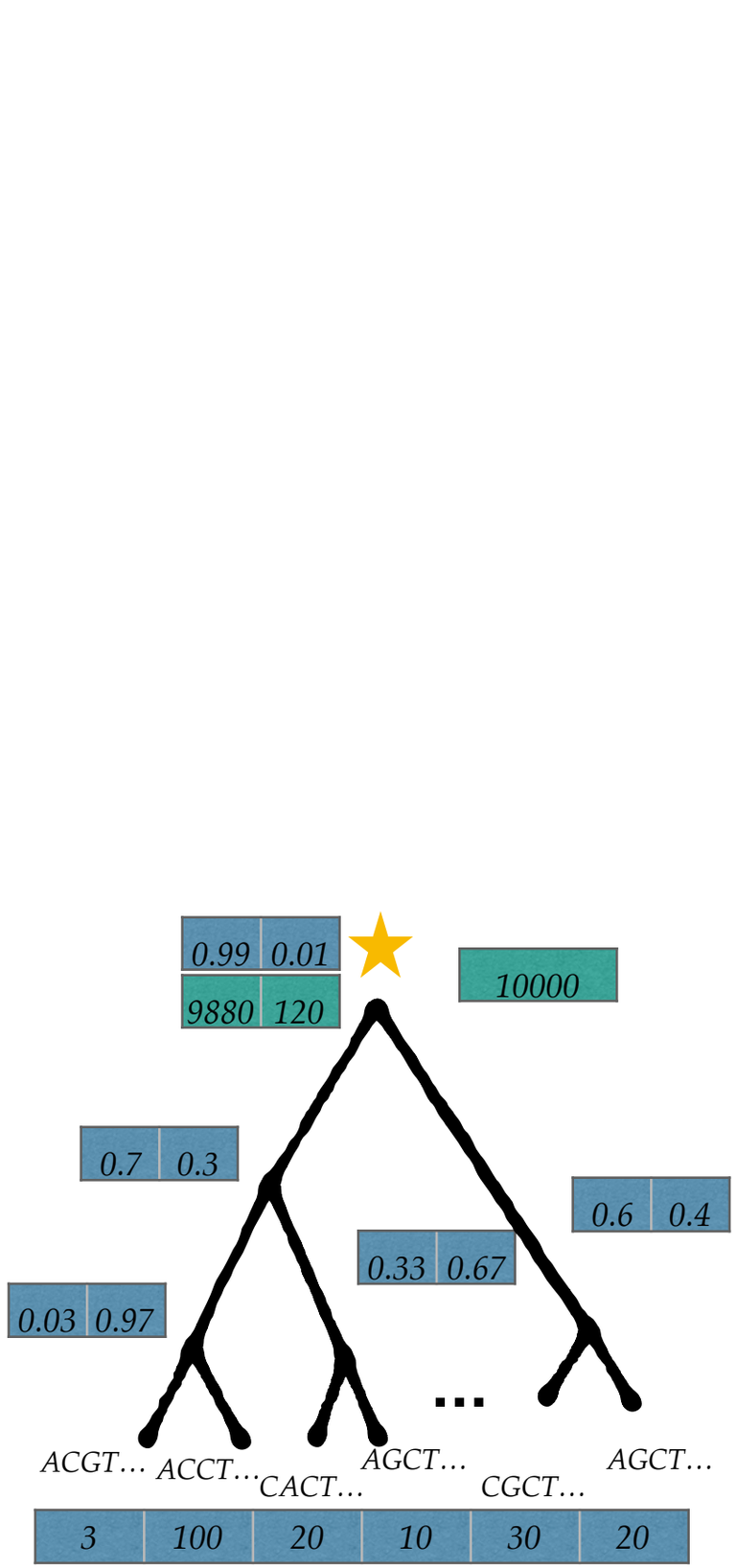

TADA-SV

Repeat:

- Start from the root
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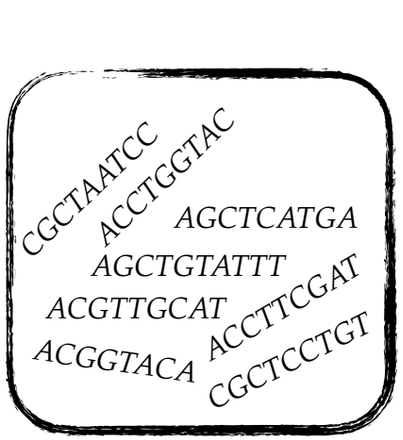
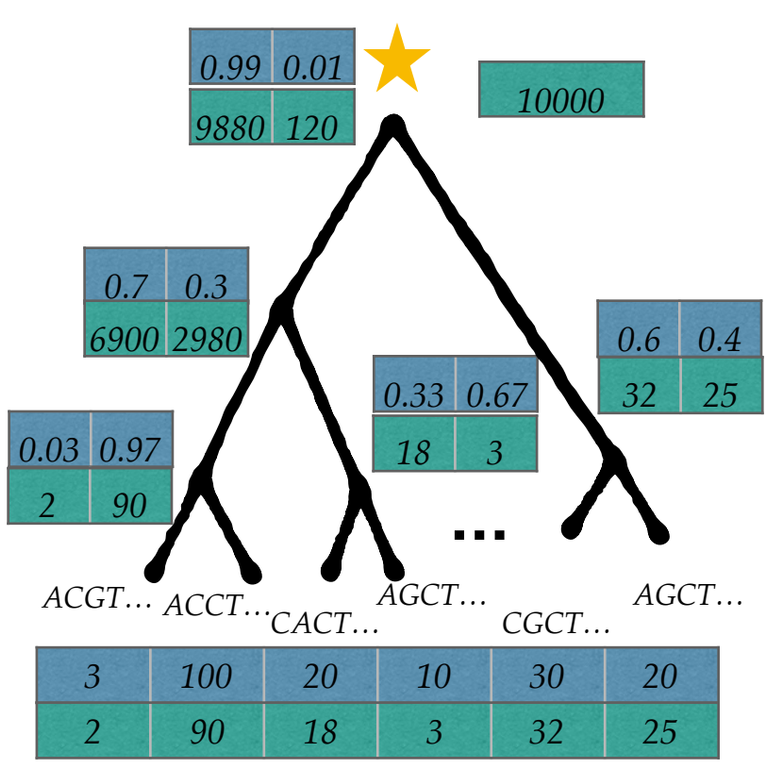
- Draw a number from Binomial distribution
- Recurse on children



ACGT…	3	2
ACCT…	100	90
CACT…	20	18
AGCT…	10	3
…	…	…
CGCT…	30	32
AGCT…	20	25



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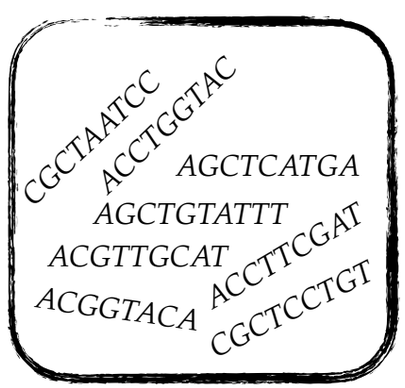
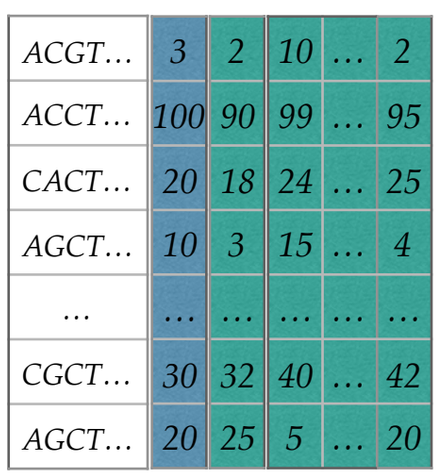
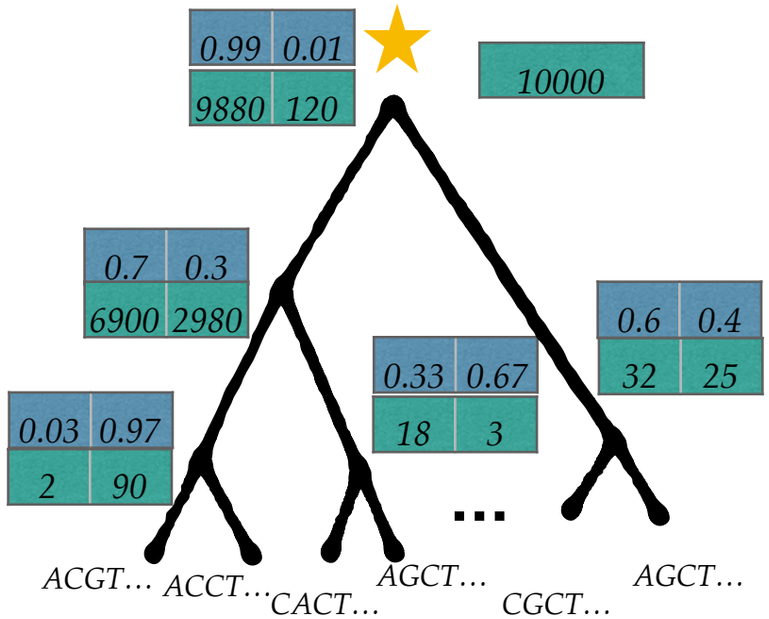




ACGT…	3	2	10	…	2
ACCT…	100	90	99	…	95
CACT…	20	18	24	…	25
AGCT…	10	3	15	…	4
…	…	…	…	…	…
CGCT…	30	32	40	…	42
AGCT…	20	25	5	…	20



3	100	20	10	30	20
2	90	18	3	32	25
10	99	24	15	40	5
…	…	…	…	…	…
2	95	25	4	42	25

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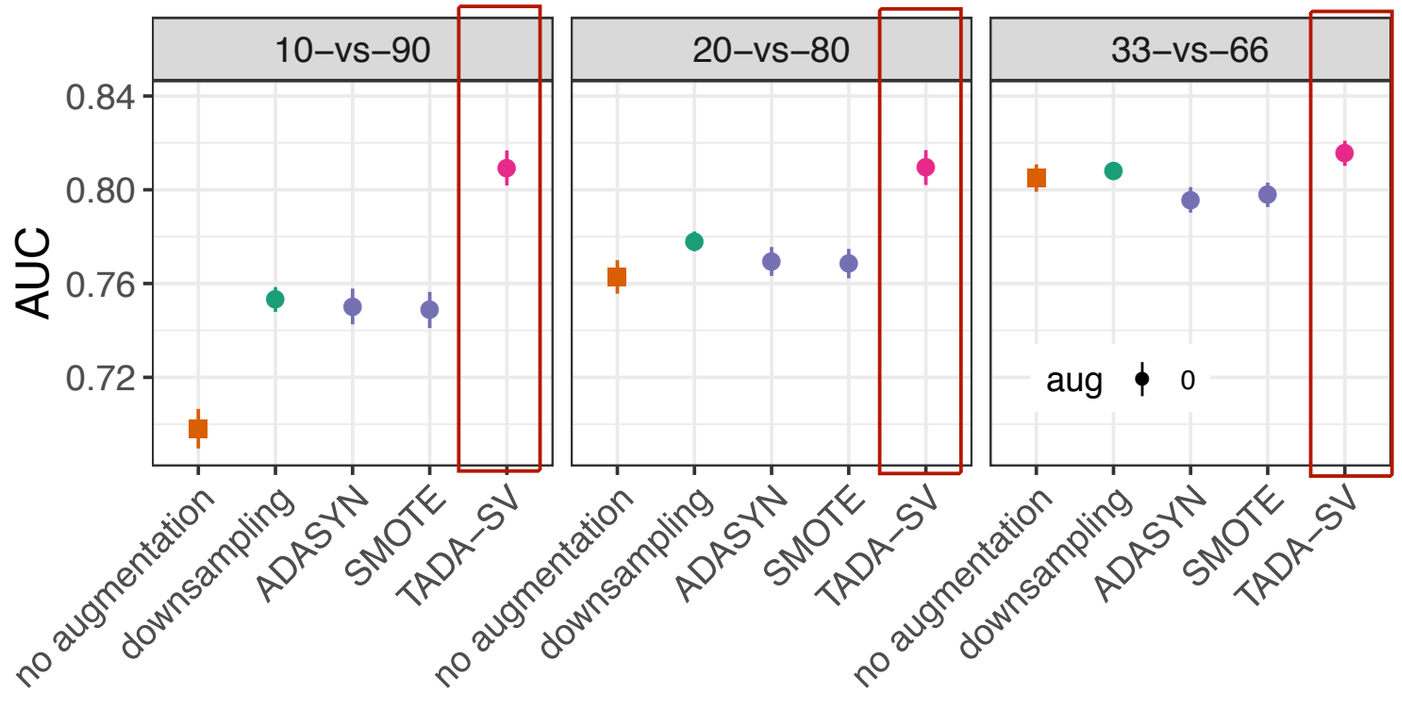
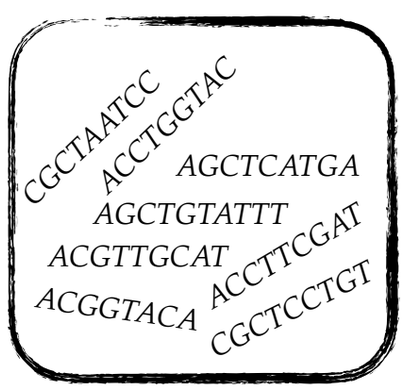
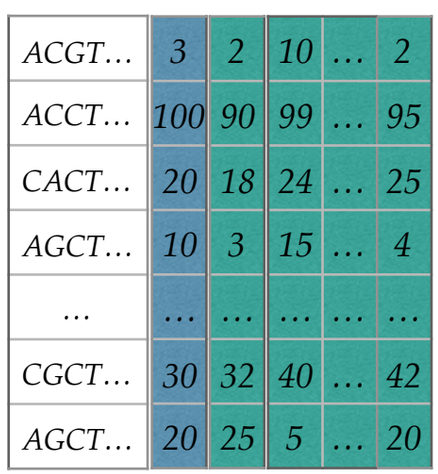
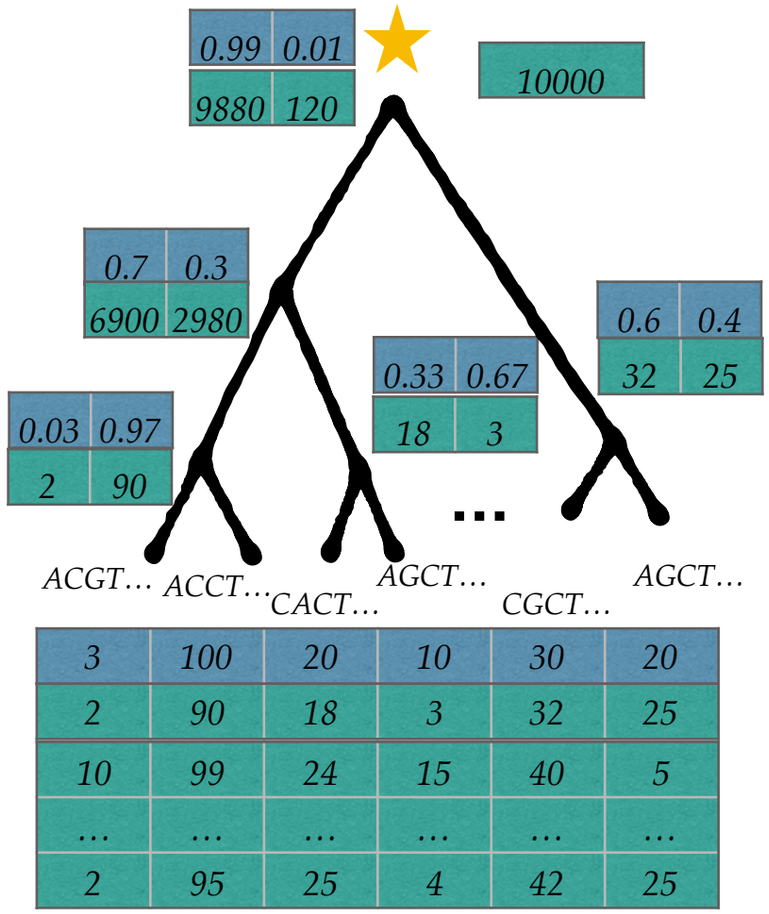






ACGT…	3	2	10	…	2
ACCT…	100	90	99	…	95
CACT…	20	18	24	…	25
AGCT…	10	3	15	…	4
…	…	…	…	…	…
CGCT…	30	32	40	…	42
AGCT…	20	25	5	…	20

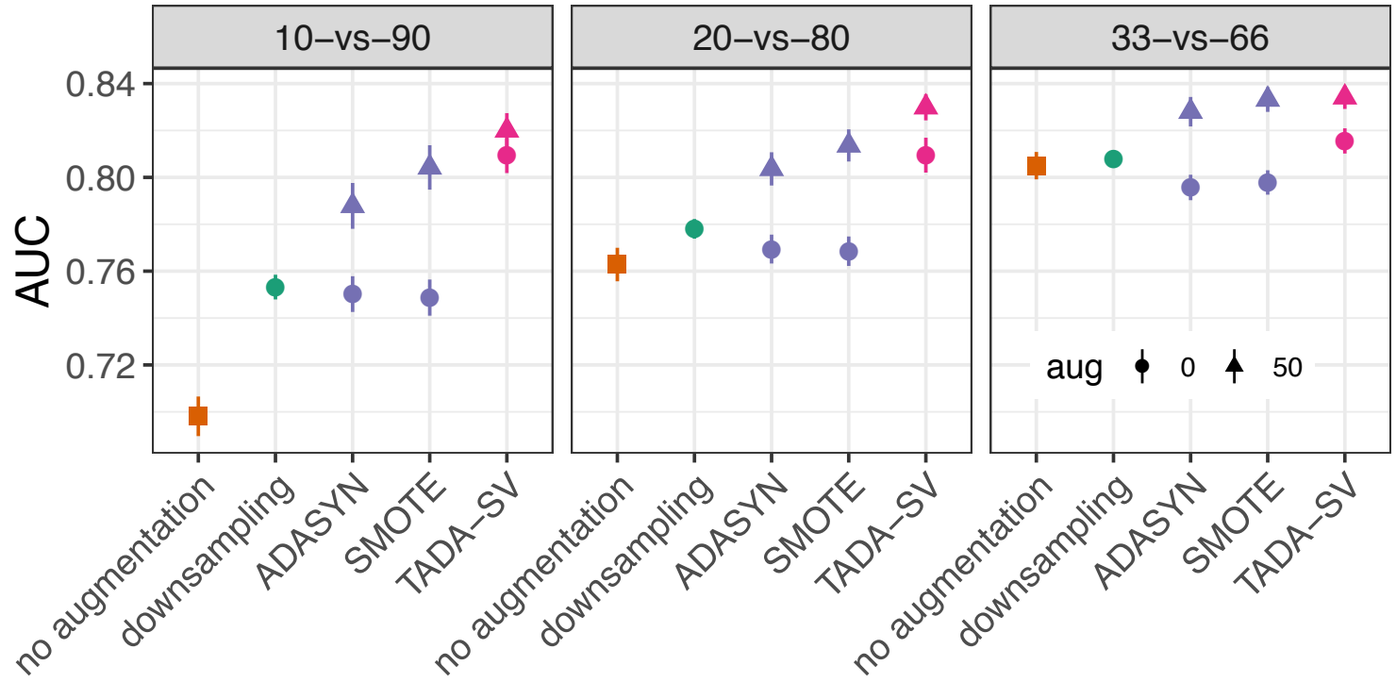


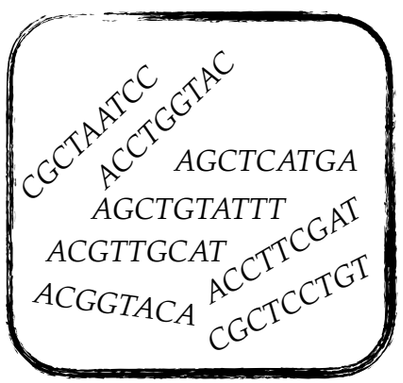
3	100	20	10	30	20
2	90	18	3	32	25
10	99	24	15	40	5
…	…	…	…	…	…
2	95	25	4	42	25

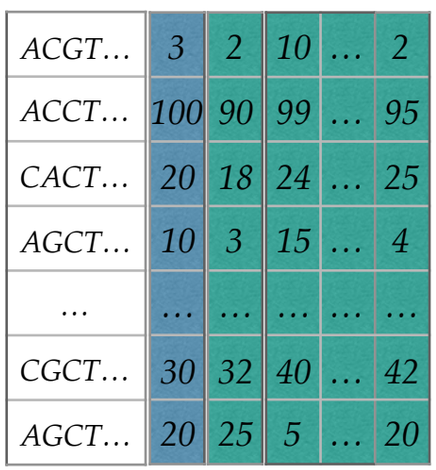
TADA-SV

Repeat:

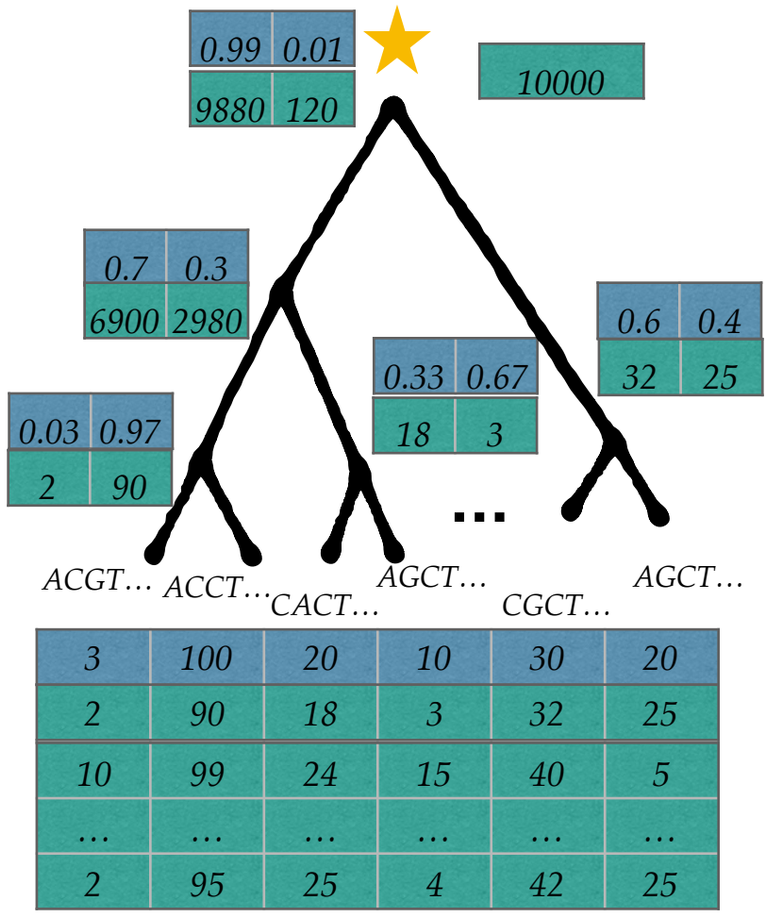
- Start from the root
  - Assume some total counts over the root
- Draw a number from Binomial distribution
- Recurse on children







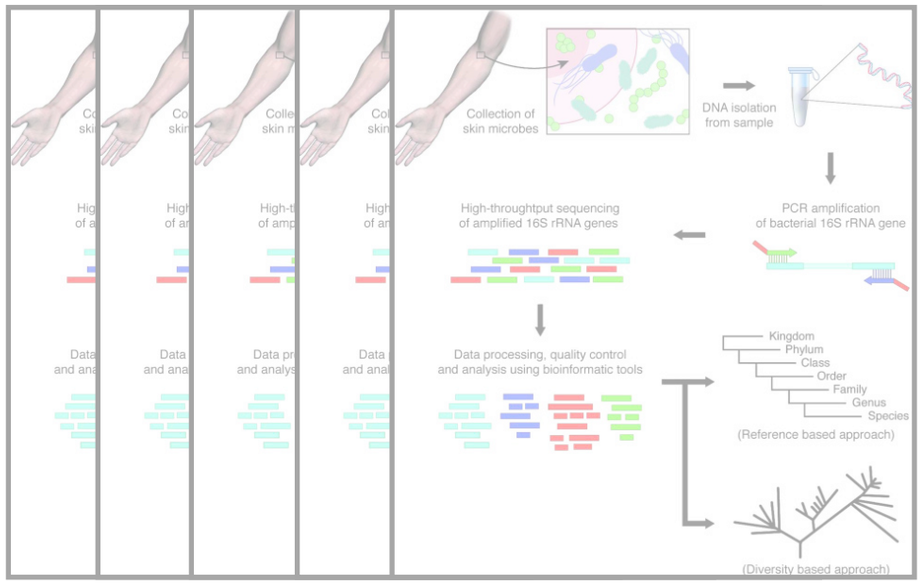
ACGT…	3	2	10	…	2
ACCT…	100	90	99	…	95
CACT…	20	18	24	…	25
AGCT…	10	3	15	…	4
…	…	…	…	…	…
CGCT…	30	32	40	…	42
AGCT…	20	25	5	…	20

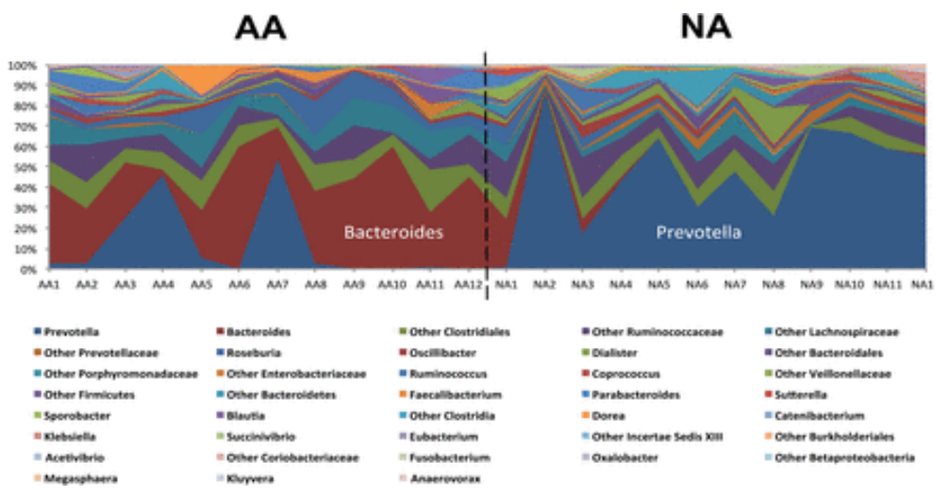


3	100	20	10	30	20
2	90	18	3	32	25
10	99	24	15	40	5
…	…	…	…	…	…
2	95	25	4	42	25

Sources of variation

- Sampling (sequencing) variation
- **Biological variation**
  - Confounding factors: ethnicity, age, gender, diet, lifestyle
  - Temporal variations
  - Not fully understood

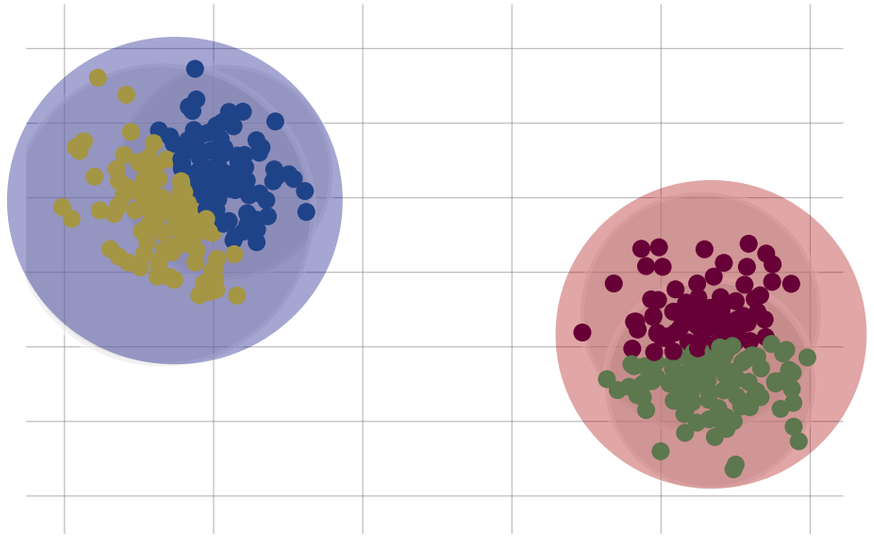


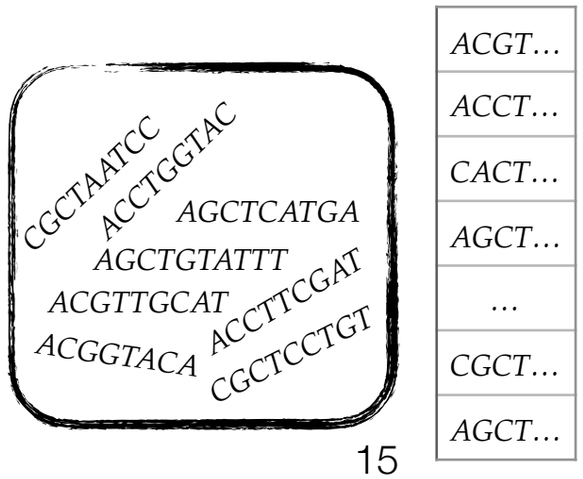


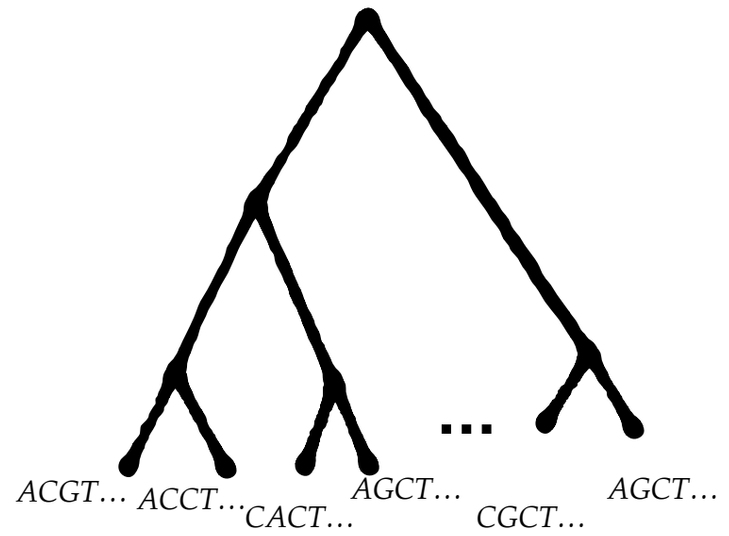
Ou et al, Am J Clin Nutr 98:111, 2013

Biological variation (BV)

- Considering variations between groups of samples







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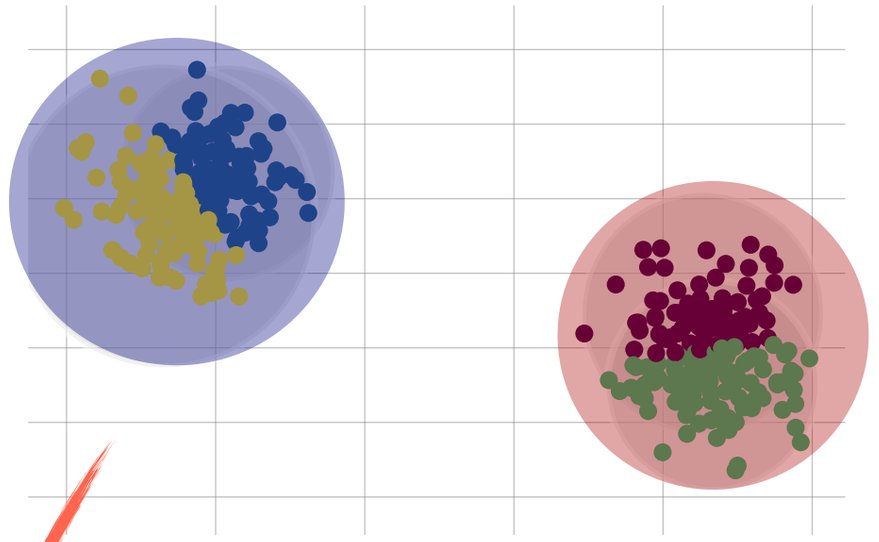
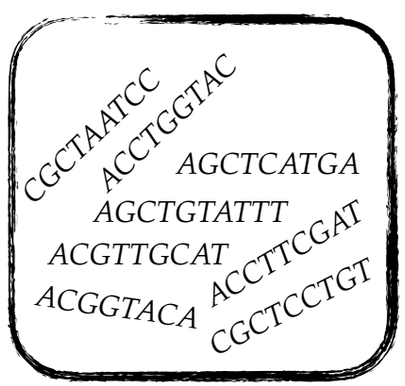
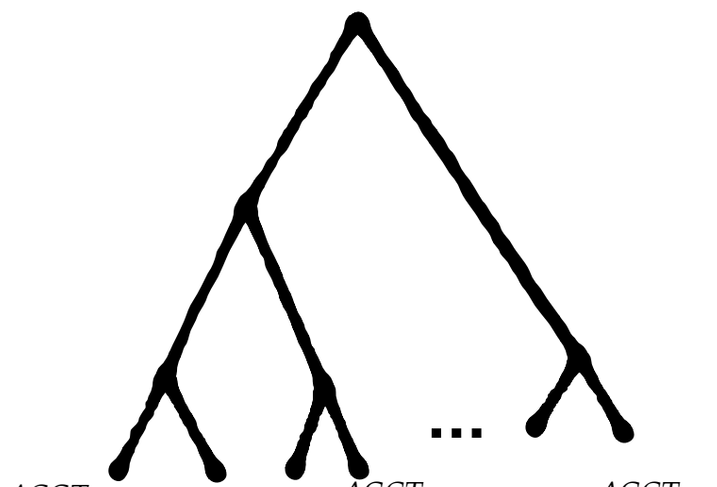




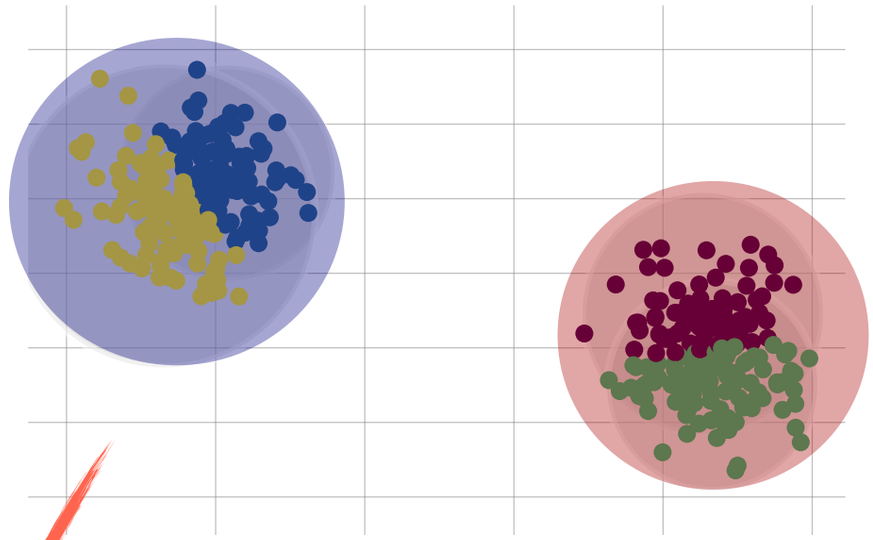
ACGT…	4	3	…	5
ACCT…	98	100	…	100
CACT…	23	20	…	25
AGCT…	23	10	…	1
…		…	…	…
CGCT…	20	30	…	10
AGCT…	19	20	…	20

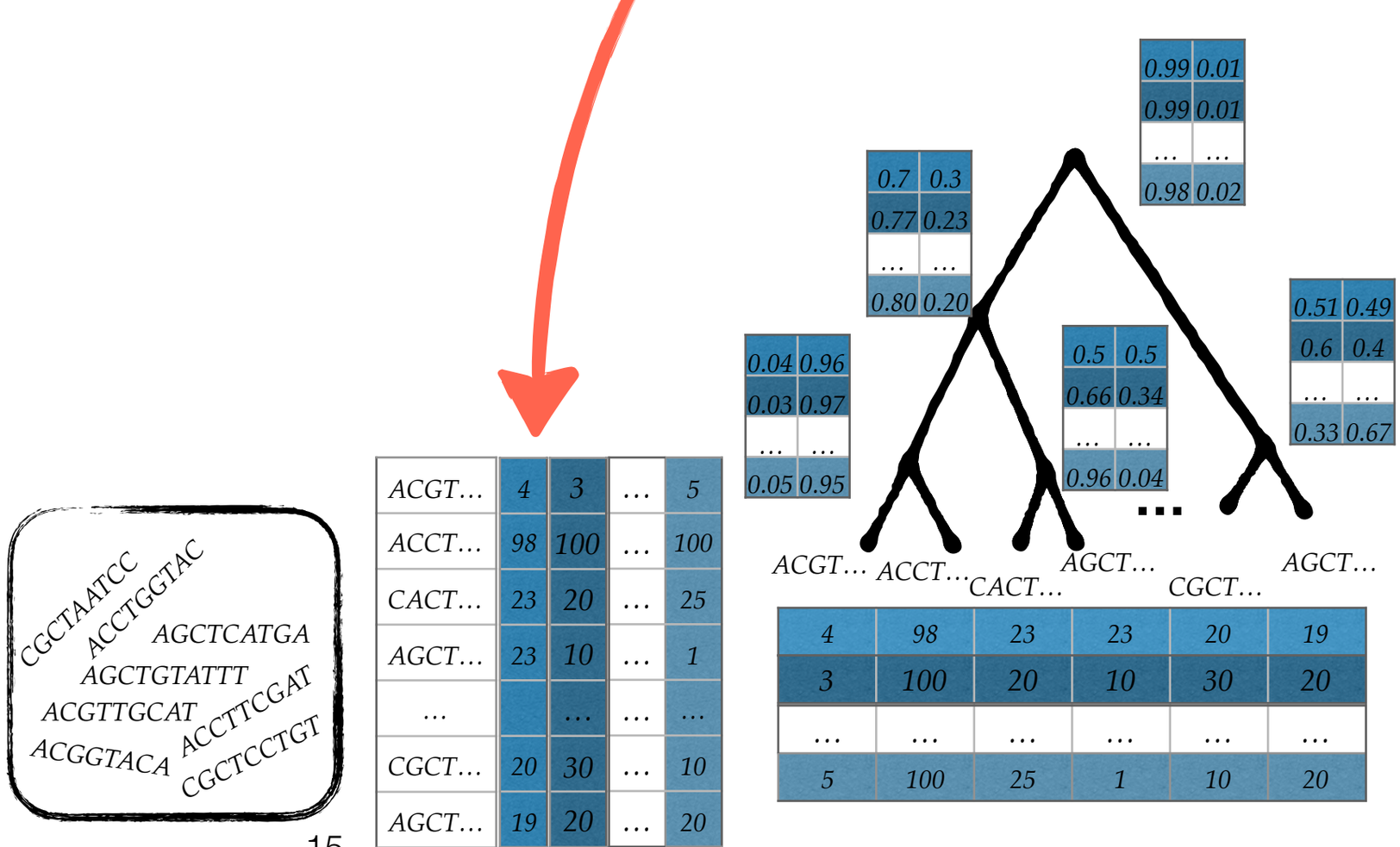


4	98	23	23	20	19
3	100	20	10	30	20
…	…	…	…	…	…
5	100	25	1	10	20

Biological variation (BV)

- Considering variations between groups of samples

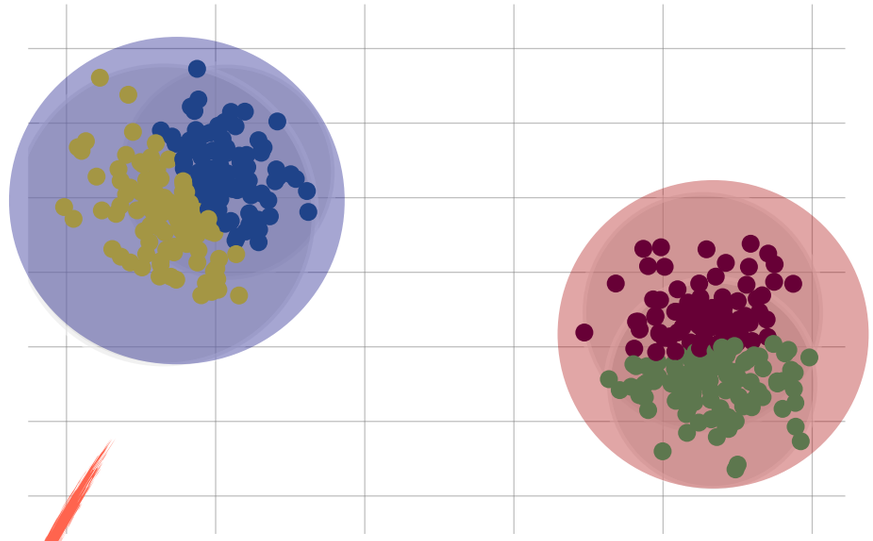


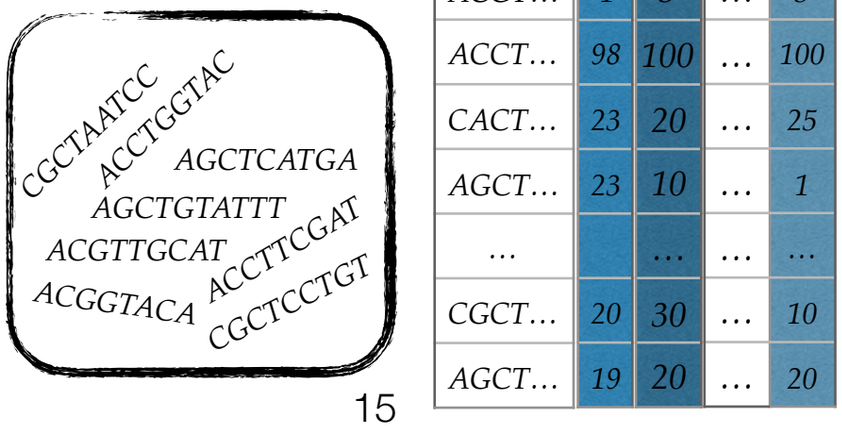


Biological variation (BV)

- Considering variations between groups of samples

- BV: Beta distributions



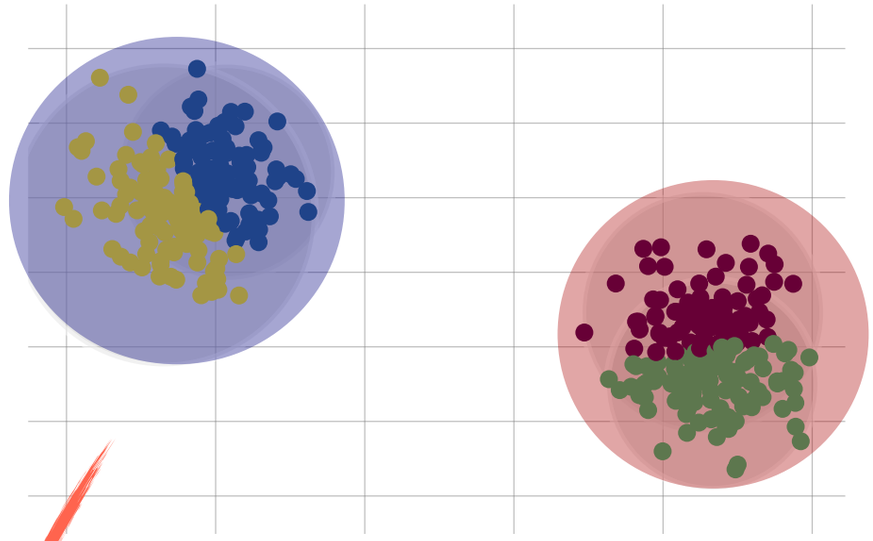


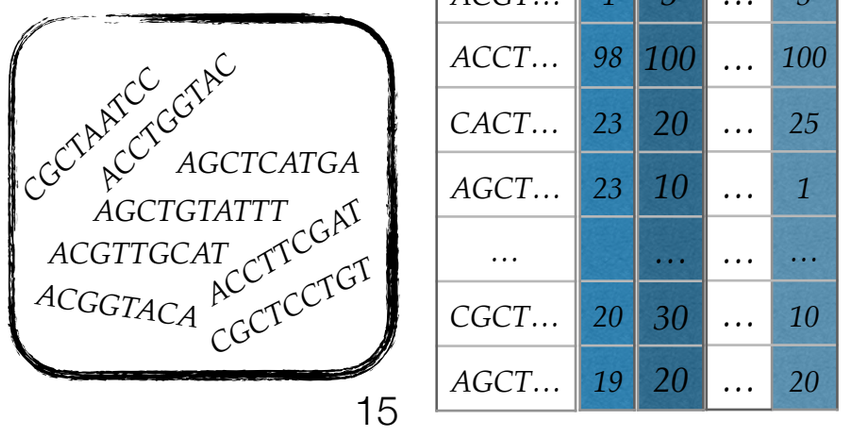
Biological variation (BV)

- Considering variations between groups of samples

- BV: Beta distributions

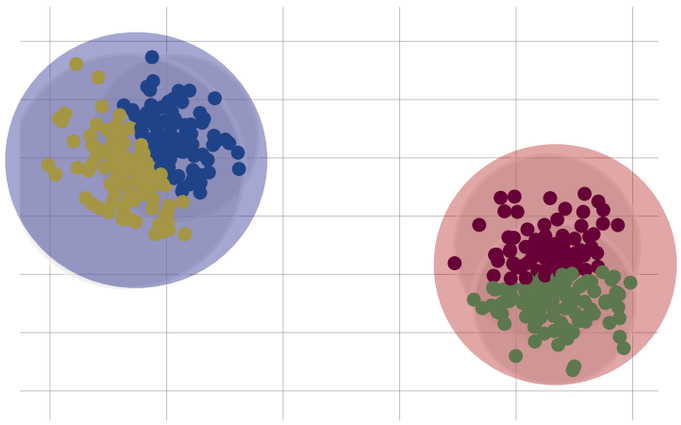
- SV: Binomial distributions

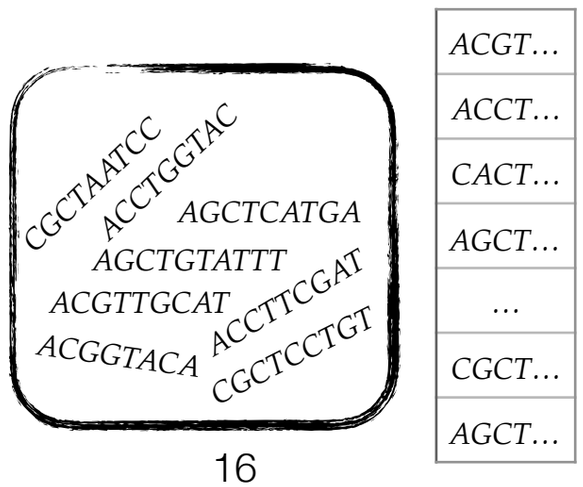


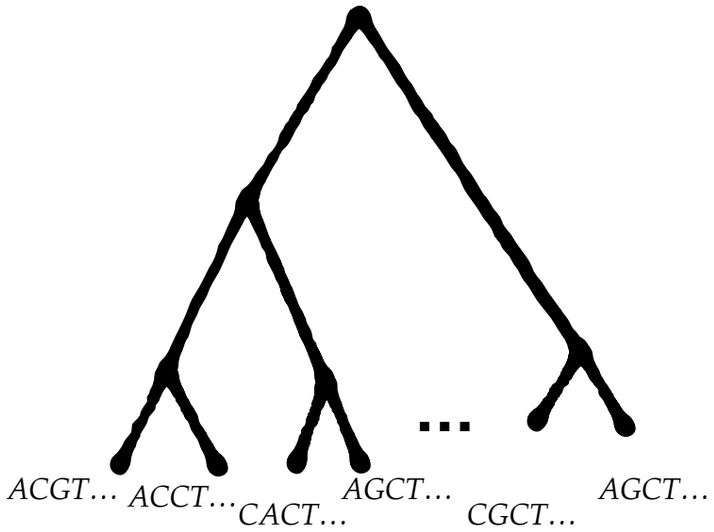


TADA-BV+SV

1. Group samples — Use class labels
   — Further cluster samples in each class







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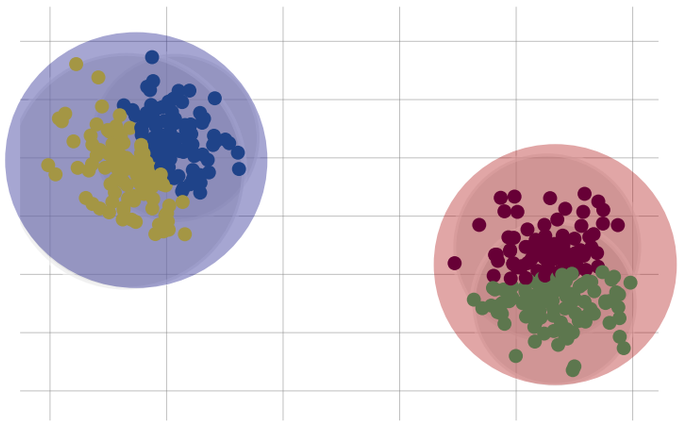

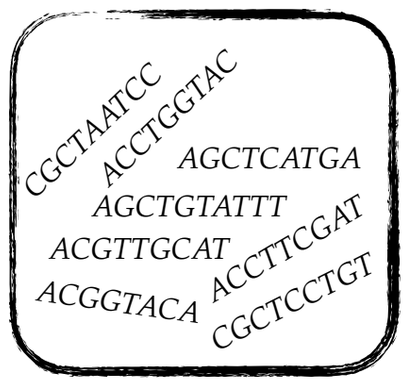

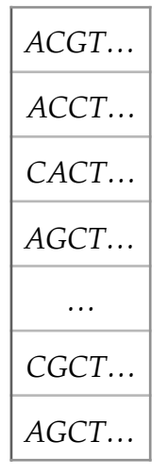

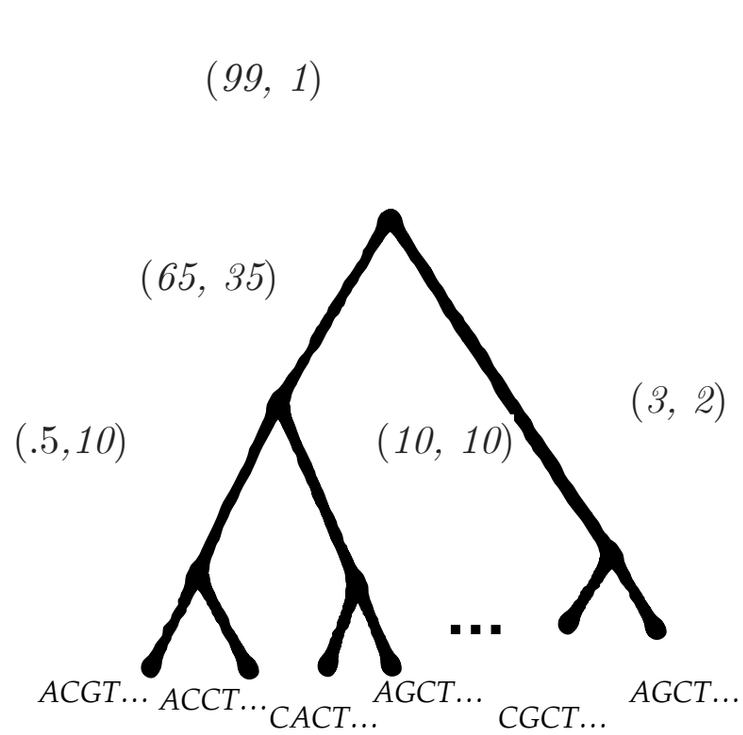

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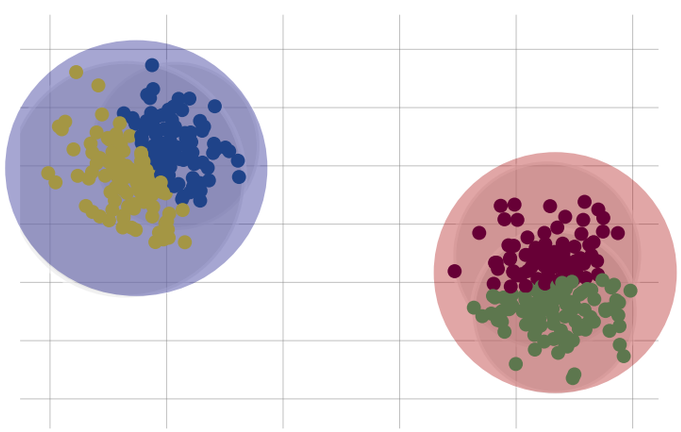

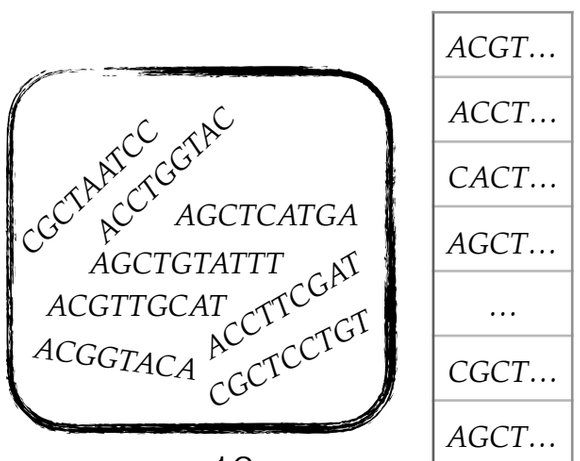

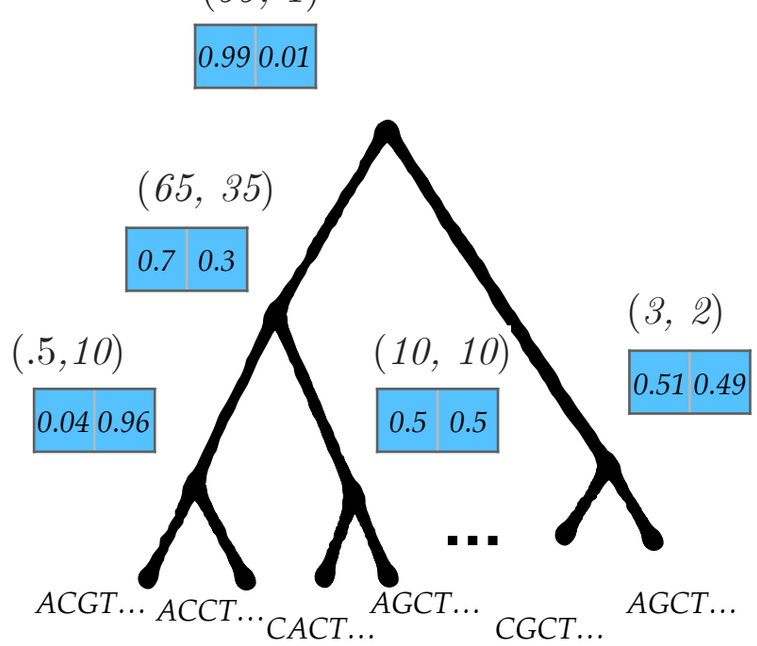

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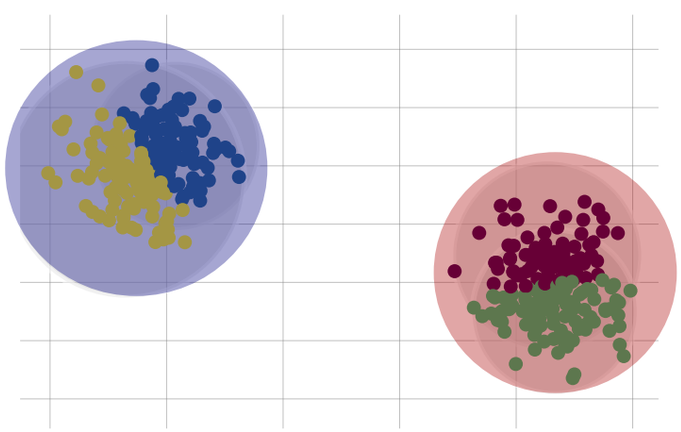

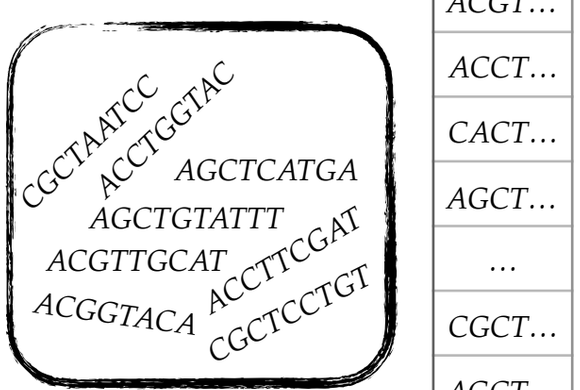

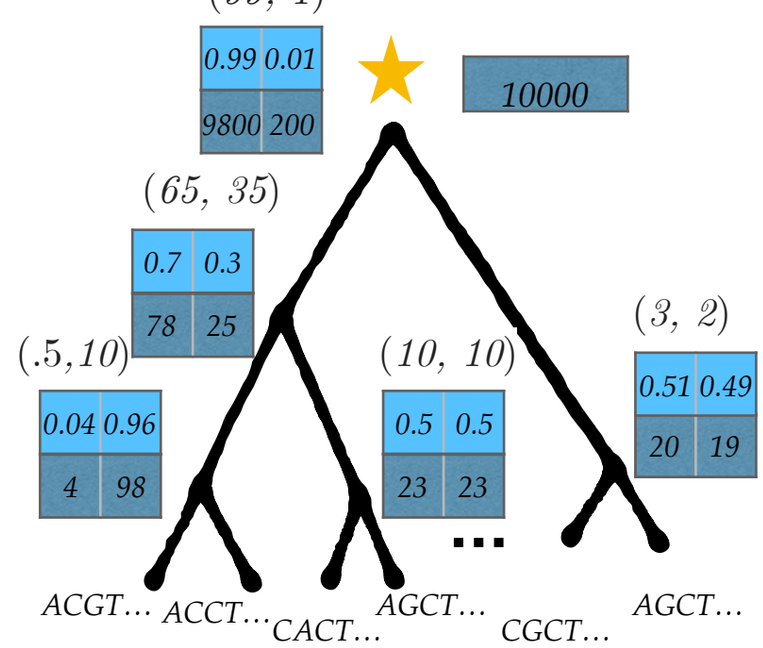

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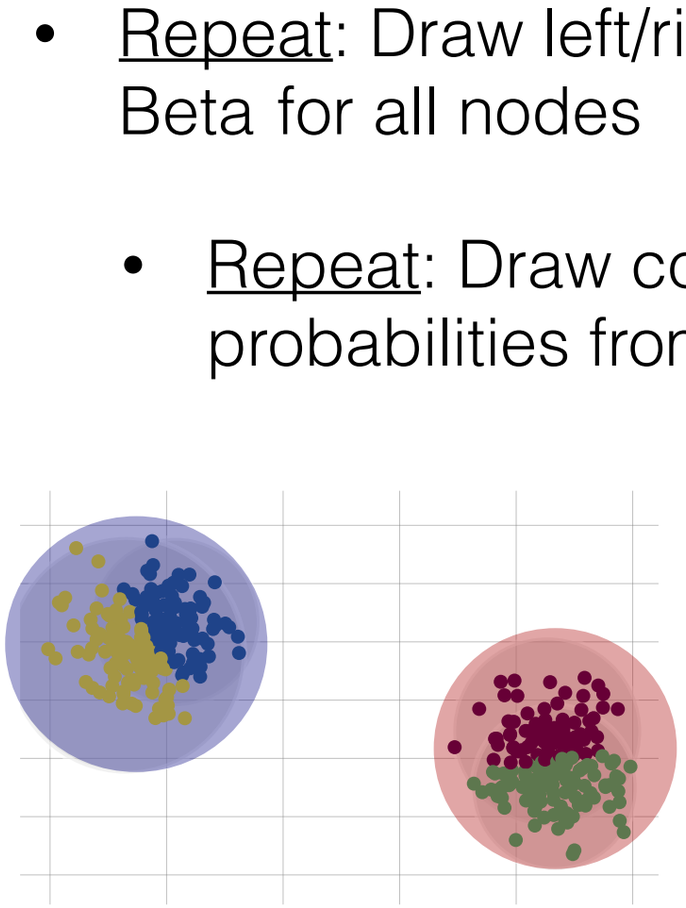

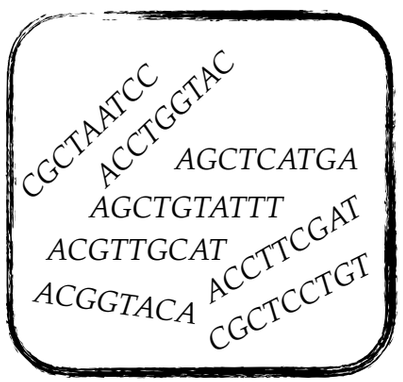

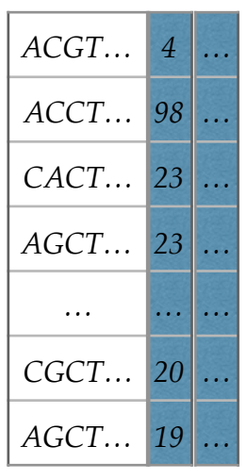

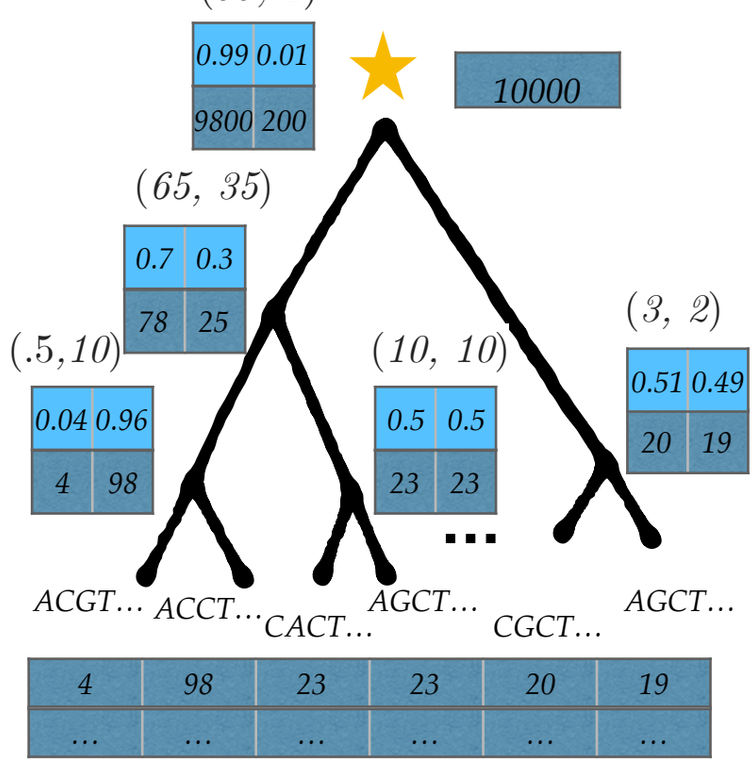

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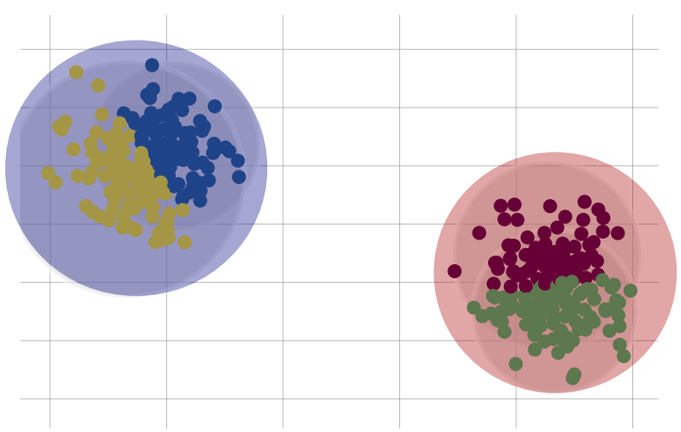

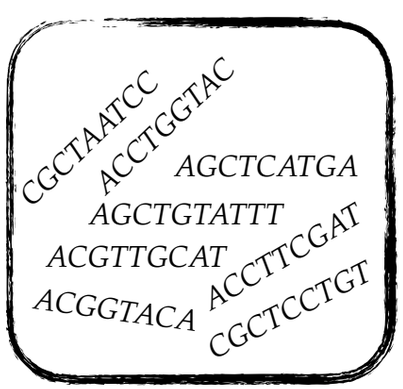

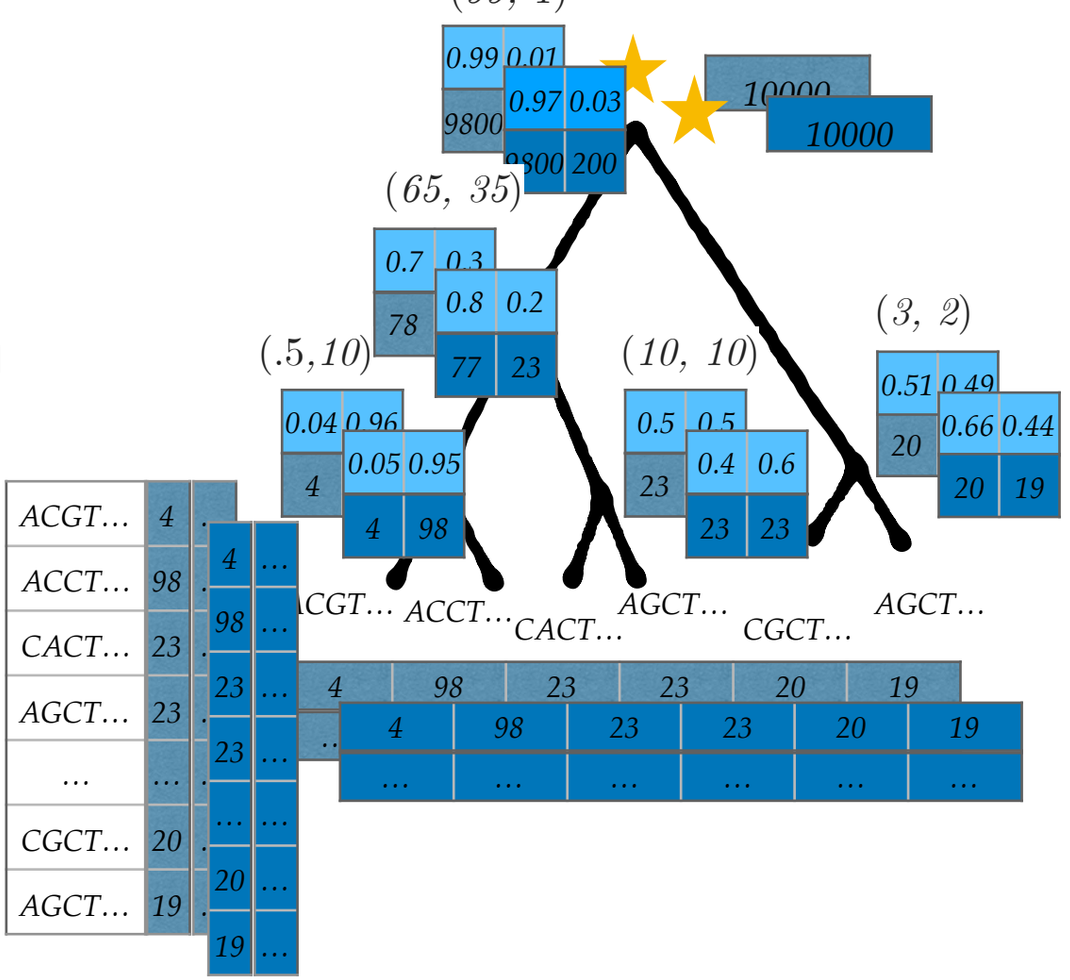

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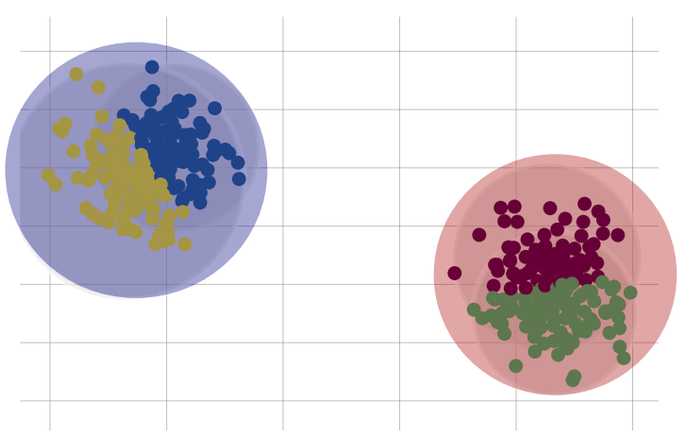

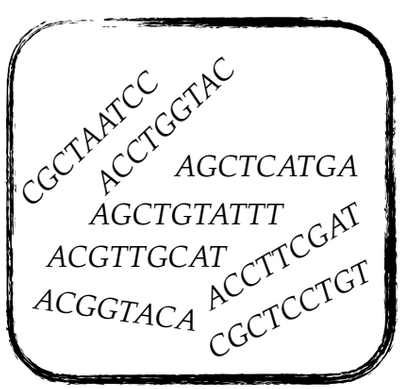

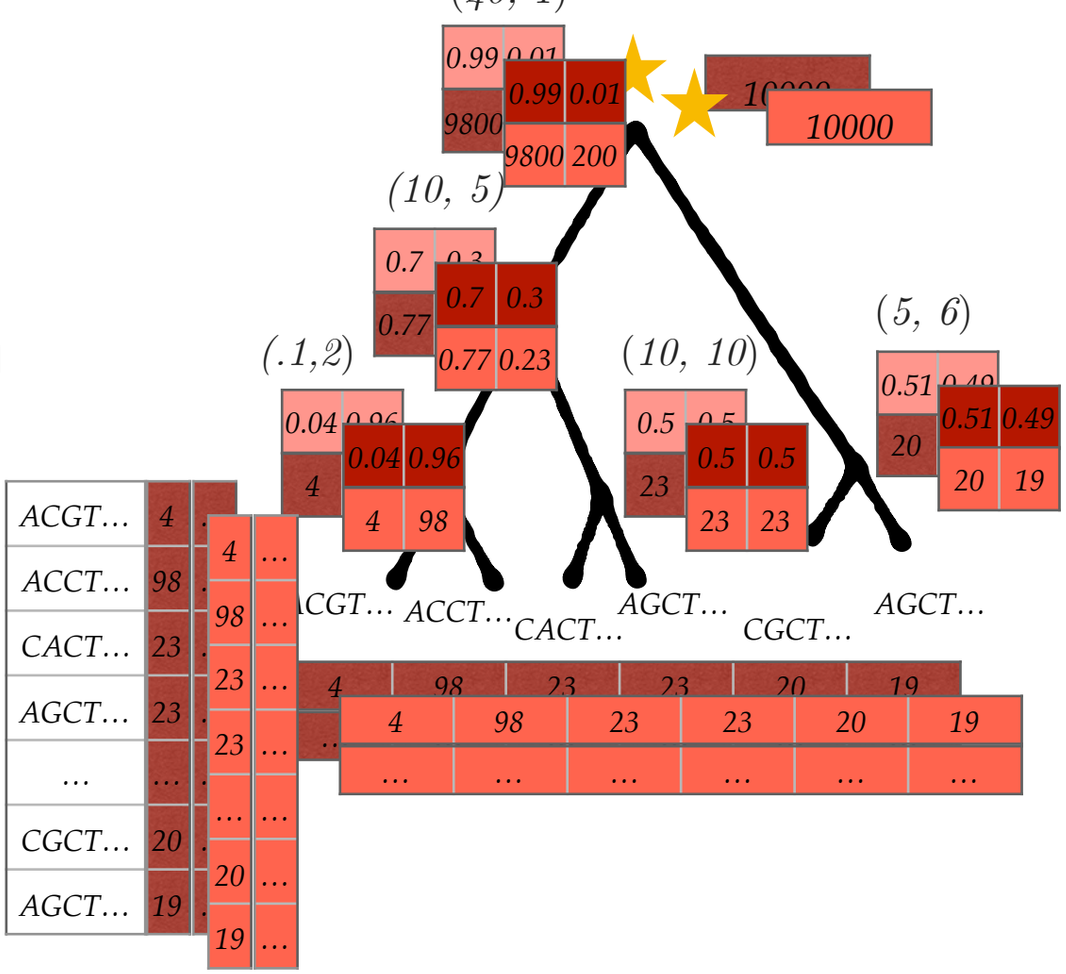

TADA BVSV+clustering

- 33% healthy vs 66% IBD
- Medium sized training dataset (712 samples)

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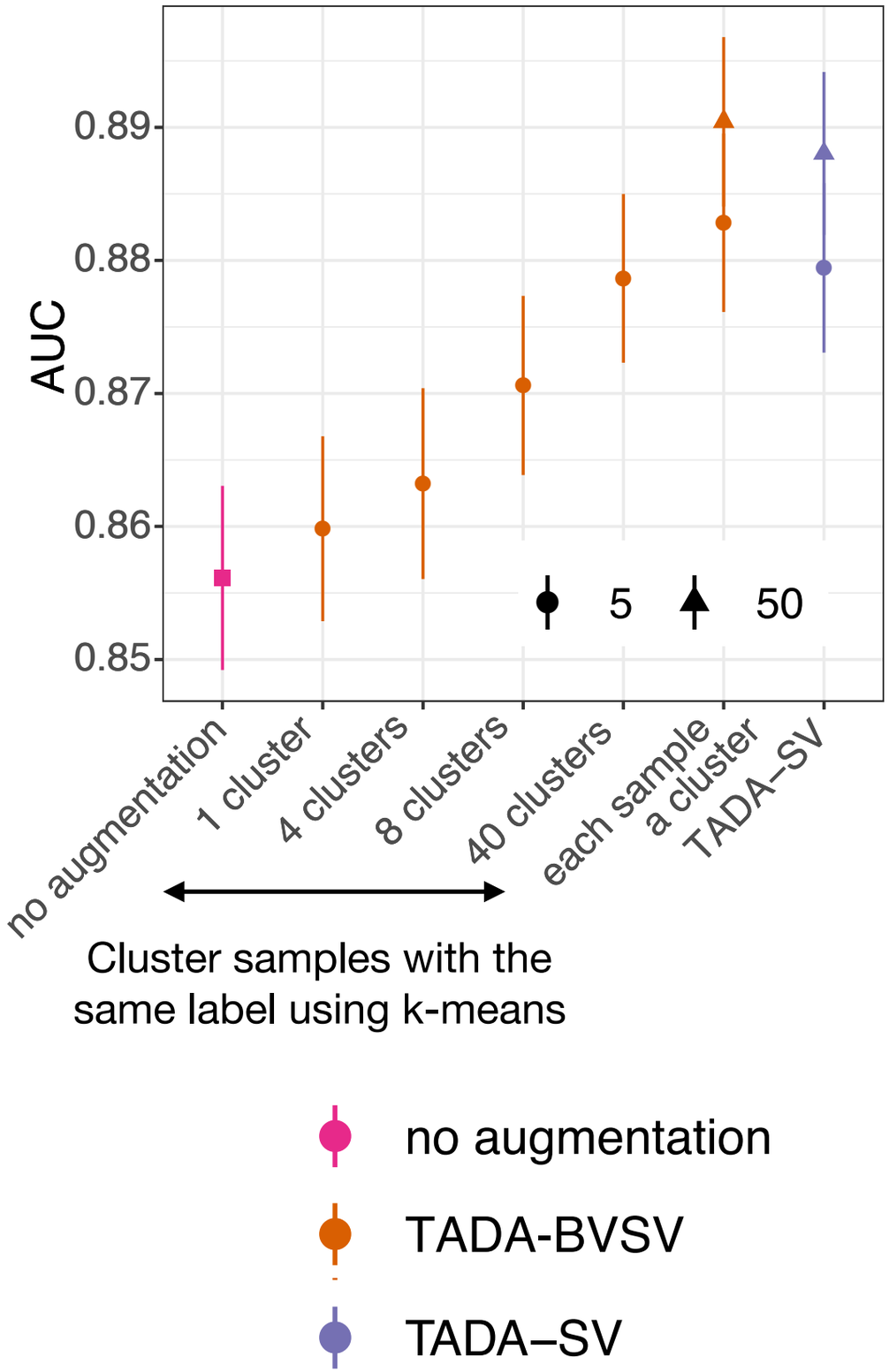

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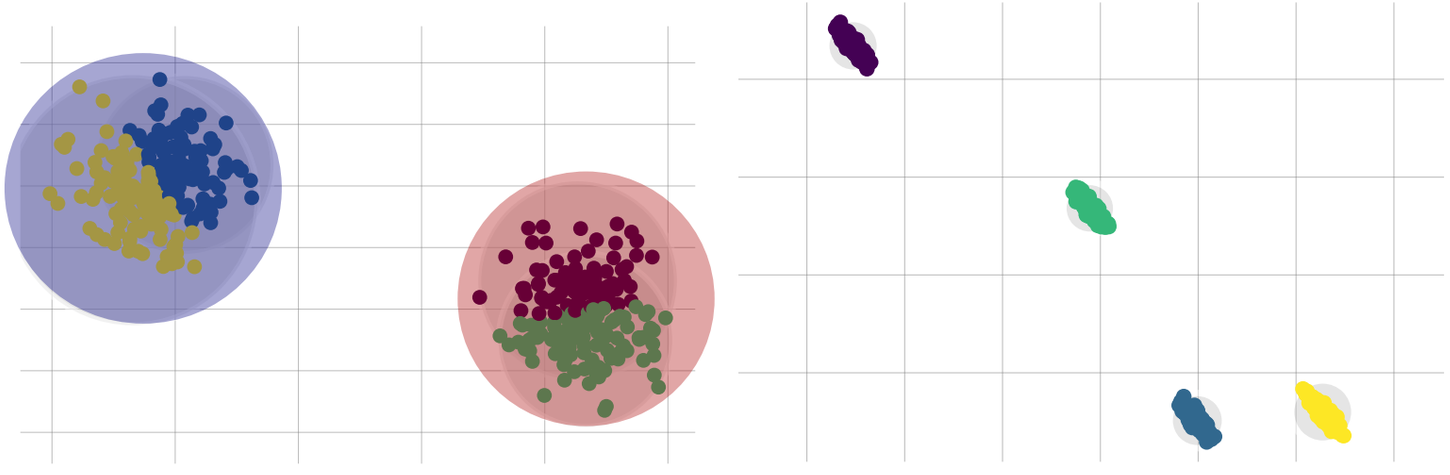
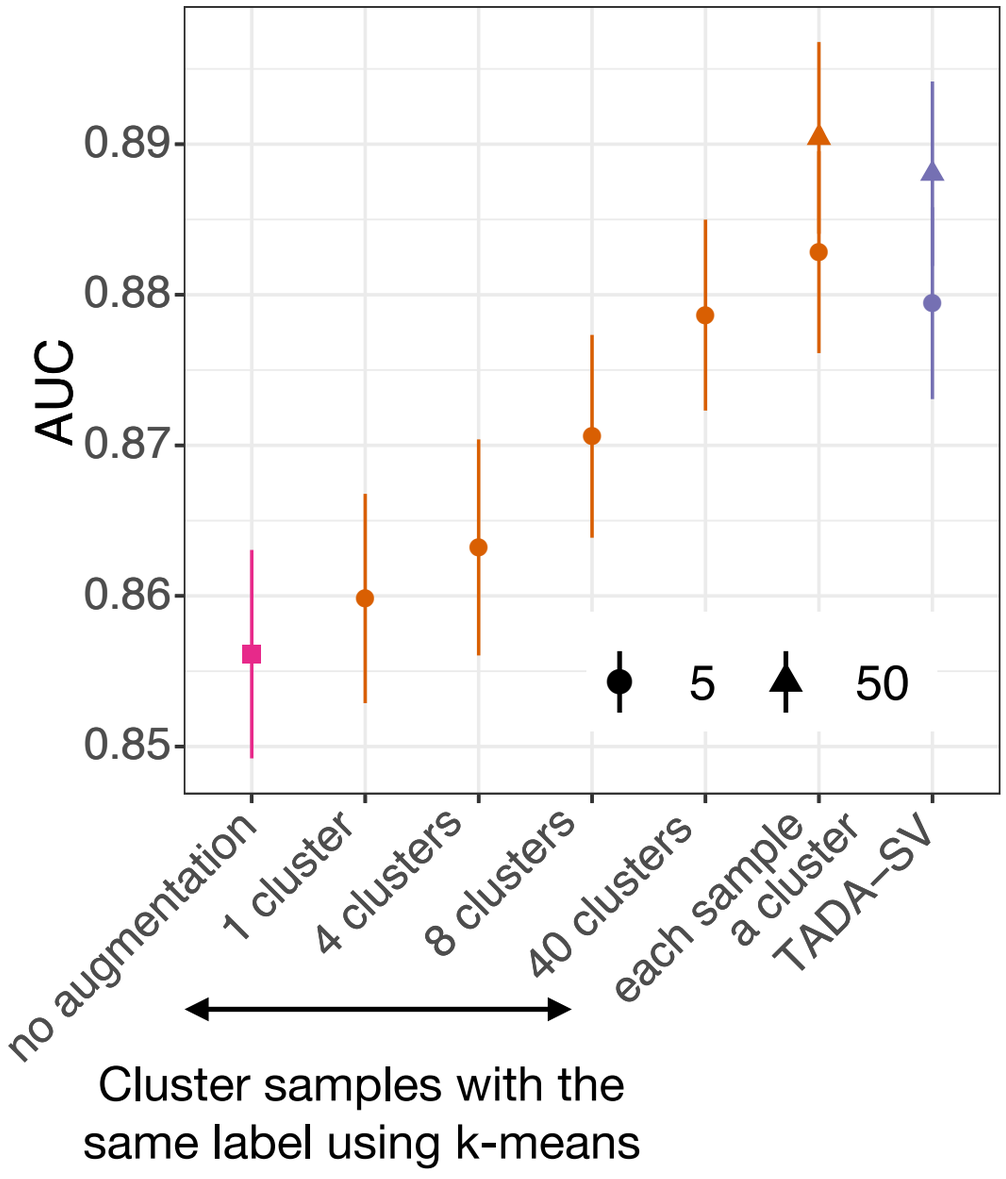




See the paper for …

Sayyari E., Kawas B., Mirarab S. 2019. TADA: phylogenetic augmentation of microbiome samples enhances phenotype classification. Bioinformatics. 35:i31–i40.

- Details of the TADA algorithm
- Many more experiments on the IBD data
  - Biased (but balanced) sampling
- Results using NN
- Results on a second dataset (American Gut) for a different trait (BMI)

Code and data

- They will be made available in a couple of days (hopefully) on GitHub

- https://github.com/tada-alg/TADA

- Apologies the code is not uploaded yet!!!

Acknowledgments

Prof. Rob Knights

Dr. Austin Swafford

Dr. Se Jin Song

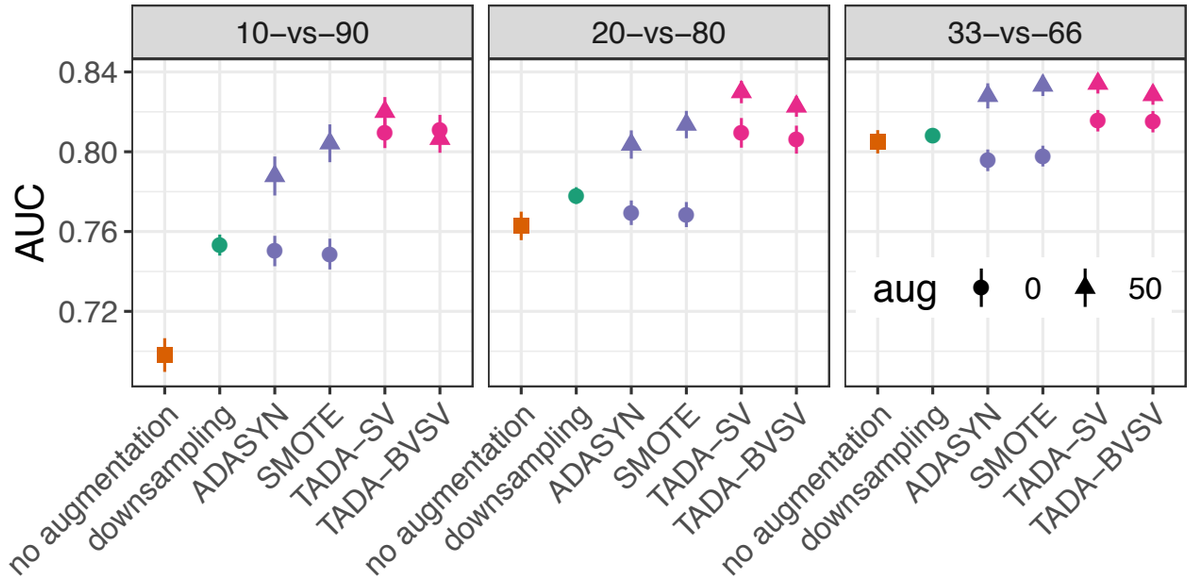
Dr. Sandrine Miller

Dr. Ho-Cheol Kim

Thank you

Why TADA-BVSV doesn't beat TADA-SV?

- BV might be sufficiently captured



Why TADA-BVSV doesn't beat TADA-SV?

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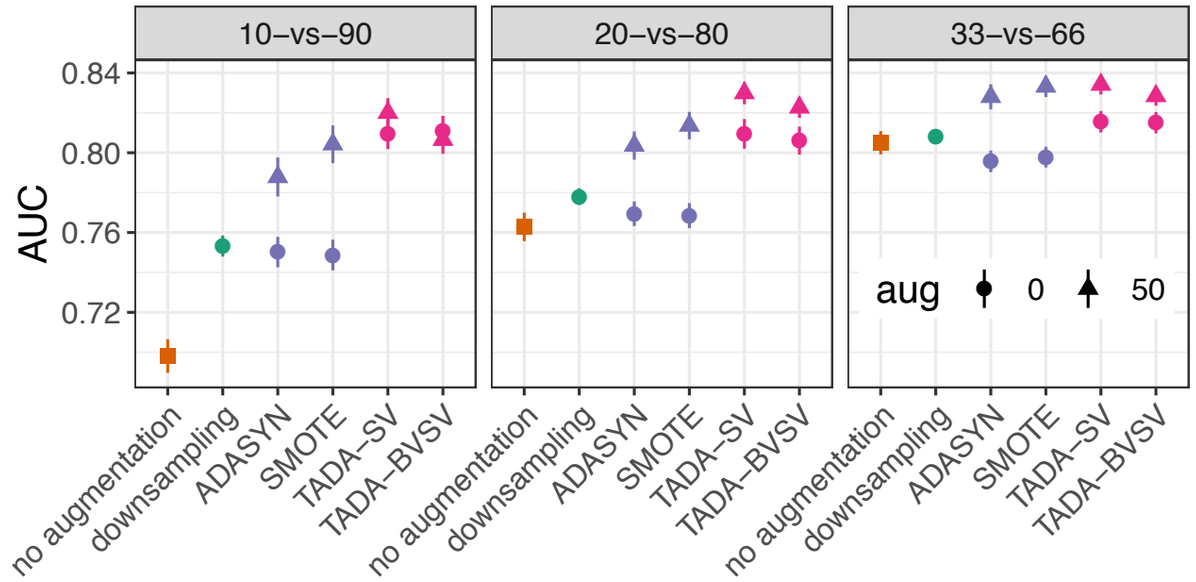
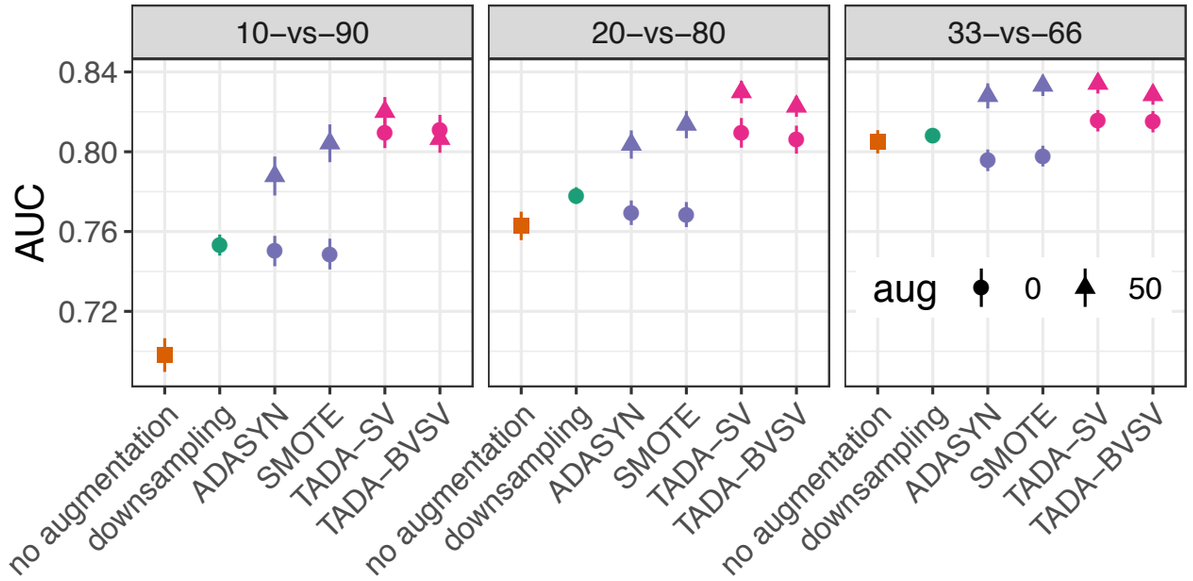


Why TADA-BVSV doesn't beat TADA-SV?

- BV might be sufficiently captured
- Beta distribution might not be the correct choice
- Conditional independence assumptions on the phylogenetic tree might not match the biology
    - Example: Horizontal gene transfer



Parameters of Beta distribution

- Beta distribution has two parameters
  - Mean and variance
- Learn them using the samples of the same cluster/class
  - Method of moments
  - Did not work well
- With few samples, learning all parameters from data is not feasible
  - We want a procedure to consider each point as the center of its own cluster

Heuristic solution

- If two nodes are far away from each other: our count estimates are robust, less variation

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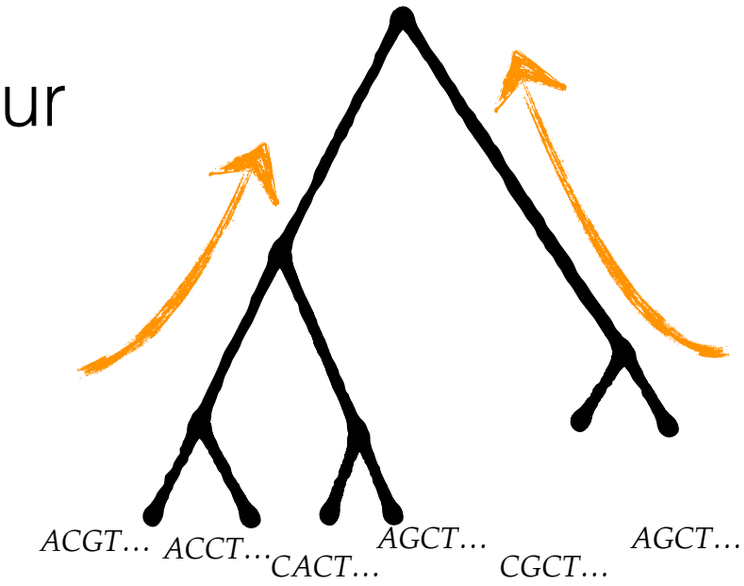

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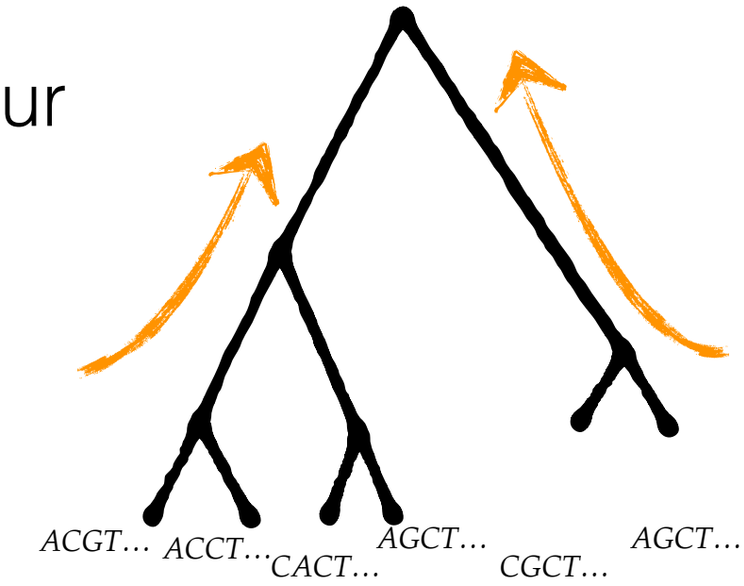

- d: average pairwise distances of leaves contained under each node

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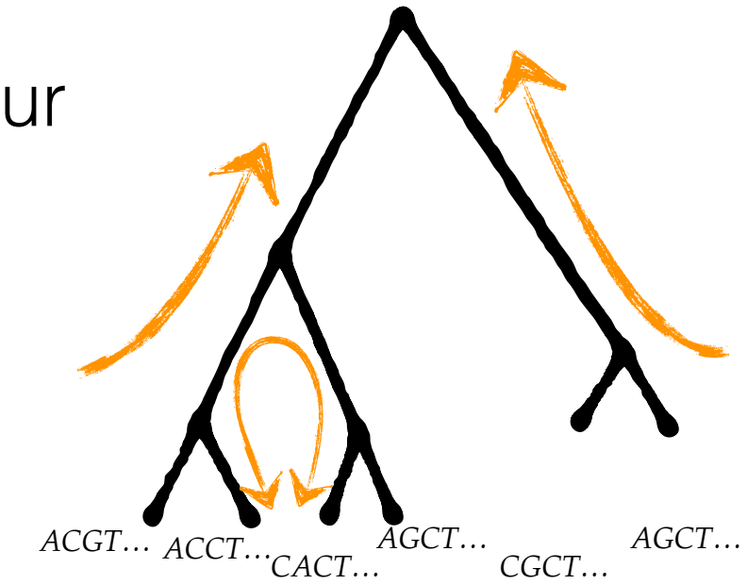

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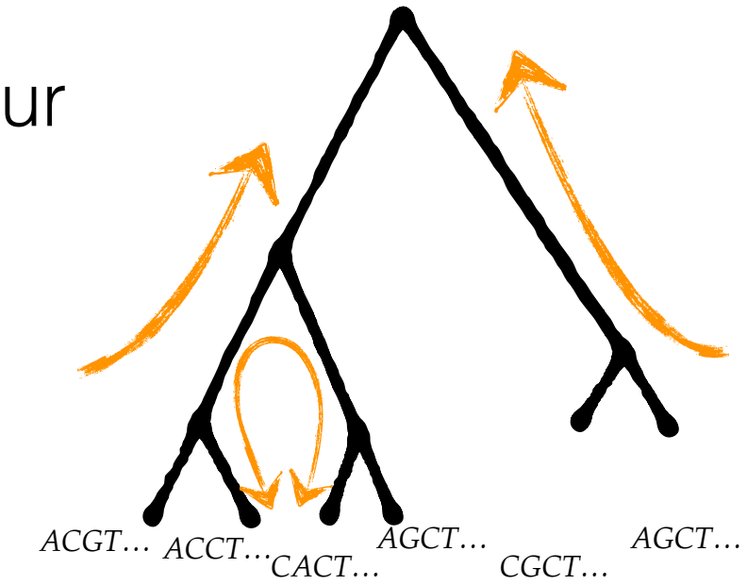

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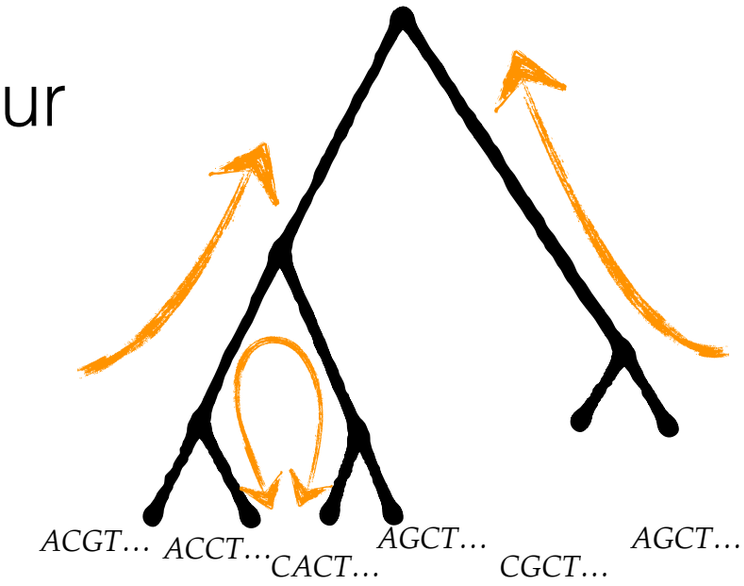

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- Mean is average proportions of right and left

Applications

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- Reduce unbalancedness with respect to class labels
  - e.g. having 20 samples for class 1 and 200 samples for class 2

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  - e.g. having only 50 samples for each class

Augmentation to reduce overfitting

- Small sized training data
  - 243 training points
- Medium sized training data
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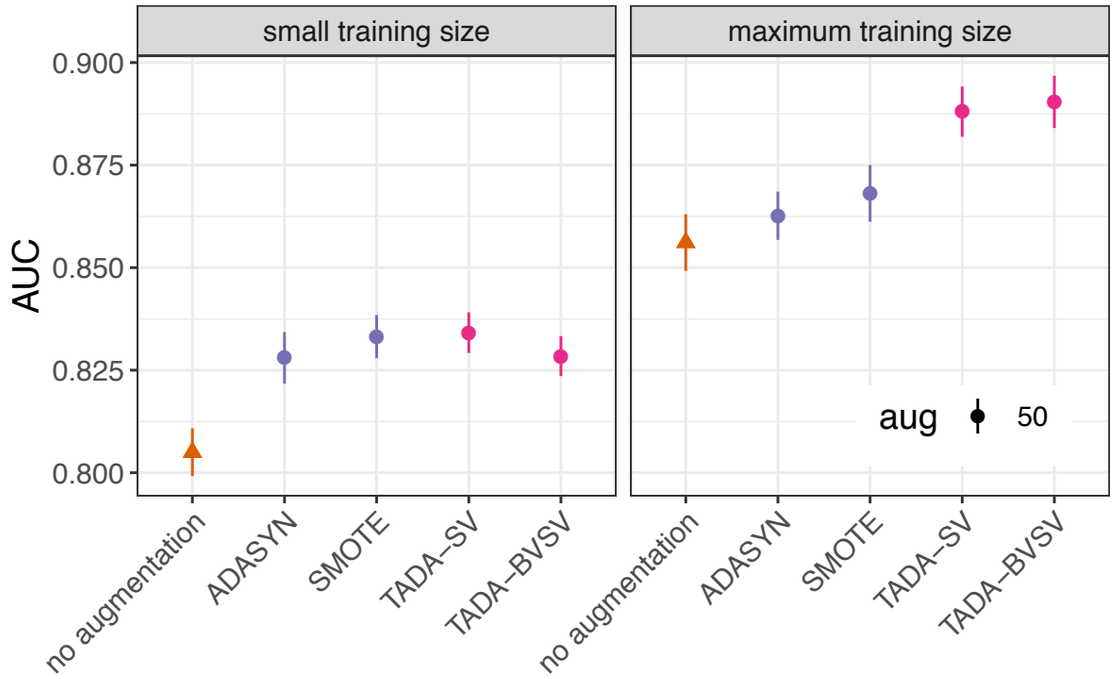
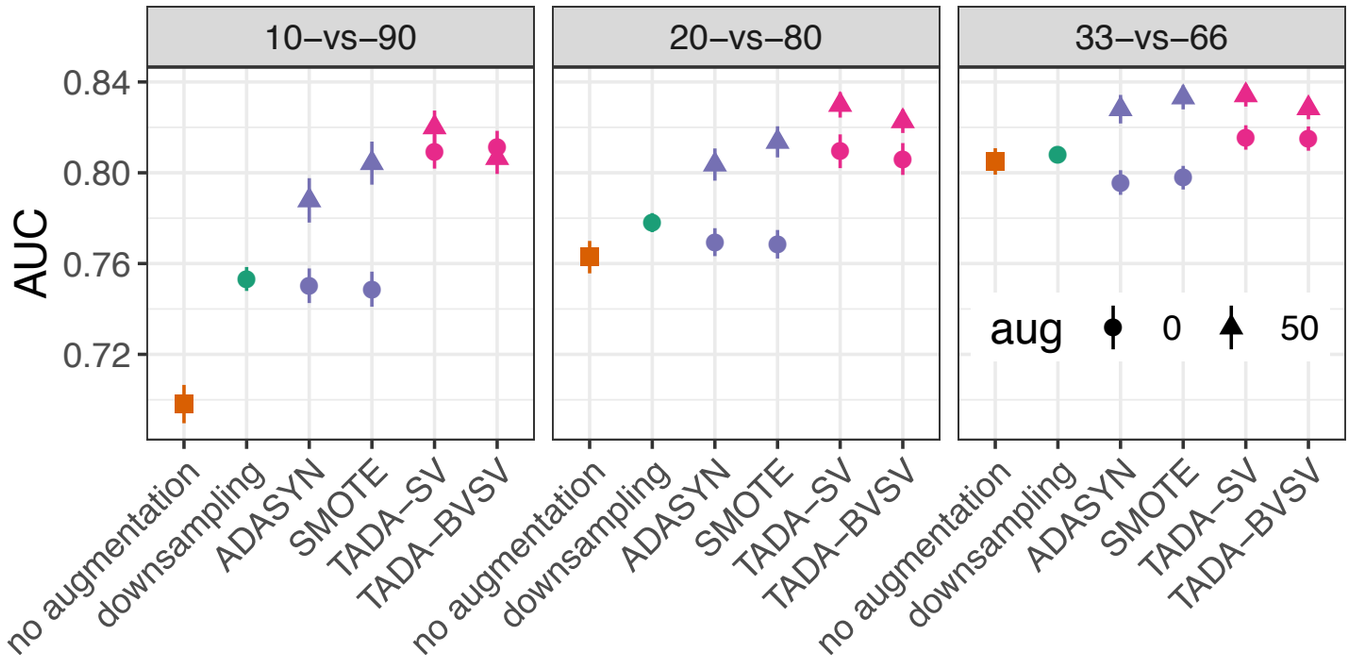


Why not helping?



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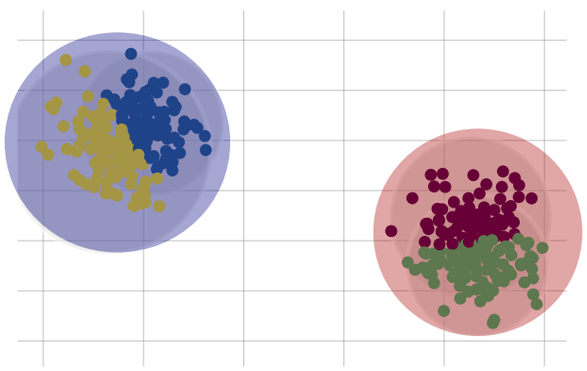

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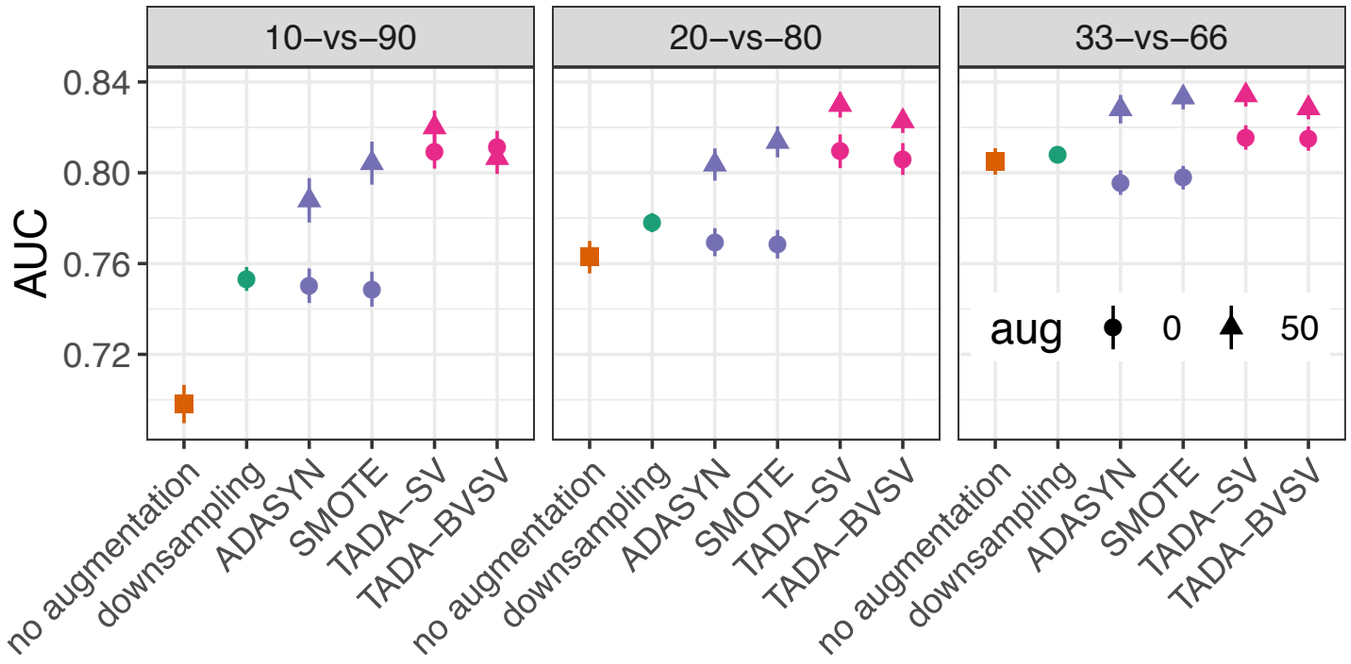

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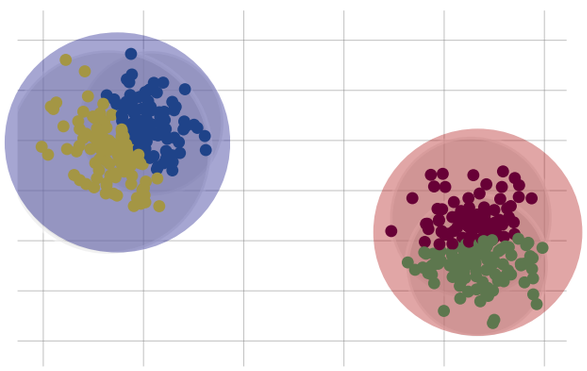

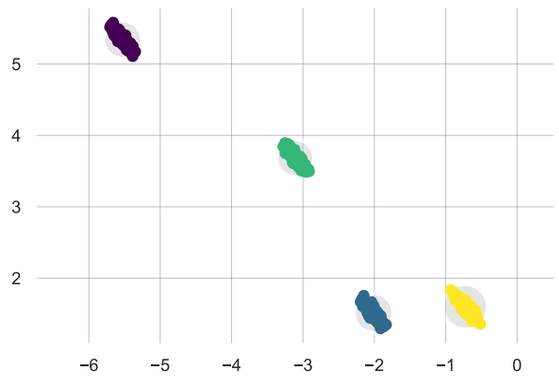

Biological variation (BV)

- Considering variations between samples

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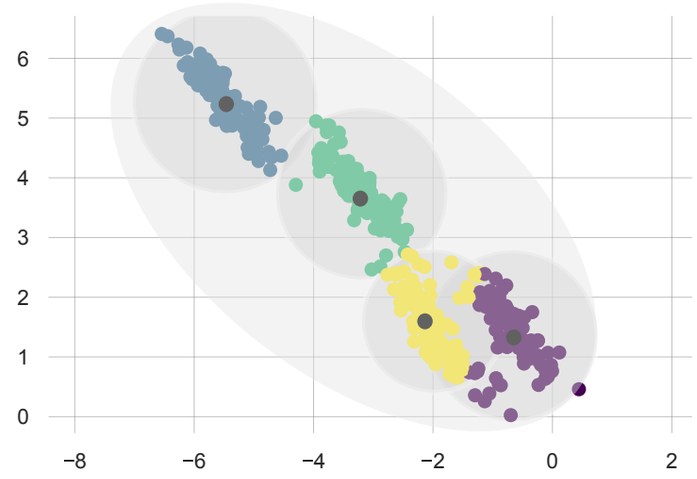

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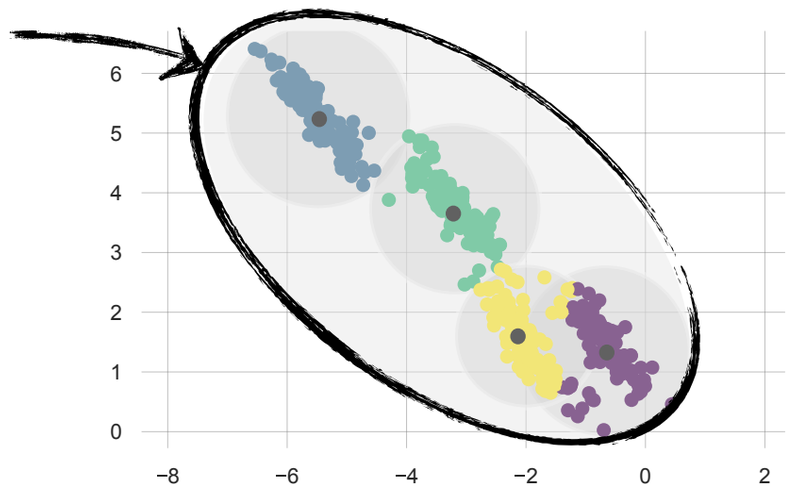

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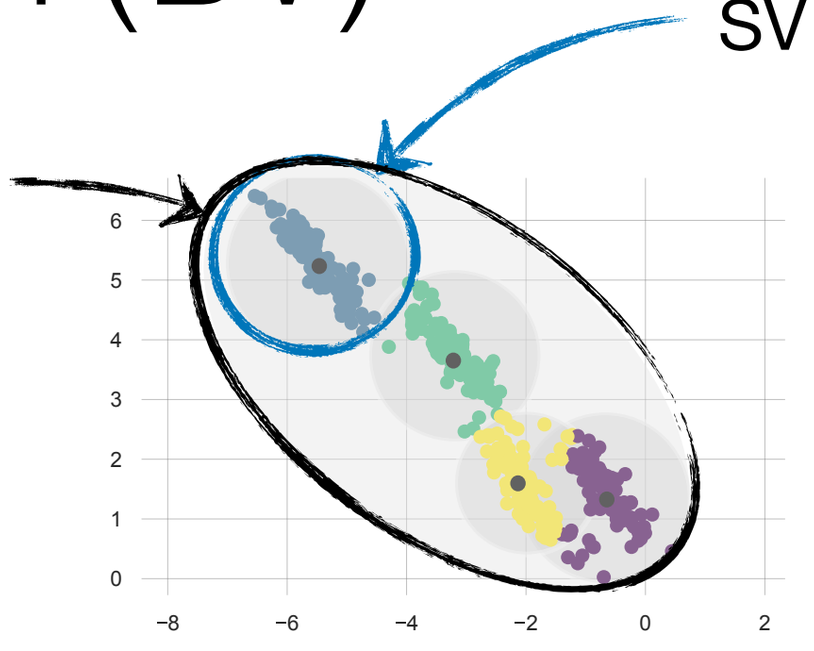

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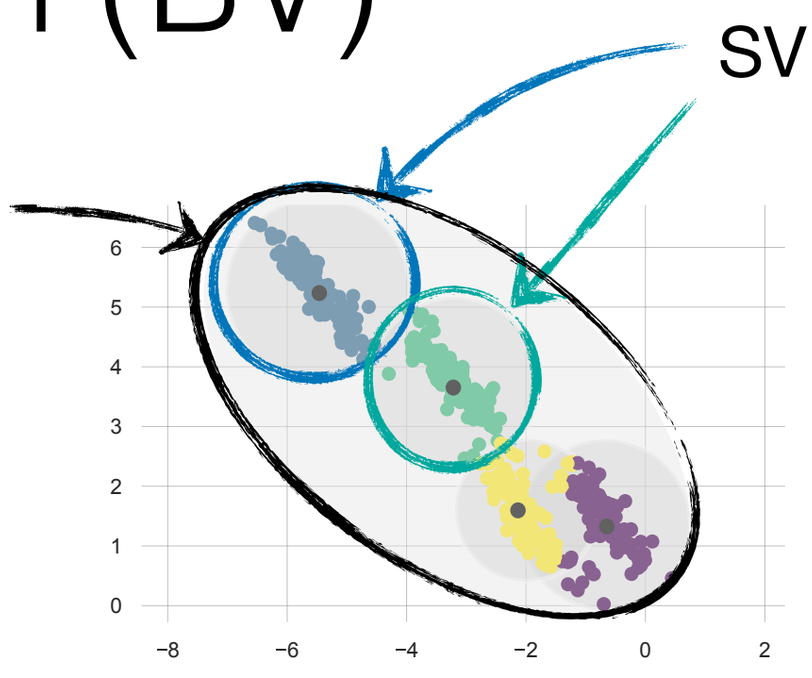

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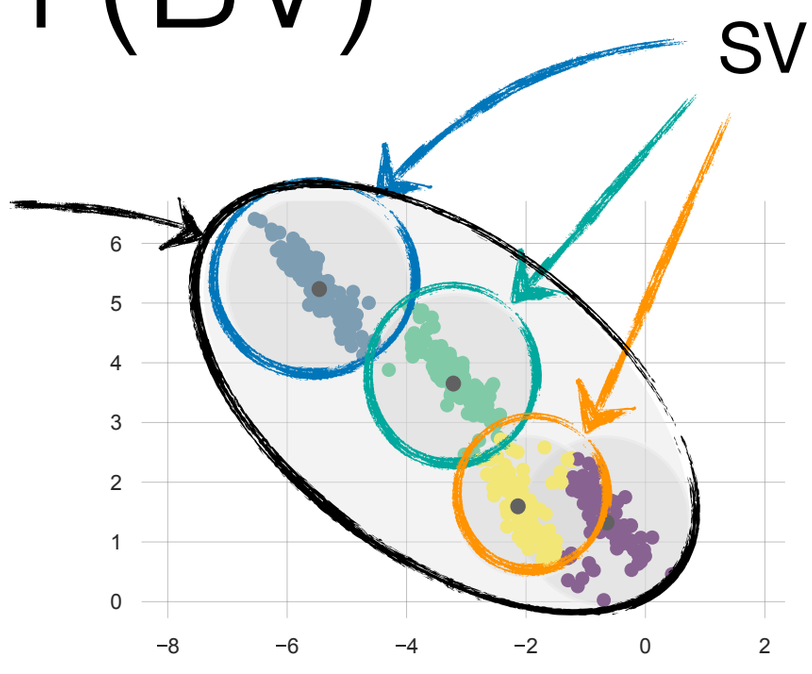

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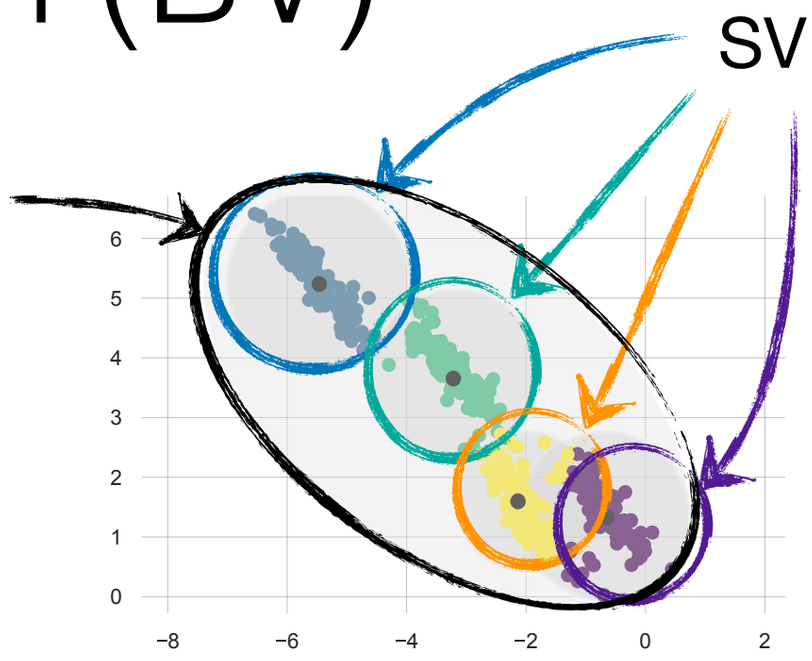

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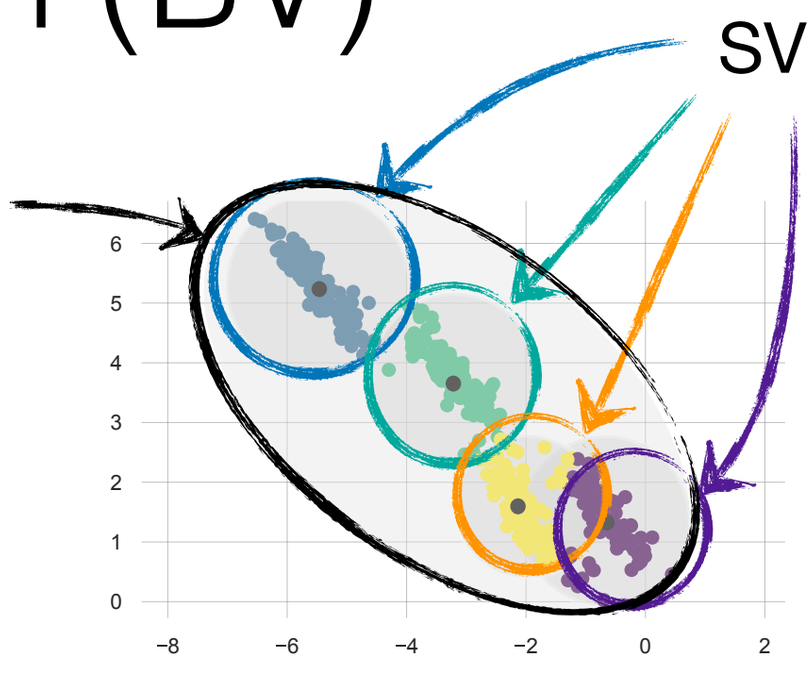

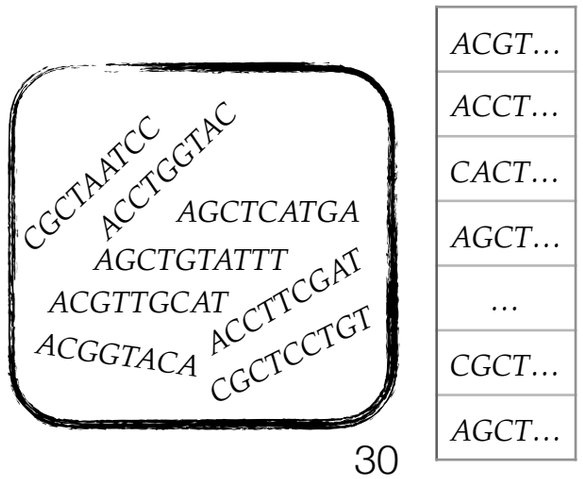

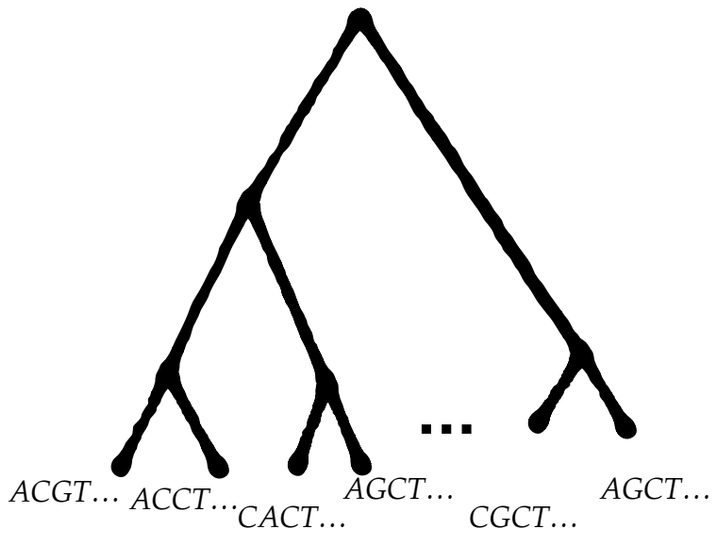

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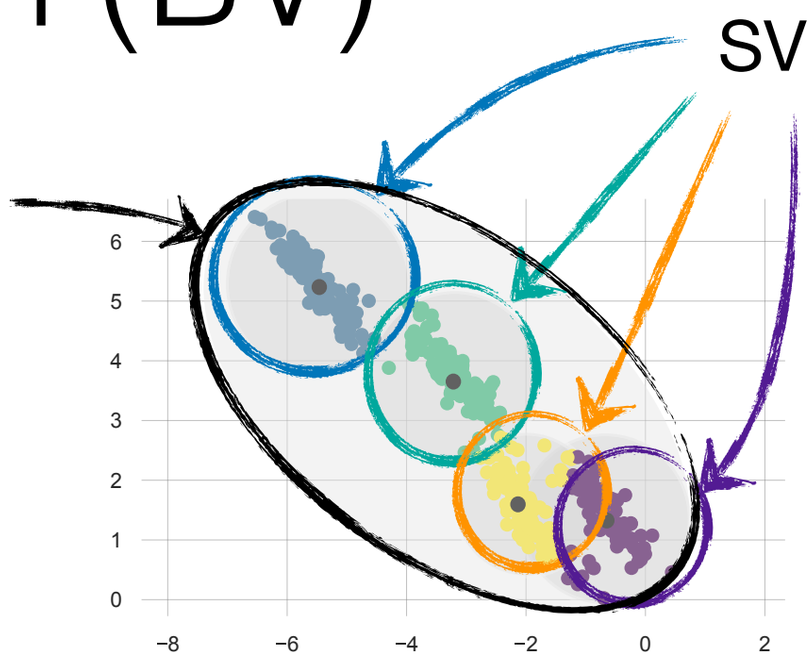
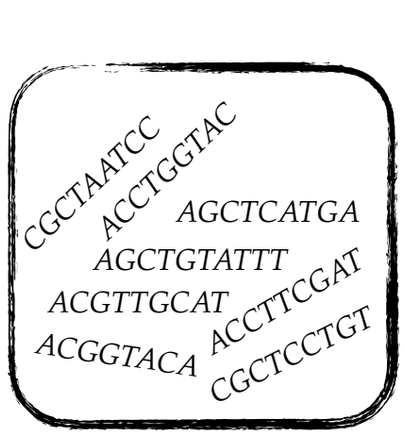
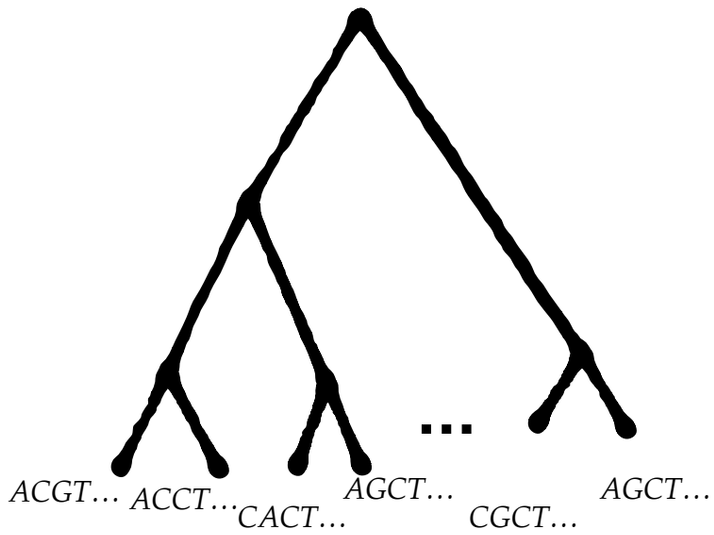




ACGT…	3
ACCT…	100
CACT…	20
AGCT…	10
…	…
CGCT…	30
AGCT…	20



3	100	20	10	30	20

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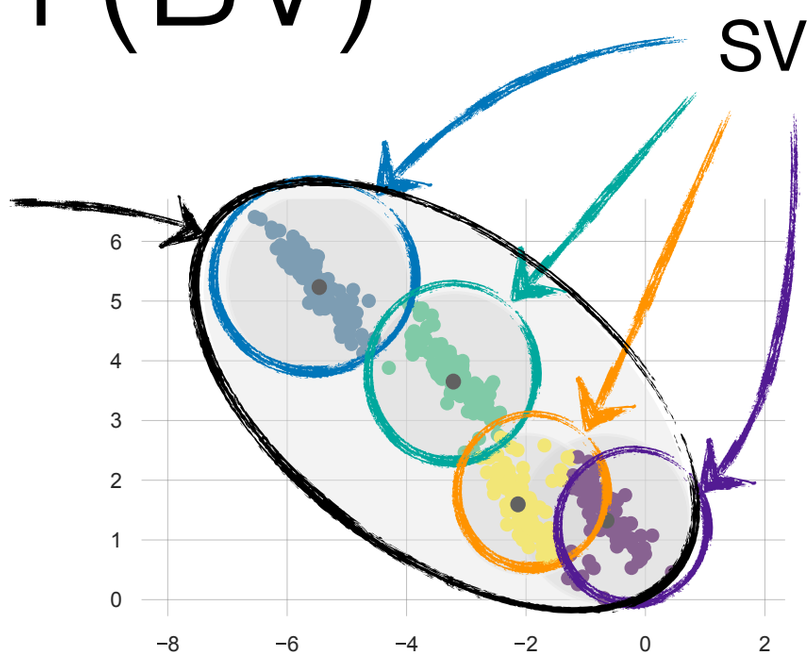
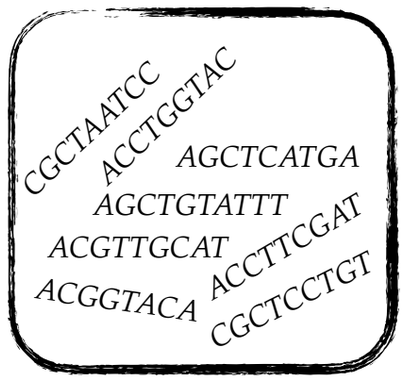
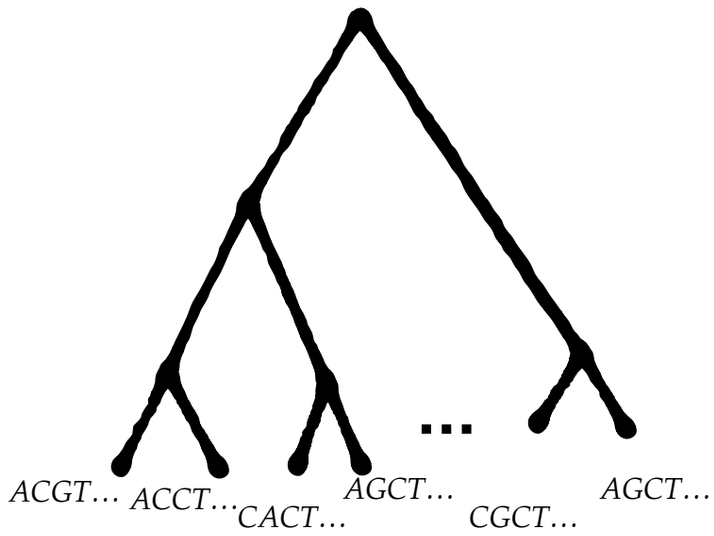




ACGT…
ACCT…
CACT…
AGCT…
…
CGCT…
AGCT…

3	…	5
100	…	100
20	…	25
10	…	1
…	…	…
30	…	10
20	…	20



3	100	20	10	30	20
…	…	…	…	…	…
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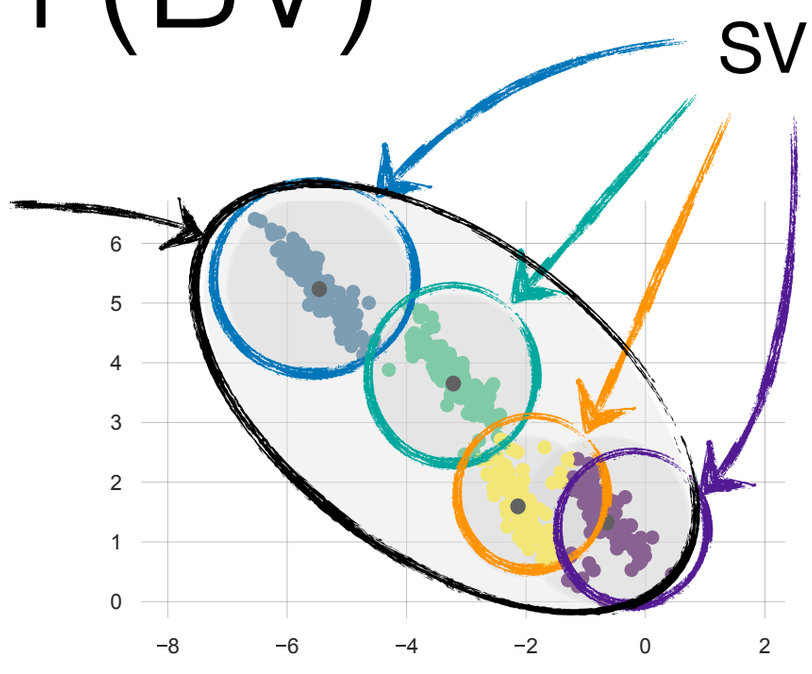

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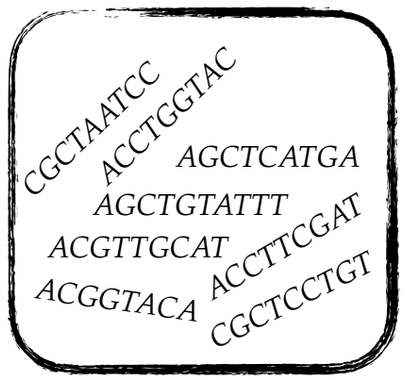
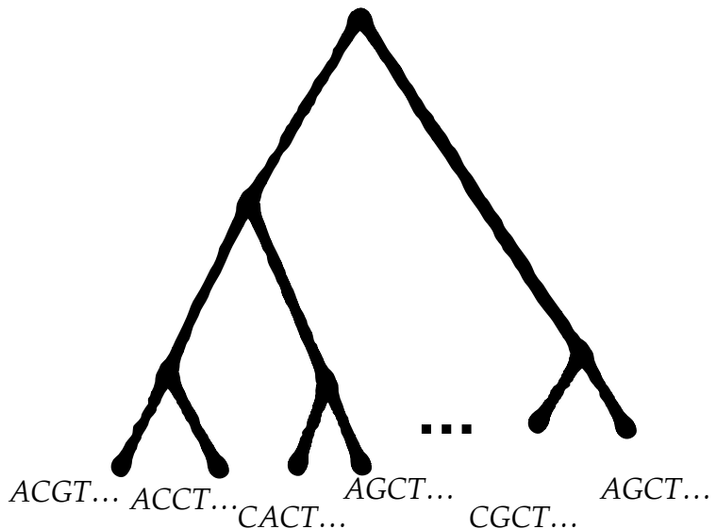


ACGT…	4	3	…	5
ACCT…	98	100	…	100
CACT…	23	20	…	25
AGCT…	23	10	…	1
…		…	…	…
CGCT…	20	30	…	10
AGCT…	19	20	…	20



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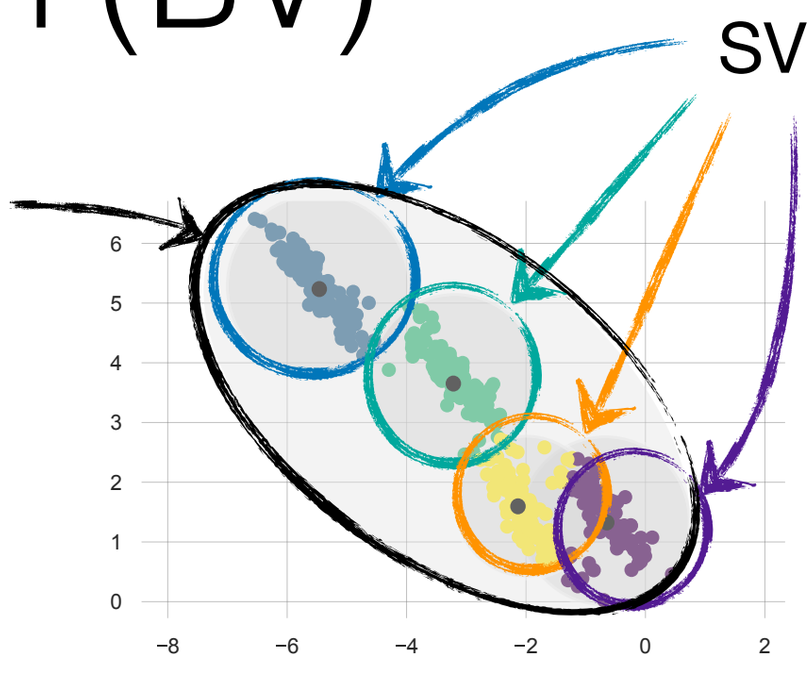

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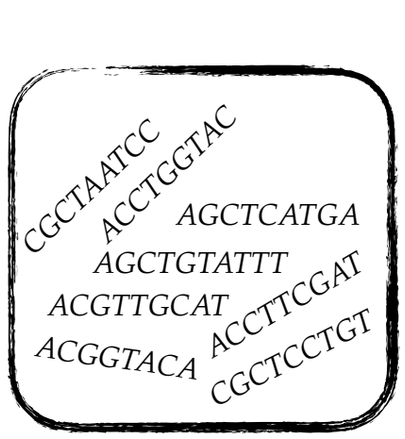

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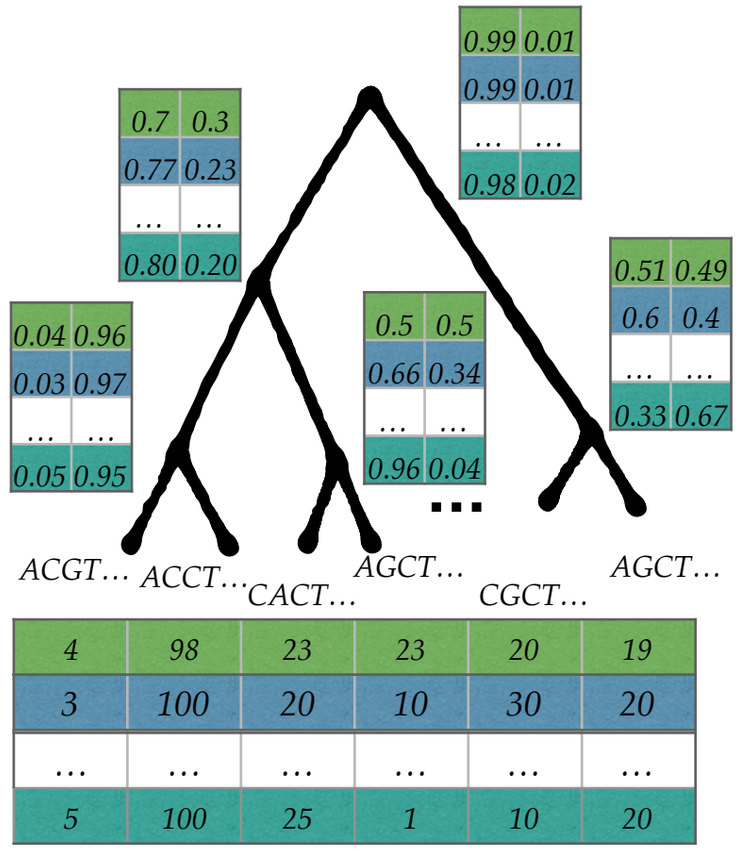

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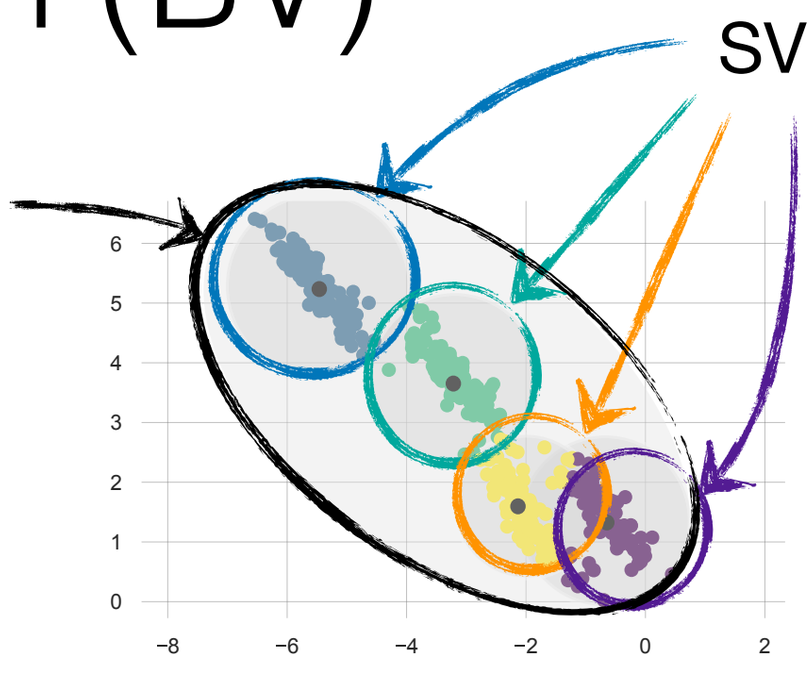
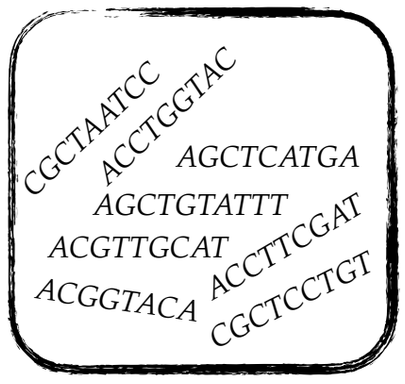
3	100	20	10	30	20
…	…	…	…	…	…
5	100	25	1	10	20

Biological variation (BV)

- Considering variations between samples
- BV: Beta distributions





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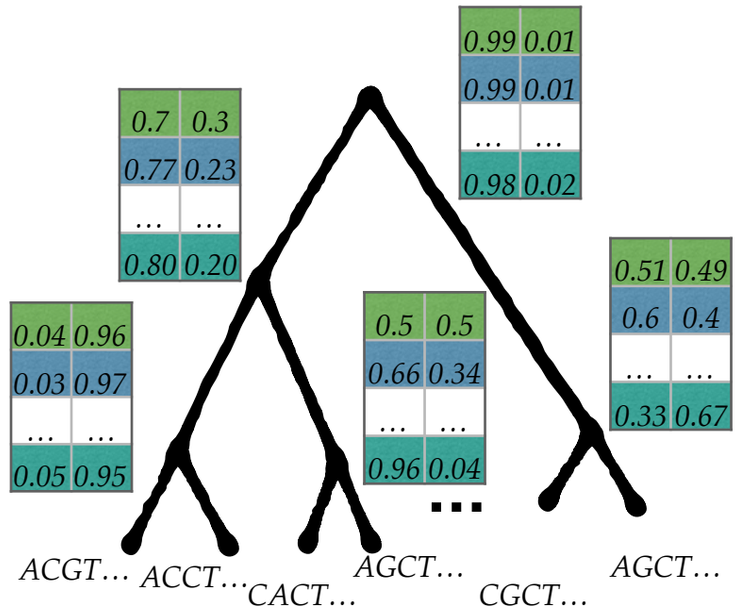

4	98	23	23	20	19
3	100	20	10	30	20
…	…	…	…	…	…
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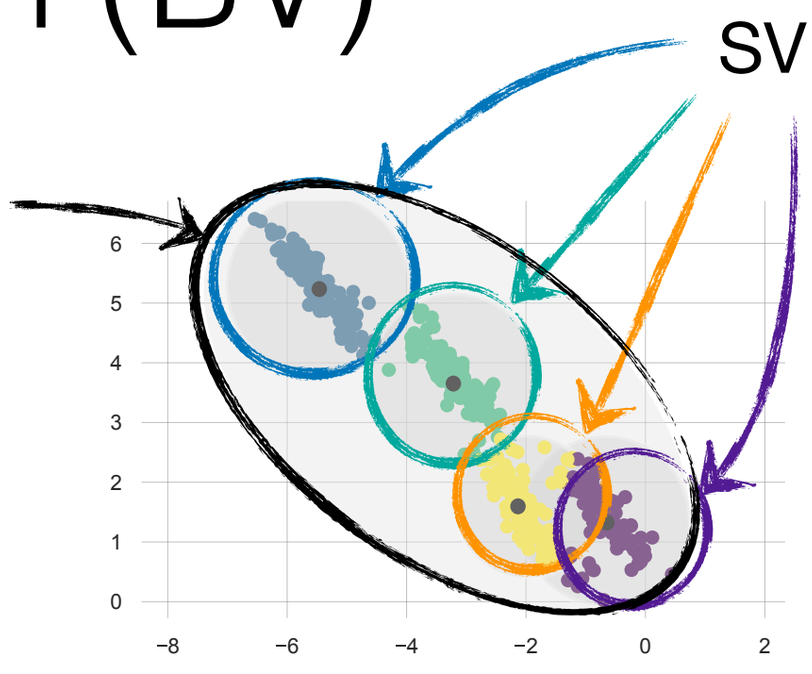
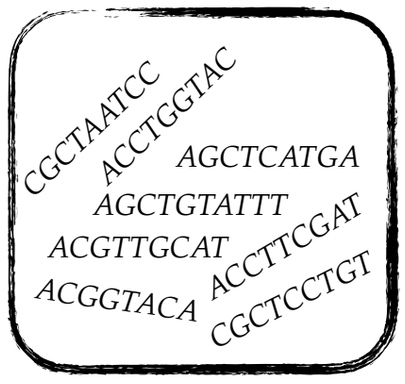
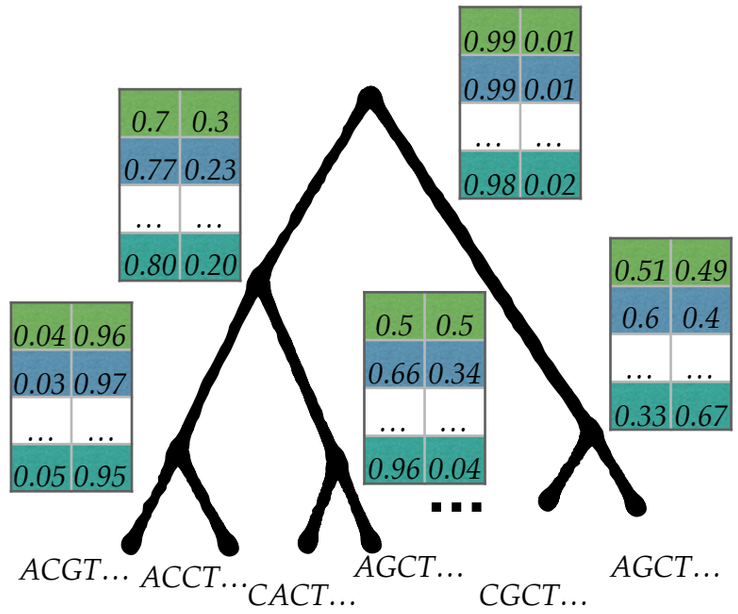
Biological variation (BV)



- Considering variations between samples
- BV: Beta distributions
- SV: Binomial distributions



ACGT…	4	3	…	5
ACCT…	98	100	…	100
CACT…	23	20	…	25
AGCT…	23	10	…	1
…		…	…	…
CGCT…	20	30	…	10
AGCT…	19	20	…	20



4	98	23	23	20	19
3	100	20	10	30	20
…	…	…	…	…	…
5	100	25	1	10	20