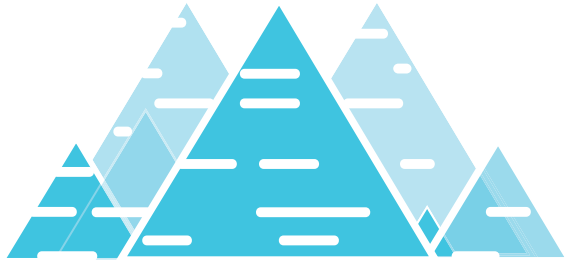

PERSONALIZED MEDICINE

SUMMIT

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Personalized medicine: a women's health perspective

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WOMEN'S HEALTH RESEARCH INSTITUTE AT BC WOMEN'S

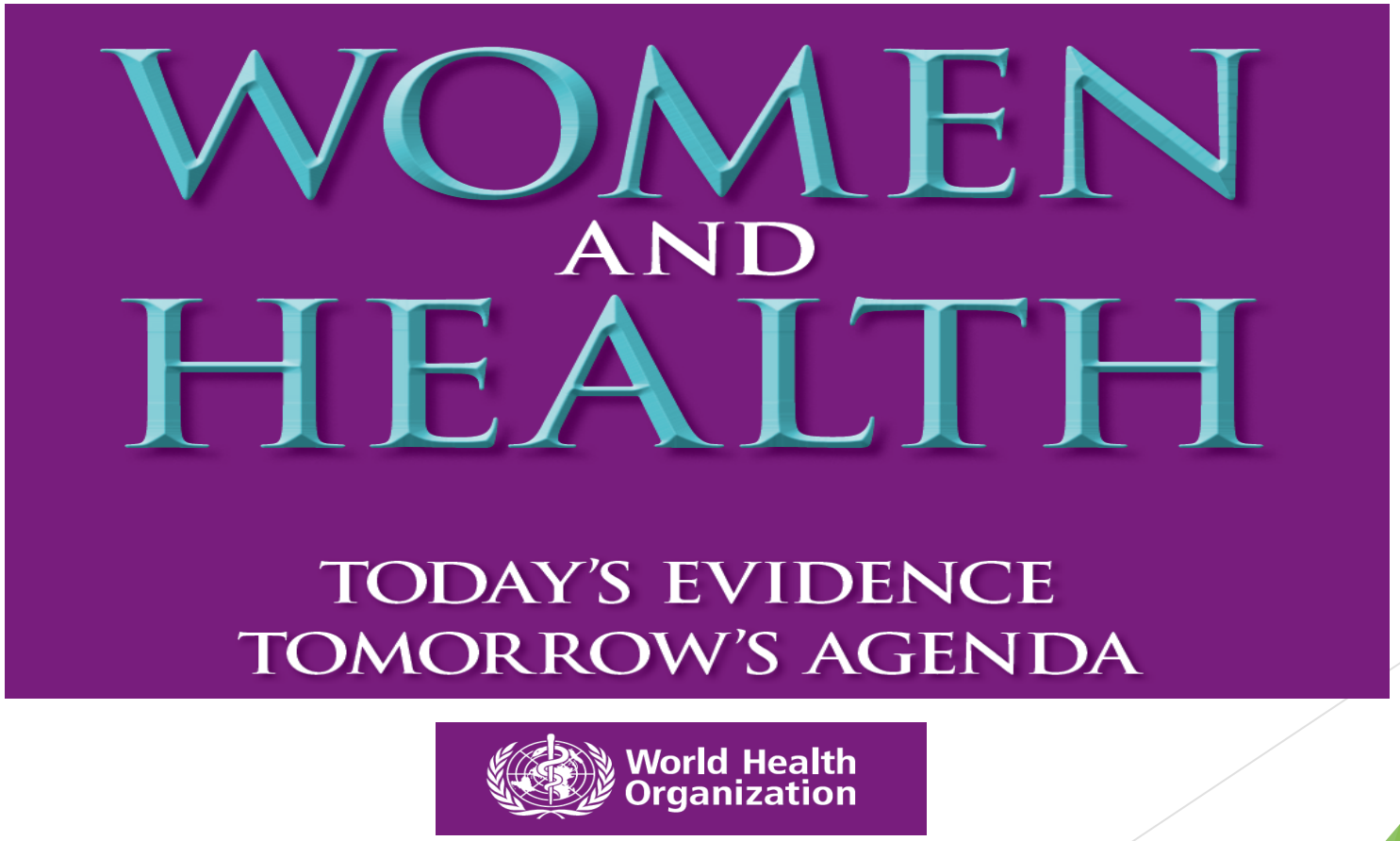
UBC

Genome BritishColumbia

Outline

- Why does a women's health perspective matter?
- Examples of the value of PMI for improved women's health
- Some examples of application of personalized medicine utilizing genomic tools to profile the vaginal microbiome

Why does a focus on women's health matter? WHO Priority for Global Health



Key findings: Women and Health

- Widespread and persistent inequities in health for women
- Sexuality and reproduction are central to women's health
- Increasing toll of chronic diseases, injuries and mental ill-health
- A fair start is critical for health of women
- Societies and health care systems are failing women

World Health Organization. Women and health: Today's evidence, tomorrow's agenda. 2010

Key findings: Women and Health

- Paying attention to women = investment in the society

Personalized Medicine:

- One of the greatest opportunities for personalized medicine is to close the equity gap for women
- In 1985, the Public Health Service Task Force on Women's Health Issues concluded that "the historical lack of research focus on women's health concerns has compromised the quality of health information available to women as well as the health care they receive."
- Despite this – slow uptake of sex and gender analyses in studies and few women in clinical trials with almost no data in pregnancy

Pharmacogenomics – an example of a positive benefit for women and infant

- Prevention of MTCT of HIV :
  - Prior to therapy and at time of changes in therapy virus is mapped for mutations that confer drug resistance – permits individual tailored therapy = 'Virtual Phenotype'
  - Patients are typed for presence of HLA-B5701 allele – if positive high risk of hypersensitivity reaction to Abacavir
  - Allows for selection of the most effective drug with least toxicity in pregnancy – future mapping for other toxicities particular to pregnancy

Other examples of women-specific benefit:

- Ovarian and endometrial cancers
  - moving from generic therapy to stratified therapy to individualized mutation based classification
  - Need to understand molecular phenotypes
  - Classic histopathologic and clinical staging may become unimportant
  - Lower toxicity of treatments

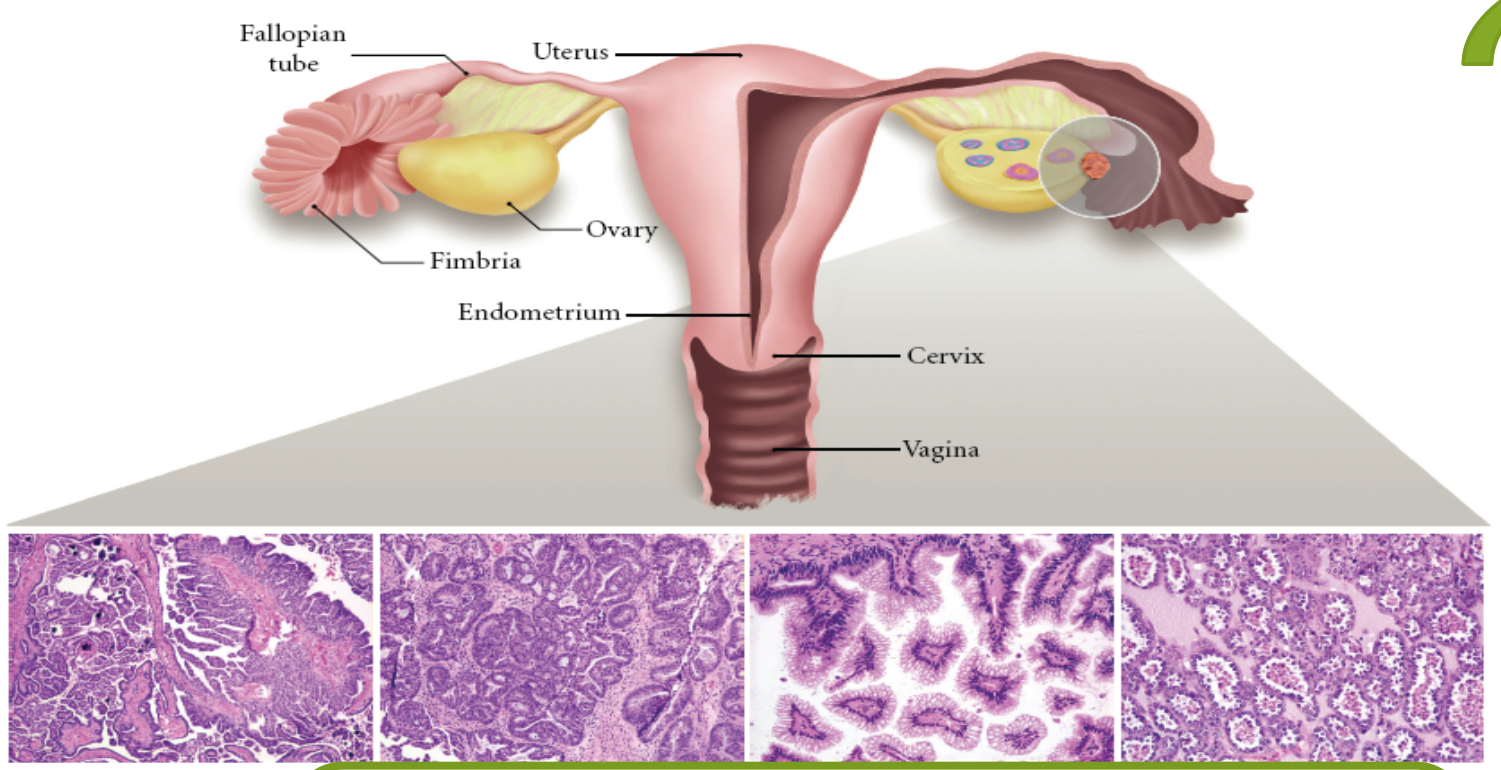
How do we categorize women with endometrial cancer and decide how to treat now?

- Based on (unreliable) histology classification
- Based on a POST-SURGICAL SAMPLE

→ LOW, INTERMEDIATE, and HIGH-RISK groups

- Rx variation within and across cancer centers & globe

Working from the successful model of ovarian cancer=distinct diseases



Serous | Endometrioid | Mucinous | Clear cell

75% | 10% | 5-10% | 5-10%

*High grade — "Type II"

Low grade — "Type I"

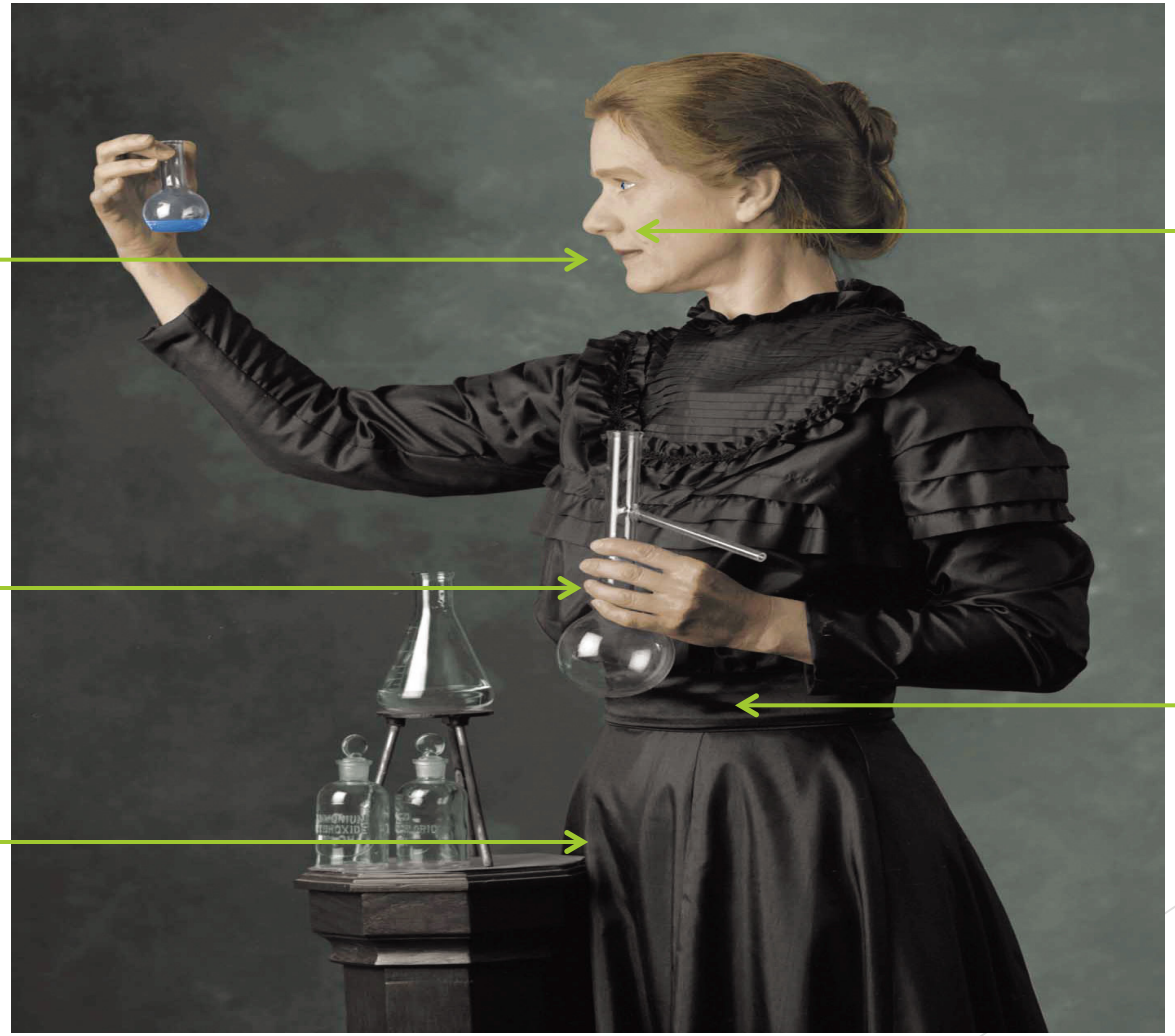
KRAS, BRAF, PIK3CA, PTEN, CTNNB1

EM Team

The study of the microbiome- to make a difference in women's health

The Microbiome

The Microbiome



International Microbiome projects

- The Human Microbiome Project – HMP – US based – multi-site – well funded
- Mega HIT – European based project
- The Canadian Microbiome Project – Funded teams to study the gut, skin, respiratory tract and the bioinformatics as well as the Vaginal Microbiome Team

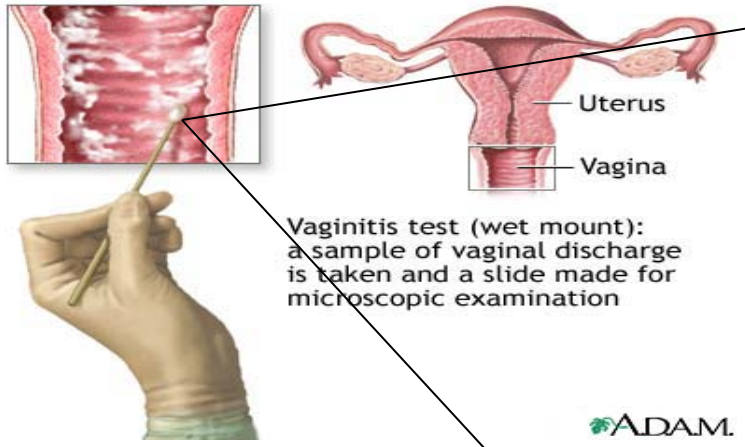
What is the importance of normal vaginal microbiota?

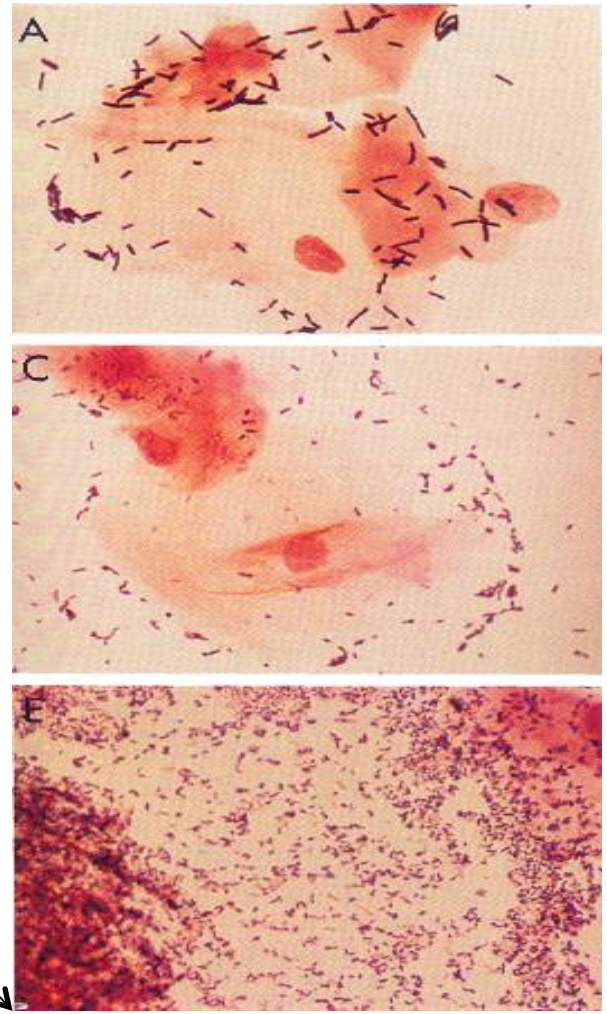
- Vaginal microbiota is the fine balance of organisms that exist in the vagina
- Normal vaginal microbiota is associated with ability to defend against vaginal/cervical pathogens
- Abnormal vaginal flora causes significant symptoms and is associated with susceptibility to many infections, risk of post surgical complications and adverse perinatal outcomes – Preterm Birth

The health burden and costs of vaginitis

- One of the MOST common causes of physician visits
- Estimated to cost 3.1 billion$ per year in the US – 500 Million in direct physician costs
- Use of health care resources that could be used more efficiently – multiple tests deployed, frequently non-conclusive
- Extensive other the counter therapies 250M$ and homeopathic therapies – 200M$ - many not effective

Current ‘gold standard’ clinical diagnosis – THE GRAM STAIN!!!





The Nugent score

- Current gold standard for evaluation of vaginal microbiota
- Highly subjective
- Frequently misinterpreted
- Under- and over-diagnosis

Gram Stain Diagnosis: Nugent's criteria (0-3 normal, 4-7 intermediate, 7-10 abnormal

Score	Lactos	Gram neg/variable	Curved rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3-4+
3	1+	3+	
4	0	4+	

Nugent et al. , J clin Micro 1991;29(2):297-301)

Current Treatment of Bacterial vaginosis – a classic example of old school medicine

- Untargeted broad spectrum anti-anaerobic therapies:
- Metronidazole oral or vaginal
- Clindamycin oral or vaginal
- Overall a 30% recurrence risk
- Likely to cause shift to gram negative dominance at first

BV associated with increased risk of STI infections

- HIV – increased risk of acquisition and level of HIV replication
- HPV - increased acquisition of HPV in HIV positive and negative women – also may be a co-factor in progression to cancer
- Chlamydia – increased susceptibility to infection with BV and/or simple loss of H2O2 producing lactobacilli
- Pelvic inflammatory disease – caused largely by organisms in the vaginal microbiome
- Infertility sequelae

Culture-based Investigations of the Vaginal Microbiota

- *Lactobacillus* spp. - predominant
  - Aid in immune/host defense against pathogens (*Sobel 1999*)
  - Influence fertility and reproductive success (*Eschenbach 1989, Hillier 1999*)
- Shift to increased diversity of mixed anaerobic bacteria (*Gardnerella vaginalis*, *Bacteroides* spp.)
  - Increased risk of STI acquisition + transmission (*Hillier 1998, Wiesenfeld 2003*)
  - Increased risk of preterm birth (*Hillier 1995*)

Culture Independent Investigations

- Large scale 16SrRNA-based studies in healthy, asymptomatic women (*Zhou 2007*, *Zhou 2009*, *Ravel 2011*, *Gajer 2012*, *Drell 2013*)
- Clusters of 4-7 defined as community state types (CST) distinguished by dominant bacterial taxa
- Most prevalent and dominant– *Lactobacillus* (*L*) *iners*, followed by *L crispatus*, *L gasseri*, *L jensenii*
- Suggestion that non-lactobacillus dominant communities may be “healthy” in some women

Defined Vaginal Community State Types

Ravel et al 2011	
I	*Lactobacillus* (*L*) *crispatus*
II	*L gasseri*
III	*L iners*
IV	Heterogenous mix
V	*L jensenii*

Gajer et al 2012	
I	*Lactobacillus* (*L*) *crispatus*
II	*L gasseri*
III	*L iners*
IVA	*Bifidobacterium*, *Dialister*, *Streptococcus*, and *Bacteroides*
IVB	*Gardnerella*, *Prevotella*, *Megasphaera*, bacterial vaginosis-associated bacteria (BVAB), and *Mobiluncus*
V	*L jensenii*

The Vaginal Microbiome Group Initiative

Vogue study team approach

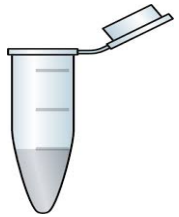
- Clinical populations studied:
  - Normal healthy women – N=300
  - Women with known recurrent vaginitis – N=100
  - Women with HIV infection N=50
  - Pregnant low risk women N=100
  - Women who have an ideopathic preterm birth – N=50
  - Women with PPROM – N=50
- Developing evolving bacteriome study methodology
- Virome analyses
- Social science surveys, focus groups

cpn60 – a target for metagenomics

- found in all virtually all bacteria
- can be PCR amplified with universal primers from any bacterial genome
- meets all the criteria for being a good metagenomic target and surpasses 16S rRNA in one important category: it's more informative
- more discriminating power for closely related species

Metagenomic analysis

Total genomic DNA extraction

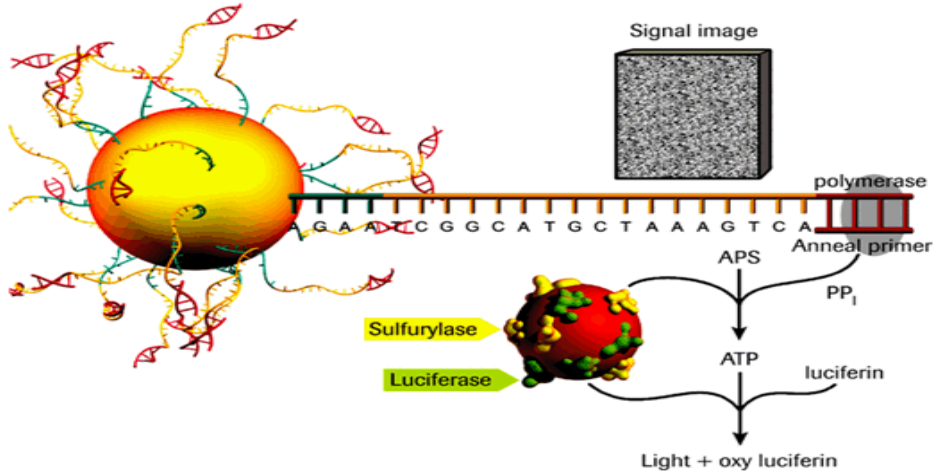


cpn60 UT PCR

cpn60 PCR amplicon libraries

Pyrosequencing
GS Junior
454 Sequencing System

Assembly



Assembly

Software: mPUMA

> mPUMA (microbial Profiling Using Metagenomic Assembly): a software package designed to profile microbial communities using a *de novo* assembly approach to form Operational Taxonomic Units (OTUs)

Input file: sff → fastq

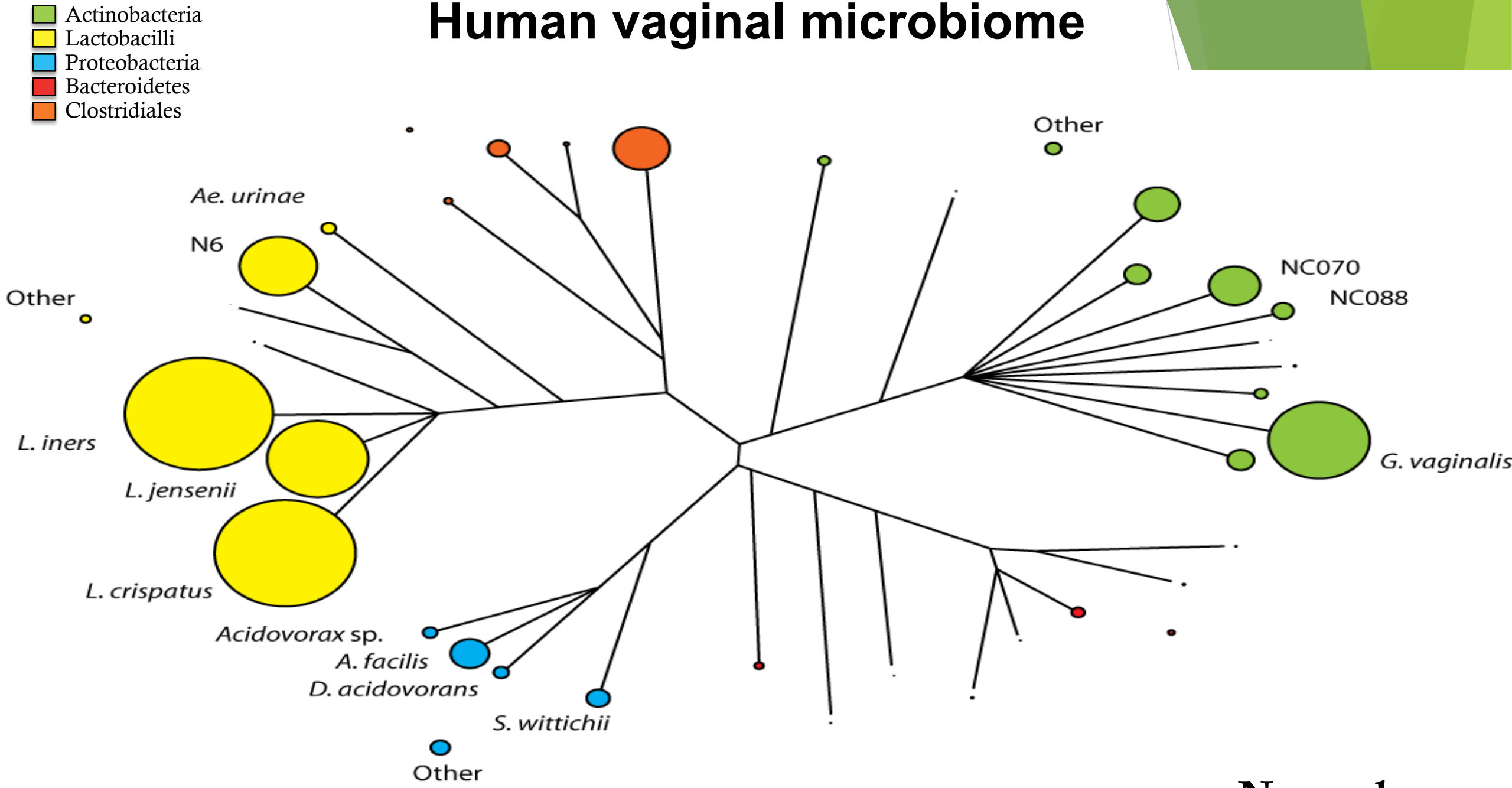
Assembly method: Trinity

Mapping method: bowtie2

Winnow option: only OTUs with >55% identity to cpnDB are included in the biom file

Output files: fasta, biom, frequency table

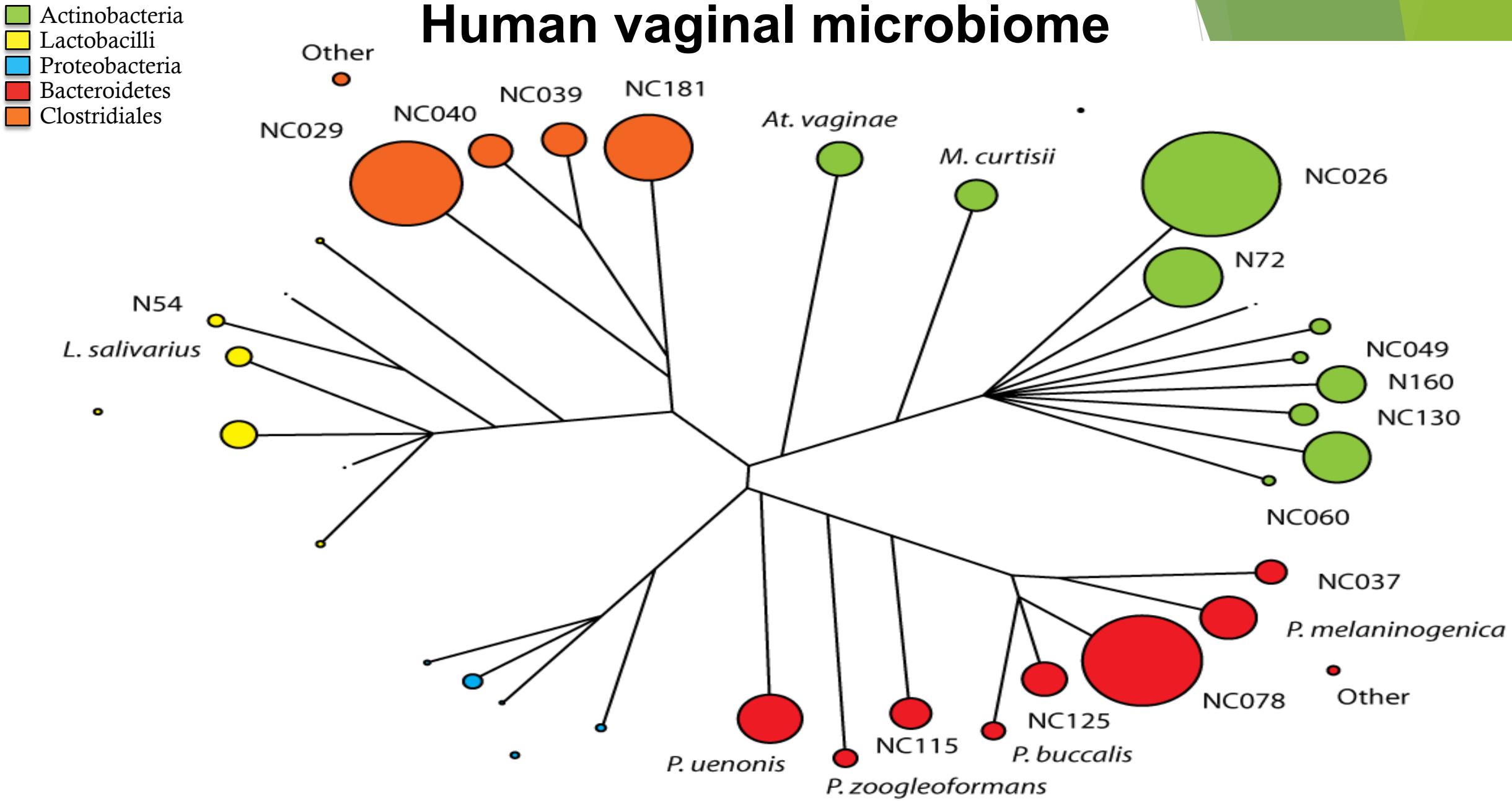
Human vaginal microbiome



Schellenberg, Links, Hill, Dumonceaux *et al.* 2010.

Normal

Human vaginal microbiome



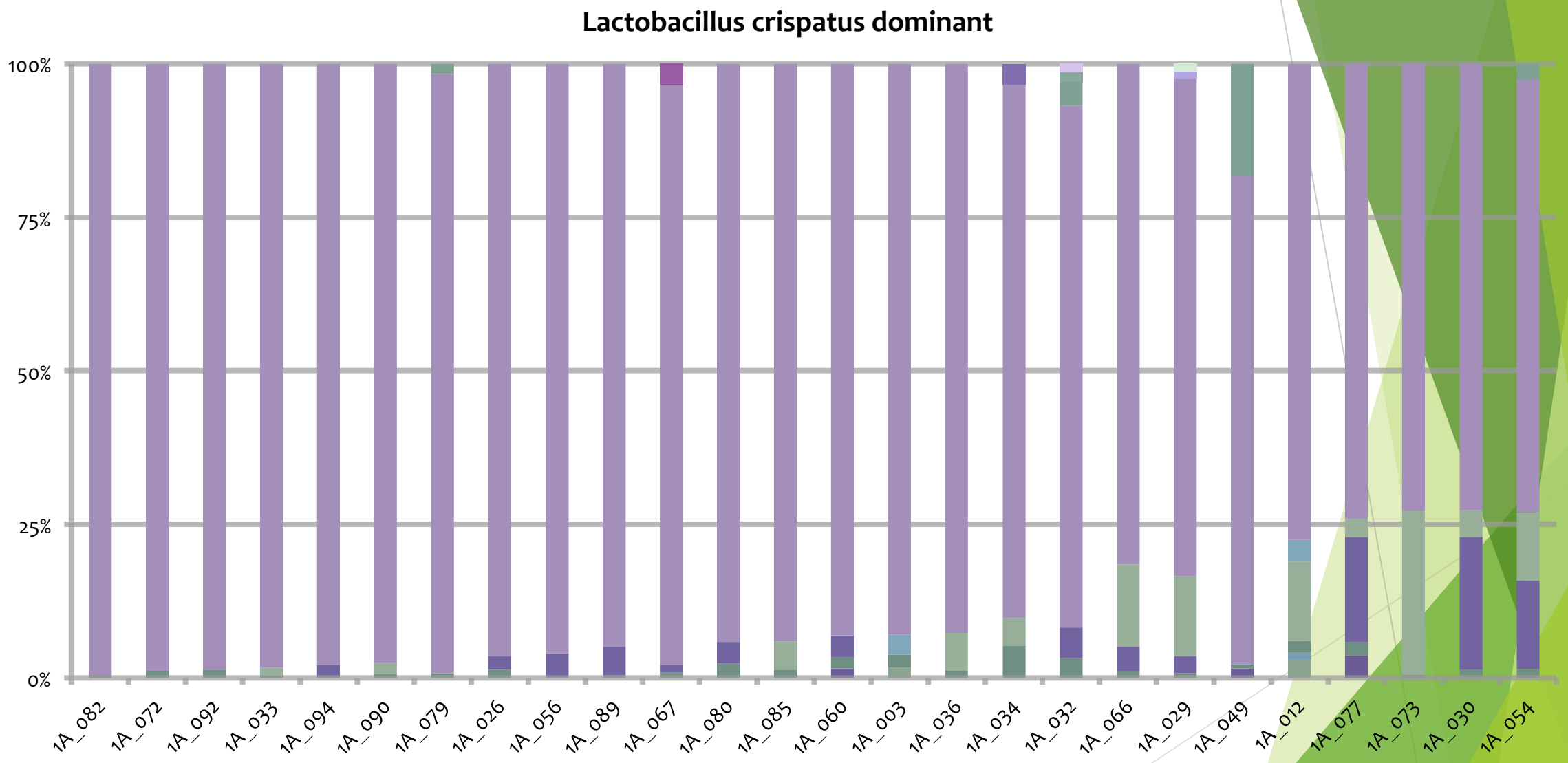
Schellenberg, Links, Hill, Dumonceaux *et al.* 2010.

Bacterial vaginosis

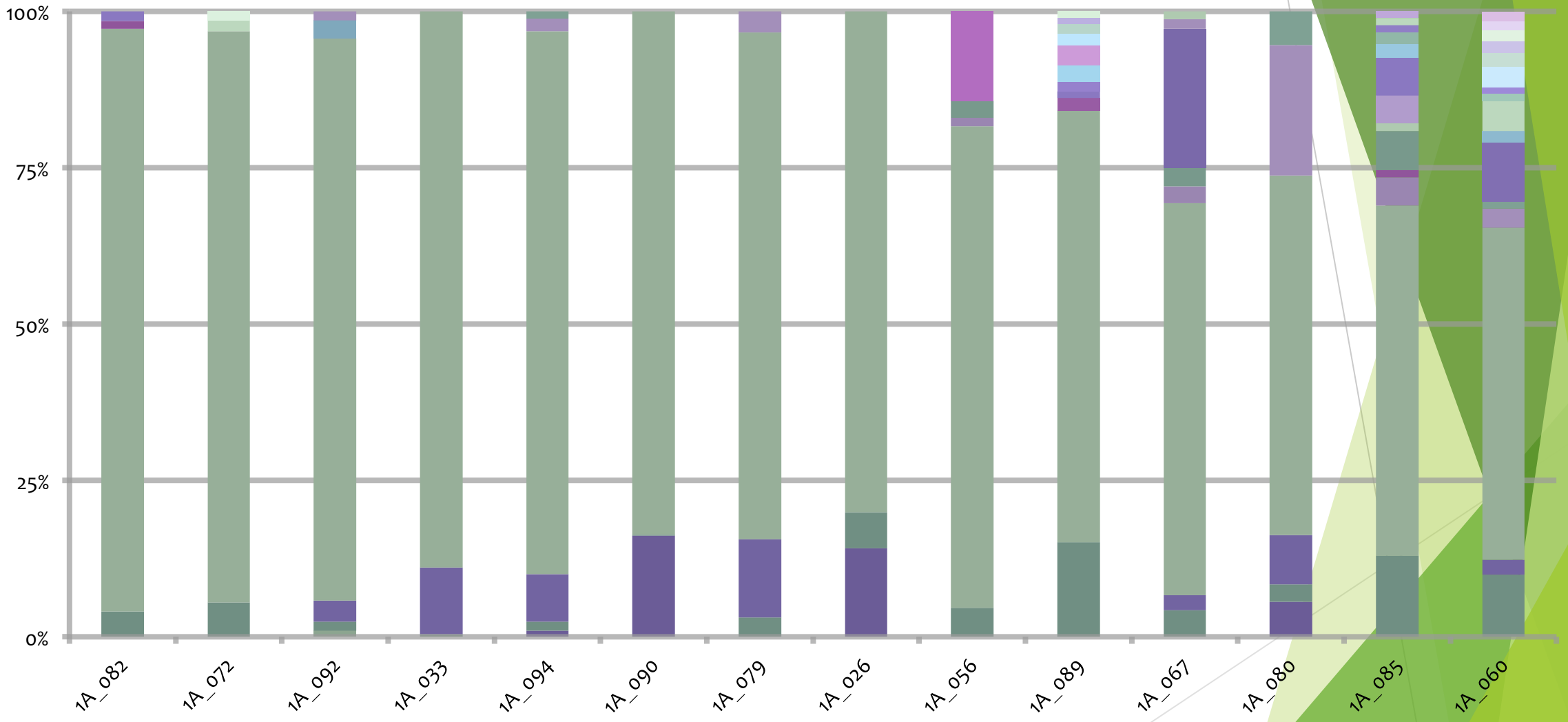
Community State Types (Money, Hill et al, submitted PlosOne)

CST	N (%)	Median Nugent Score	Shannon Diversity Index	Dominant OTU (Prevalence)
I	153 (49%)	0	0.96 ± 0.07	*L crispatus* (100%)
II	13 (4%)	0	1.09 ± 0.18	*L gasseri* (100%)
III	49 (16%)	0	1.17 ± 0.1	*L iners* (100%)
IVA	19 (6%)	2	2.48 ± 0.35	*Bifidobacterium* spp (45%), *Prevotella* spp (79%), *Atopobium vaginae* (53%), Proteobacteria (89%)
IVB	31 (10%)	8	2.26 ± 0.12	*Gardnerella vaginalis* A (100%) *Megasphaera genomsp.* (94%), *Prevotella timonensis* (87%), BVAB3 (74%)
IVC	**20 (6%)**	**5**	**1.71 ± 0.15**	***Gardnerella vaginalis* B (100%)**
V	25 (8%)	0	1.55 ± 0.15	*L jensenii* (100%)

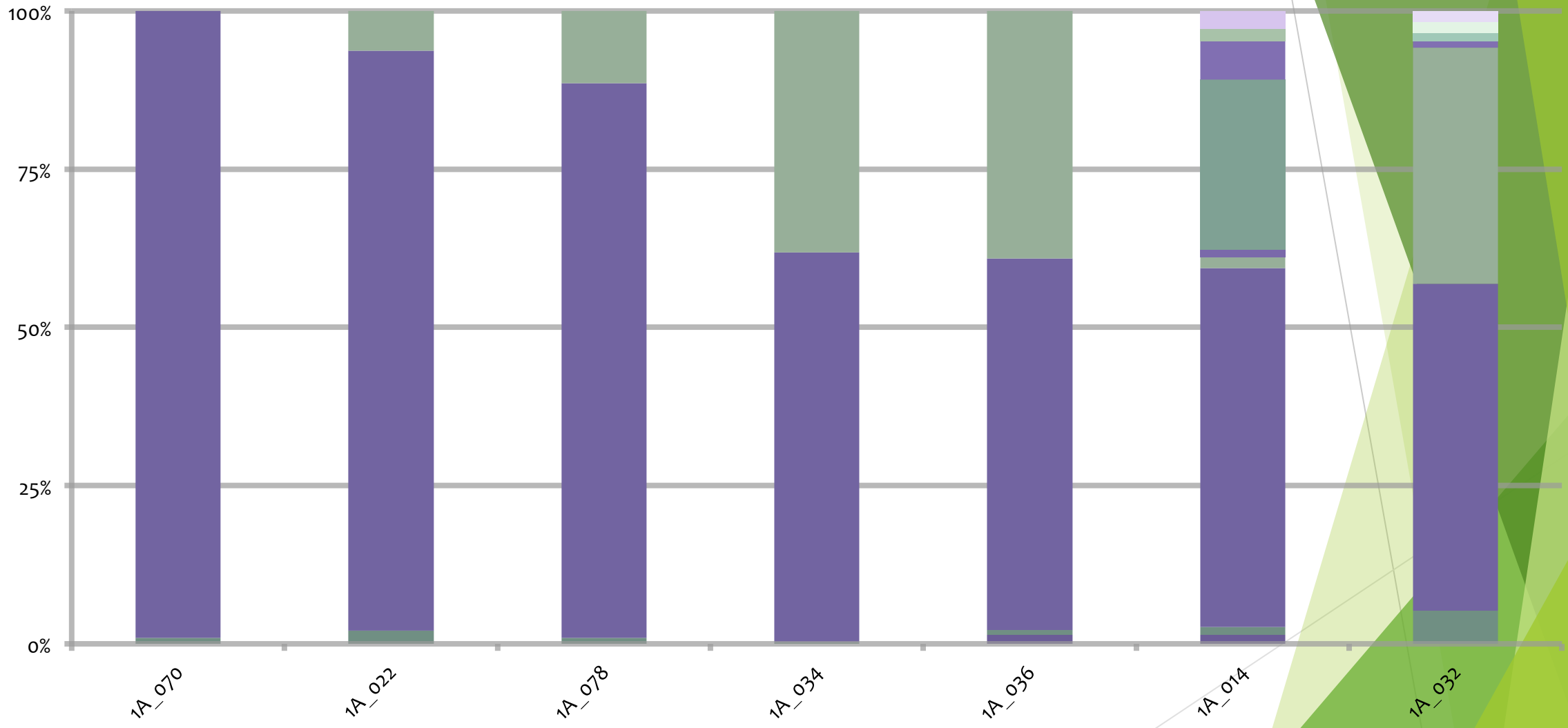
Examples of healthy women's profiles:

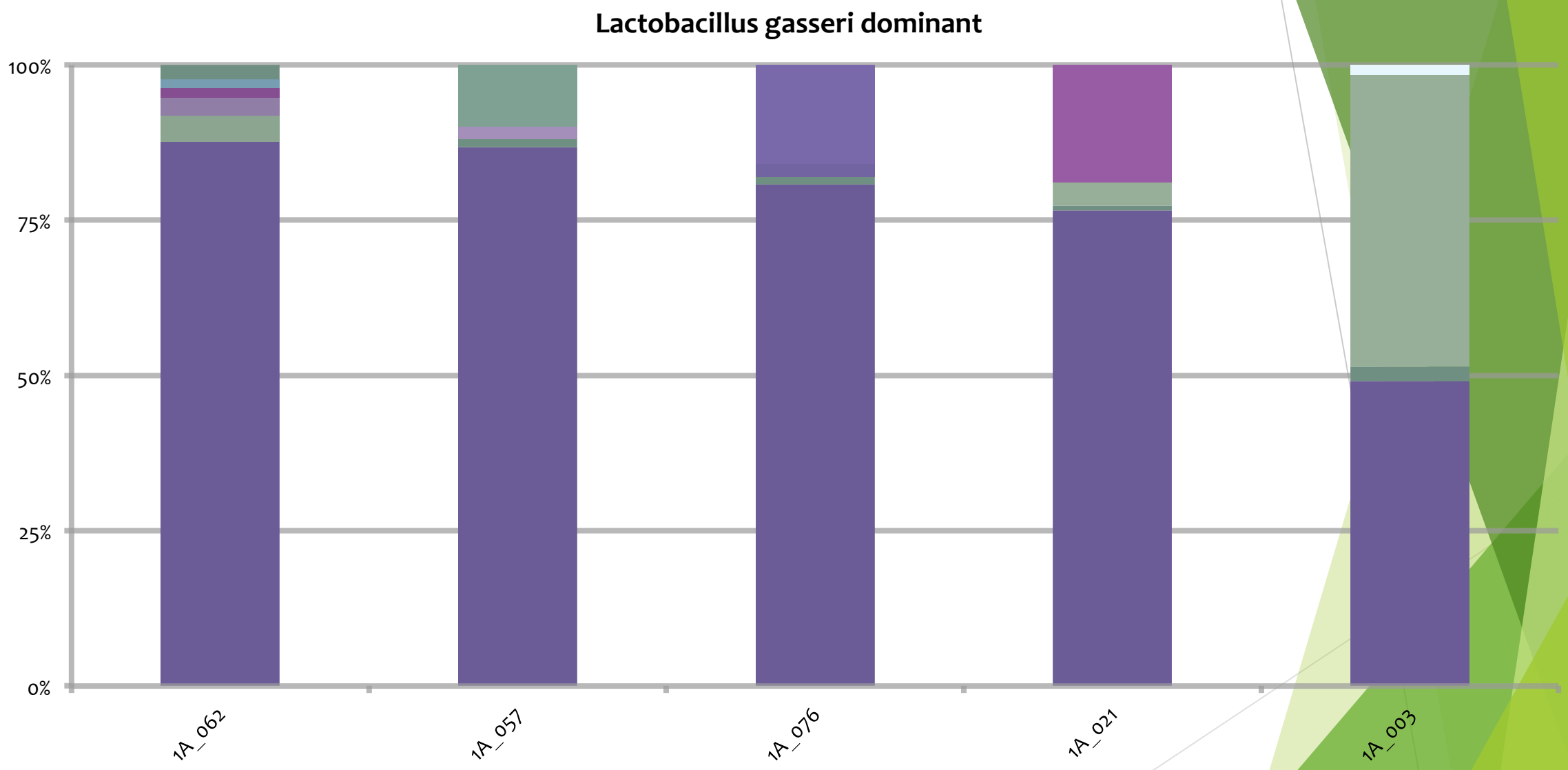


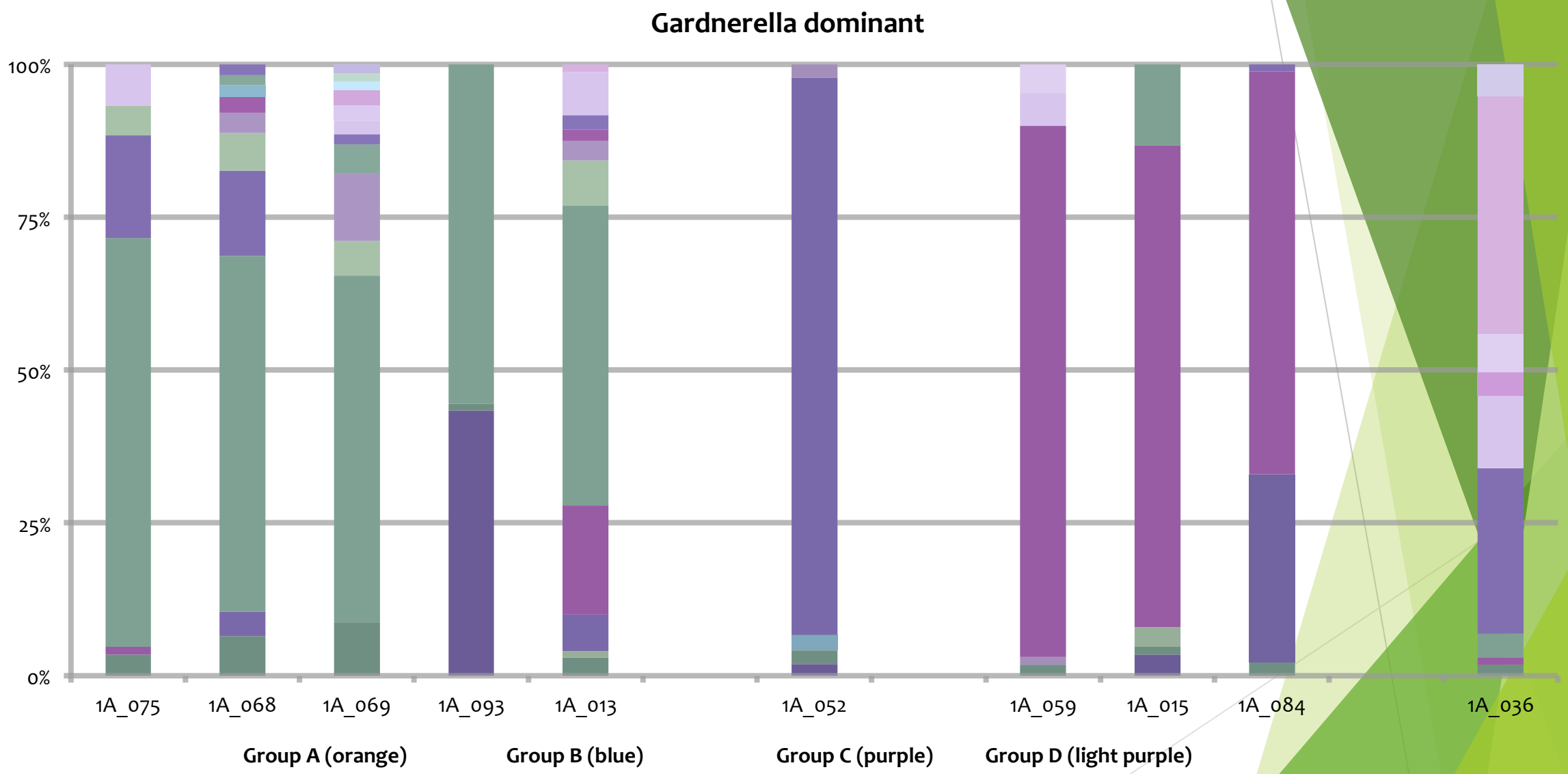
Lactobacillus iners dominant

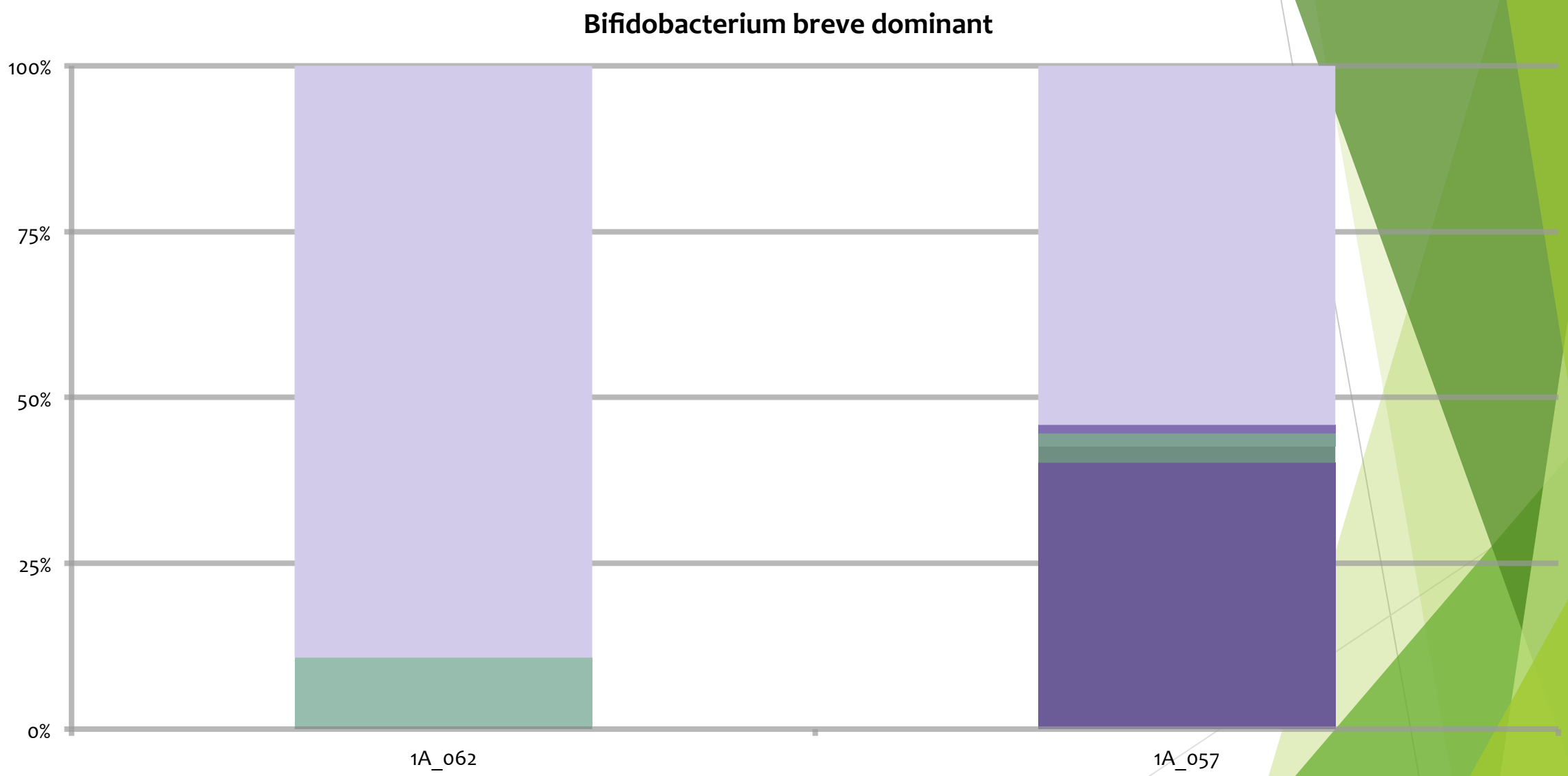


Lactobacillus jensenii dominant



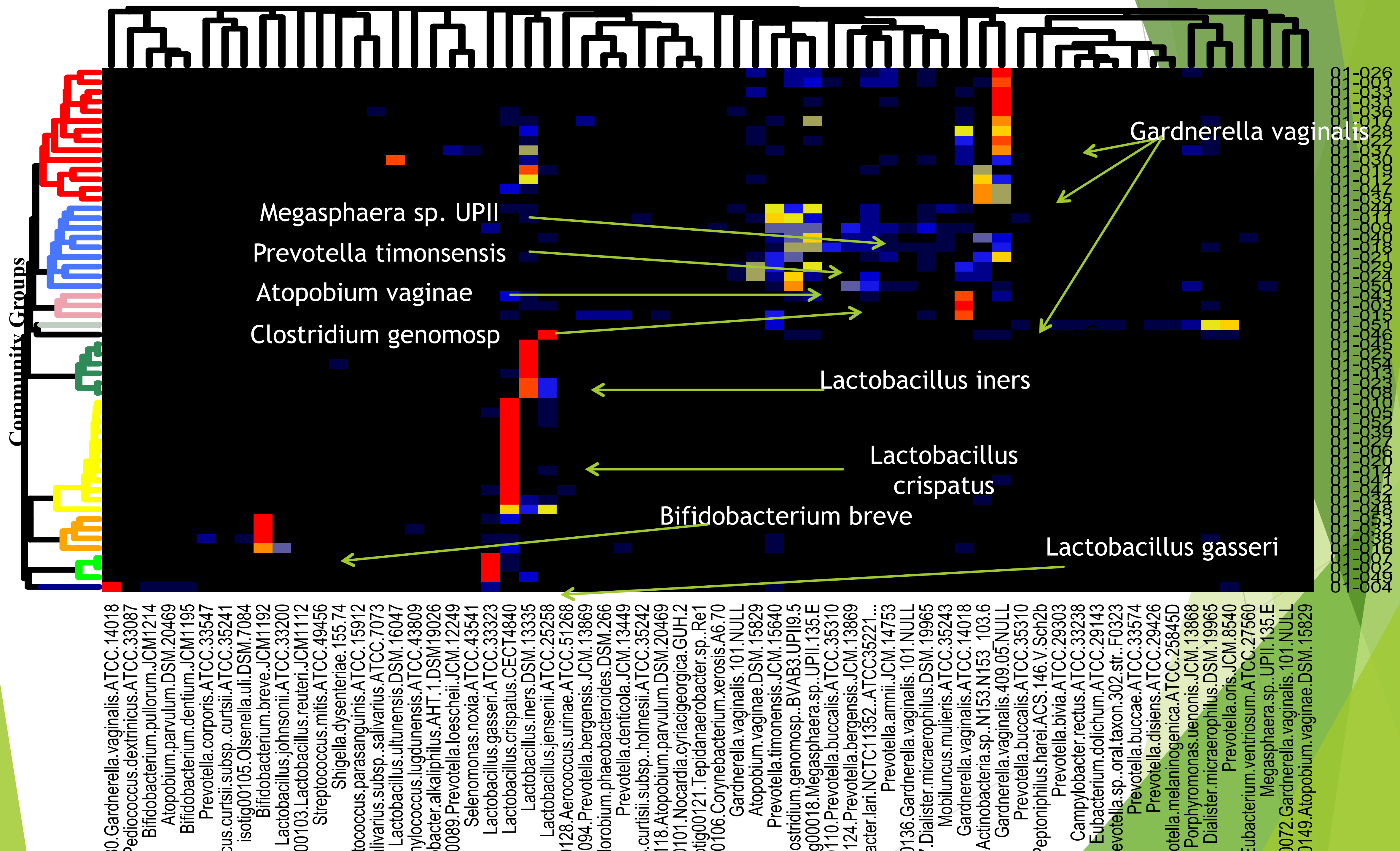

Lactobacillus gasseri dominant
100%
75%
50%
25%
0%
1A_062
1A_057
1A_076
1A_021
1A_003


Gardnerella dominant
100%
75%
50%
25%
0%
1A_075
1A_068
1A_069
1A_093
1A_013
1A_052
1A_059
1A_015
1A_084
1A_036
Group A (orange)
Group B (blue)
Group C (purple)
Group D (light purple)


Bifidobacterium breve dominant
100%
75%
50%
25%
0%
1A_062
1A_057

Characteristics of vaginal microbiome of HIV positive women

- HIV-positive women have a higher prevalence (> 30%) of bacterial vaginosis (BV) compared to HIV-negative women (Atashili *et al.* 2008)
- HIV-positive women with BV have higher genital tract HIV load (Cu-Uvin *et al.* 2001)
- Significant association between decreased vaginal lactobacilli and increased genital HIV RNA levels (Sha *et al.* 2005) and low CD4 count (Mane *et al.* 2012)

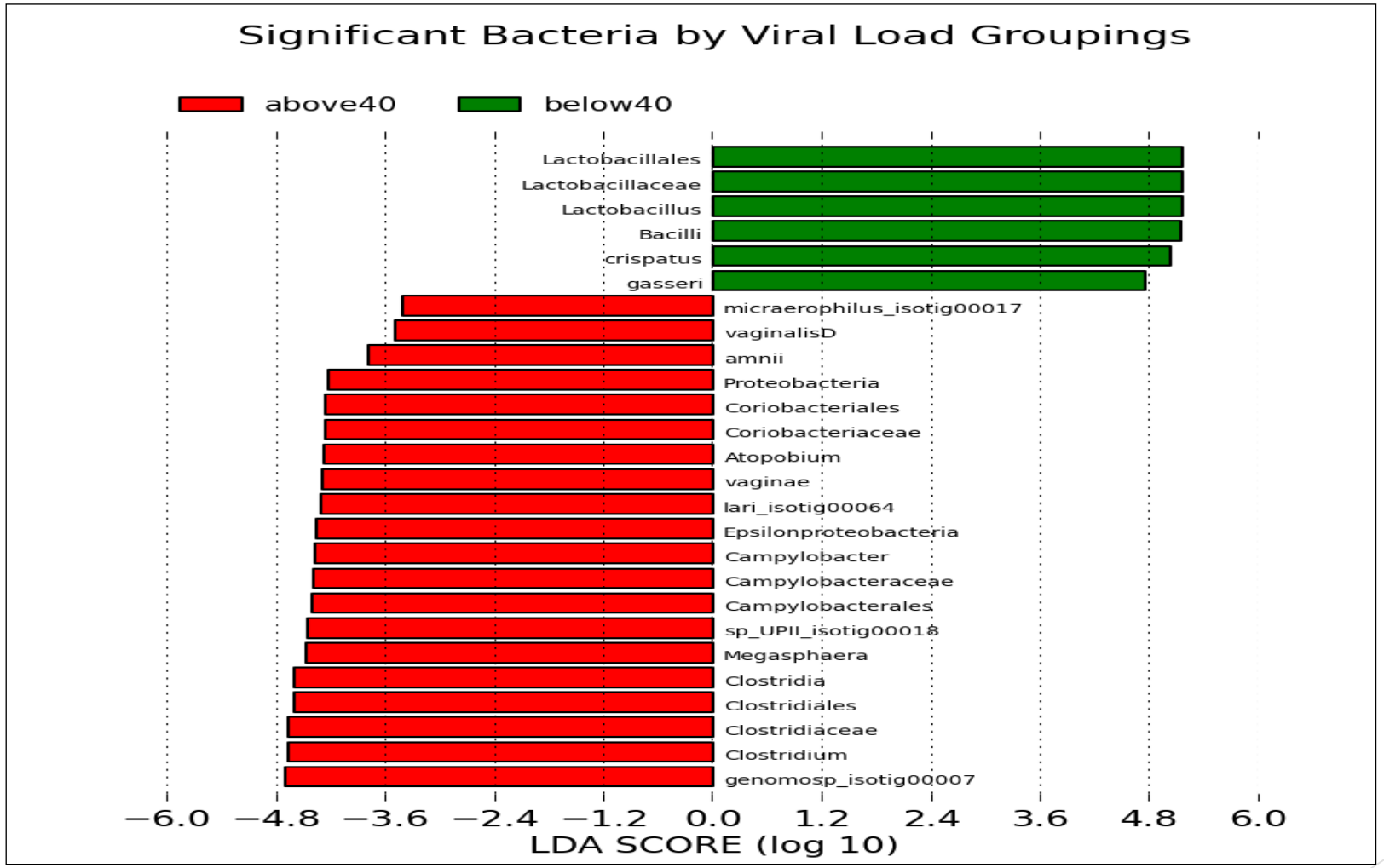


Community Groups
Megasphaera sp. UPII
Prevotella timonsensis
Atopobium vaginae
Clostridium genomosp
Gardnerella vaginalis
Lactobacillus iners
Lactobacillus crispatus
Bifidobacterium breve
Lactobacillus gasseri

Bacterial Taxa Diversity in HIV positive women

- Atopobium parvulum
- Atopobium vaginae
- Bifidobacterium breve
- Bifidobacterium dentium
- Bifidobacterium pullorum
- Campylobacter rectus
- Dialister micraerophilus
- Eubacterium dolichum
- Gardnerella vaginalis A, B, C, D
- isotig00007 Clostridium genomosp.
- isotig00017 Dialister micraerophilus
- isotig00018 Megasphaera sp. UPII
- isotig00030 Gardnerella vaginalis C
- isotig00064 Campylobacter lari
- isotig00072 Gardnerella vaginalis D
- isotig00089 Prevotella loescheii
- isotig00093 Mobiluncus curtsii
- isotig00094 Prevotella bergensis
- isotig00096 Prevotella sp. oral
- isotig00101 Nocardia cyriacigeorgica
- isotig00102 Chlorobium phaeobacteroides
- isotig00103 Lactobacillus reuteri
- isotig00105 Olsenella uli
- isotig00106 Corynebacterium xerosis
- isotig00110 Prevotella buccalis
- isotig00118 Atopobium parvulum
- isotig00119 Dethiobacter alkaliphilus
- isotig00121 Tepidanaerobacter sp. Re1
- isotig00124 Prevotella bergensis
- isotig00128 Aerococcus urinae
- isotig00136 Gardnerella vaginalis D
- isotig00140 Eubacterium ventriosum
- isotig00149 Atopobium vaginae
- Lactobacillus crispatus
- Lactobacillus gasseri
- Lactobacillus iners
- Lactobacillus jensenii
- Lactobacillus johnsonii
- Lactobacillus ultunensis
- Megasphaera sp. UPII
- Mobiluncus curtisii
- Mobiluncus mulieris
- Pediococcus dextrinicus
- Peptoniphilus harei
- Porphyromonas uenonis
- Prevotella amnii

Bacteriome by HIV viral suppression

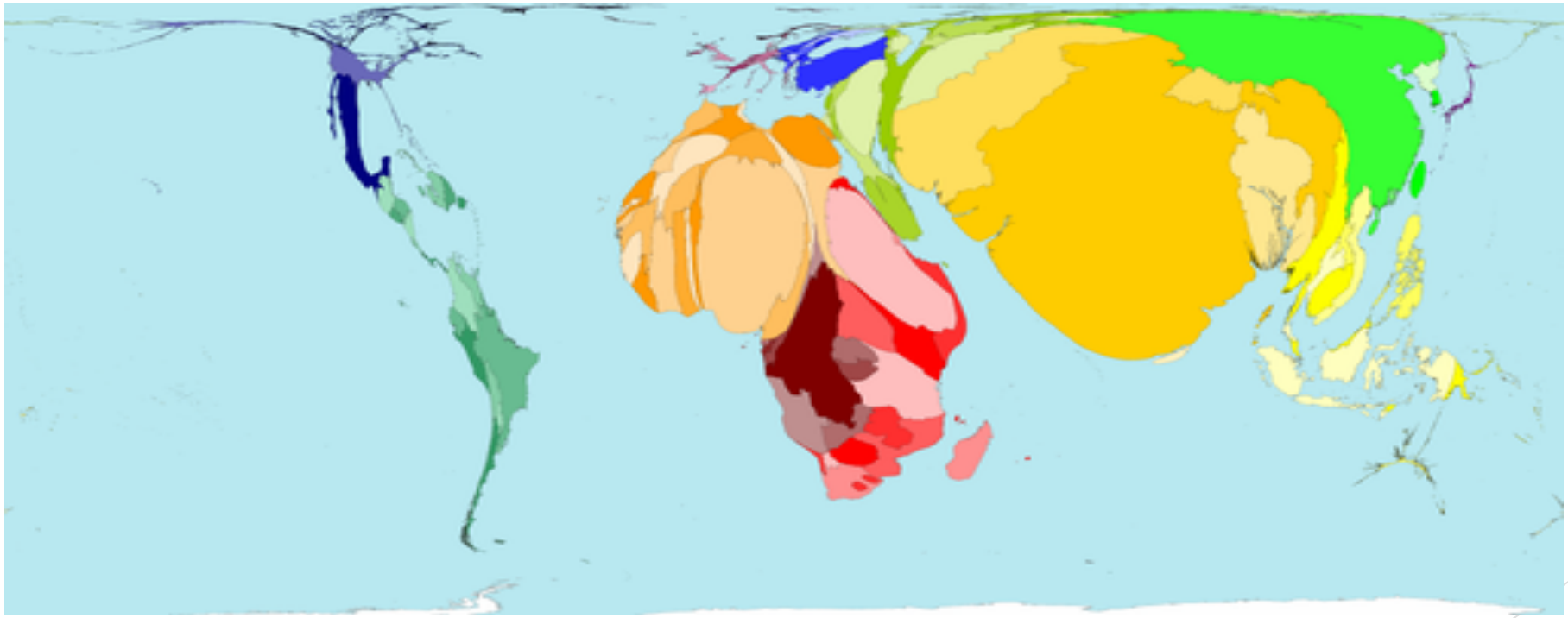


LDA Scores of Significant Bacteria by Viral Load Groupings ($p<0.02$ for all species)

Preterm delivery

- 7.1% of all deliveries are preterm
  - 24,000 per year in Canada
- Increasing in Canada (higher in US at 13% and rising) – NICU crisis
- Accounts for 75% of neonatal morbidity and mortality including long term neurologic morbidity
- Cost to the health care system: $1-3 billion per year in Canada

Infant mortality associated with preterm birth globally



Causes of preterm birth

- 30% indicated births – maternal or infant disease
- 40-50% due to idiopathic preterm birth – related to infection/inflammation
- 15-30% secondary to PPROM

- i.e. up to 55-80% due to dysbiosis

Intrauterine Infection

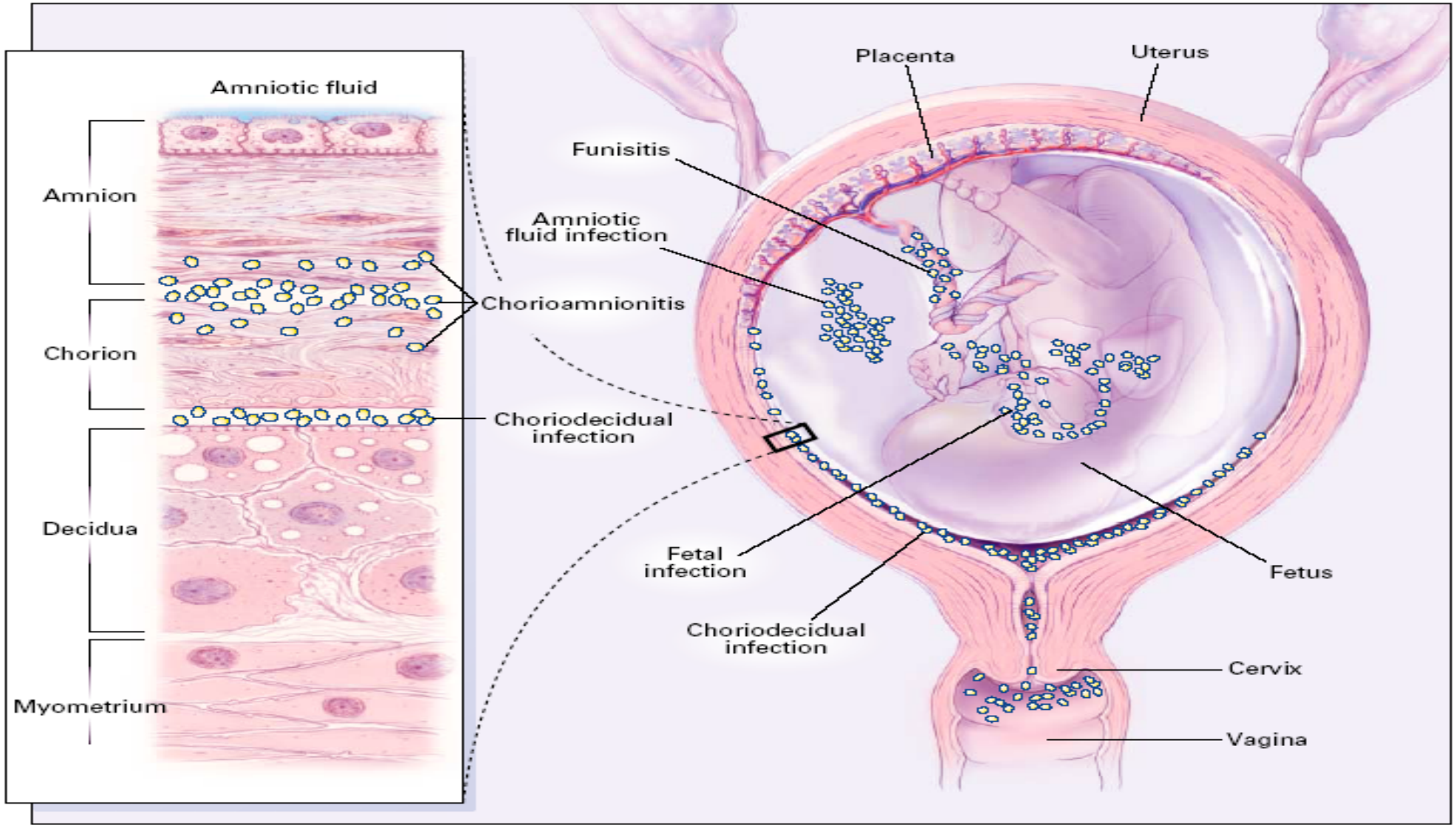


Figure 1. Potential Sites of Bacterial Infection within the Uterus.

Goldenberg et al. *NEJM*. 2000; 342: 1500-7.

Consequences of Chorioamnionitis

- More common w/ maternal BV
- ~15-30% of women with PPROM
- Neonatal complications more common in preterm infants
  - Neonatal sepsis, pneumonia, respiratory distress
- Severe neonatal morbidity with PPROM
  - 55% cases of PPROM + chorioamniotis vs 18% cases of PPROM alone
- Neurodevelopmental delay and cerebral palsy

Ramsey et al. 2005. Am J Obstet Gynecol.192:1162-6; Aziz et al. 2009. J Matern Fetal Neonatal Med. 22: 780-784;
Gaudet et al. 2005. Obstet Gynecol Surv 56:433-6.

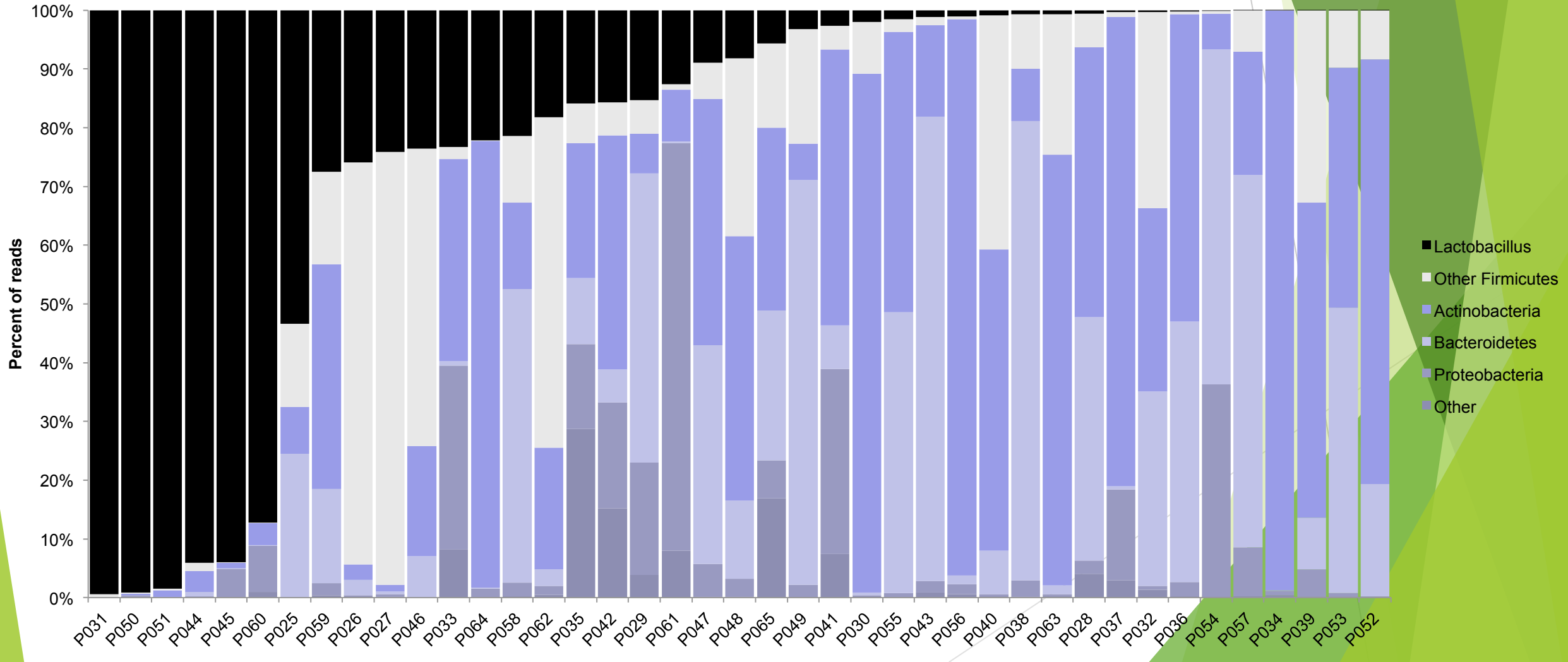
BUT prevention of PTB by screening and treating Bacterial vaginosis to date has failed – WHY?

- Wrong test applied at the wrong time
- Women at high risk not being properly selected
- Not able to detect all culprit microorganisms
- Totally unsophisticated methods to test shifts in bacteria associated with treatment?
- Treating all women as if they are the same!

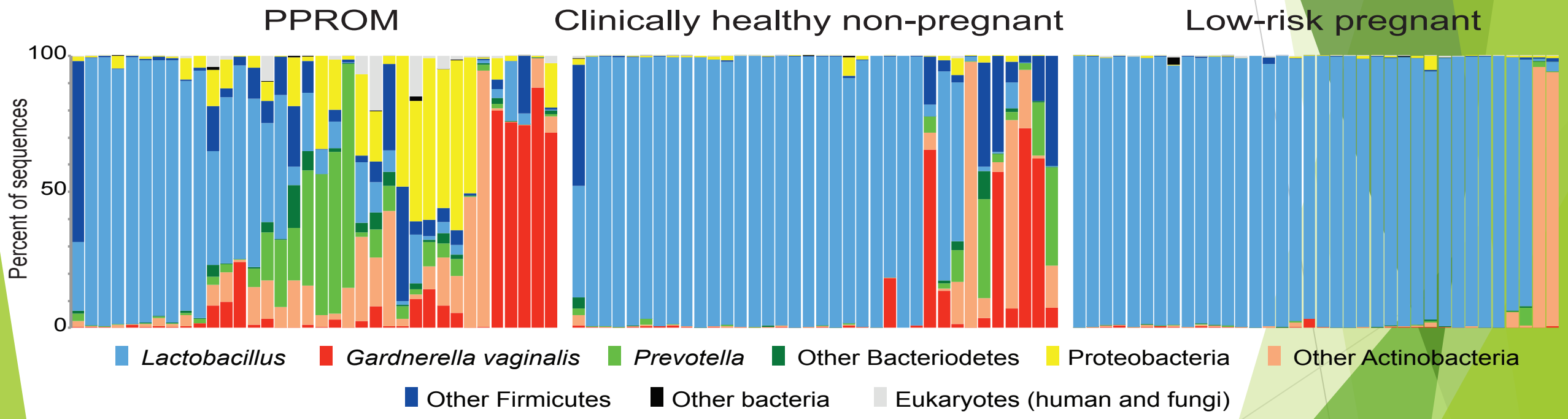
PPROM study in BC population (March of Dimes funded)

* Prospective enrollment of women at 24+0-33+6 with idiopathic PPROM
* Enrolled 52 women
* Women were consented, vaginal swabs taken at admission/time of PPROM diagnosis, weekly then at delivery and the neonate is followed for outcomes
* We hypothesized that specific microbiome clusters would correspond with latency and infectious/inflammatory morbidity in infants

Most PPROM profiles are not dominated by *Lactobacillus* and are highly diverse



Vaginal microbial profiles – overview
CPN60 based data



Summary

- Preterm birth rates are increasing with no successful approaches to prevention
- A healthy vaginal microbiome can protect against adverse pregnancy outcome
- Vaginal dysbiosis can result in adverse pregnancy outcome
- Metagenomics presents a novel new approach to understand the microbiome and the potential for new screening and prevention approaches

The future of Personalized Medicine for women

- Personalized medicine offers the opportunity for closing the equity gap in health care with tailored specific diagnostics and treatments for women
- Metagenomics offers the opportunity for specific and sensitive diagnostics of the vaginal microbiome
- The healthy microbiome is being redefined – we propose a new clustering structure
- Future testing with a simple swab may combine microbiome with HPV and STI screening for efficient specific profiles to predict risk of disease and cancer
- Reproductive success and prevention of prematurity depends on further study into profiles associated with adverse outcomes and strategies defined to modify microbiome profiles.
- Women's health depends on personalized medicine

The Vogue research team

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Thank You

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

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