A comparison of two methods to extract bacterial DNA of the digestive tract

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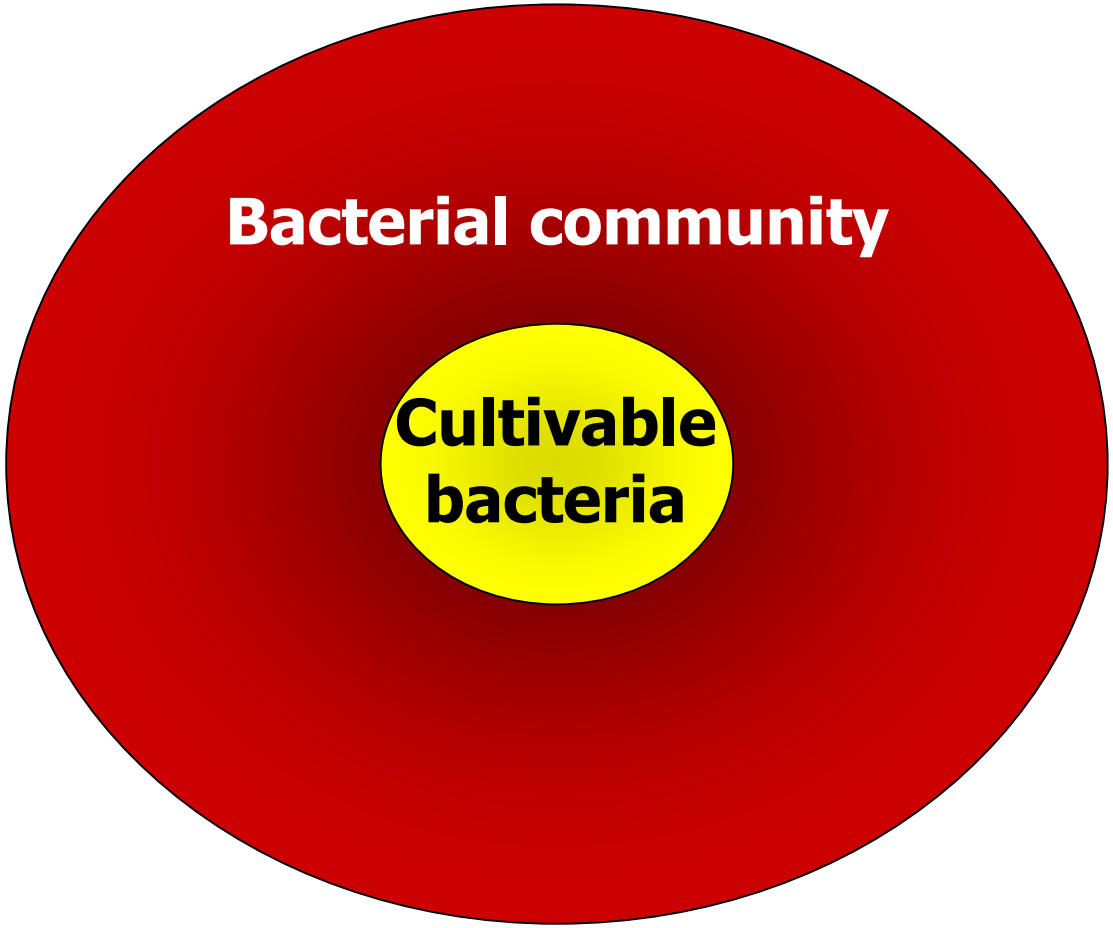
Culture independent analysis (1)



Molecular methods

= culture independent

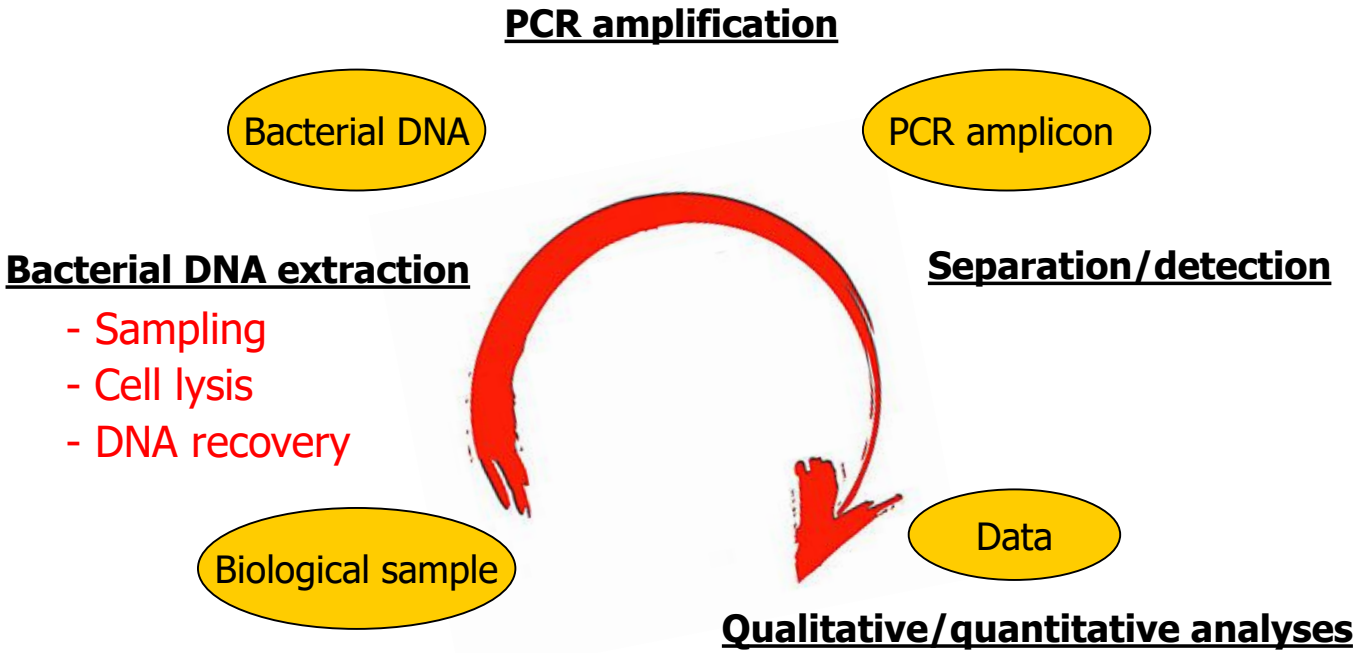
Qualitative data

- Biodiversity
- Evolution of the community

Quantitative data

- Specific groups
- Main species

Culture independent analysis (2)



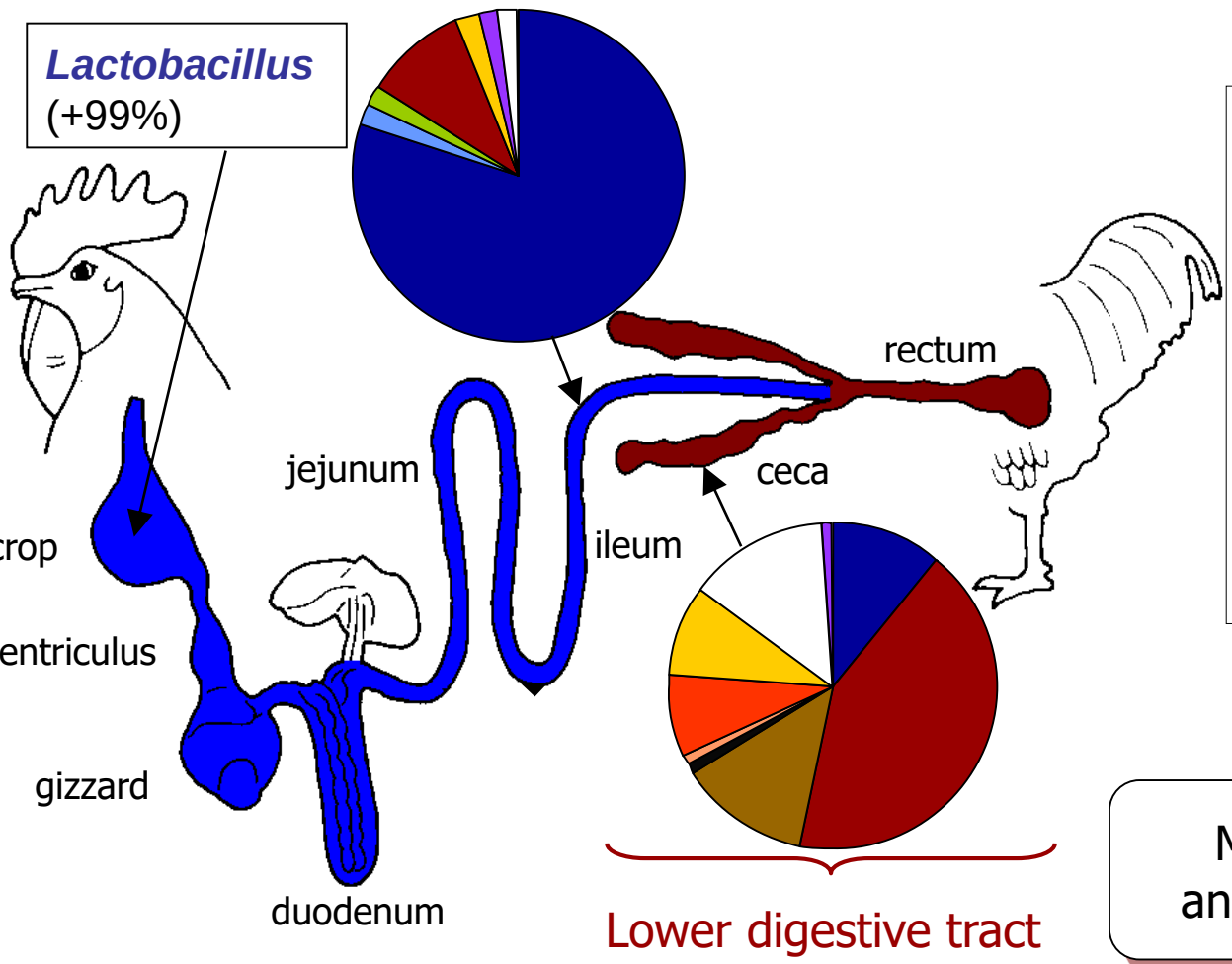
Bacterial DNA extraction can lead to bias (Zoetendal *et al.*, 2001)

Chicken digestive microbiota (lumen)

Mostly aerobes or aerotolerants

Upper digestive tract

Lactobacillus (+99%)



Mostly anaerobes

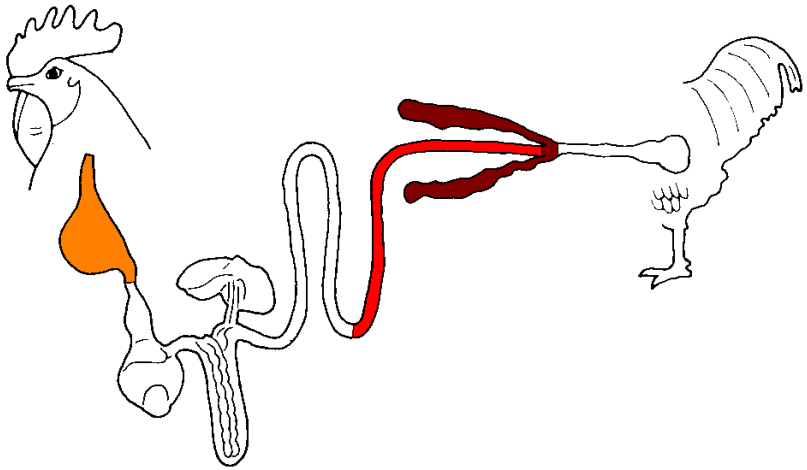
Objective

Comparison of
2 DNA extraction methods for qualitative
and quantitative analyses of chicken
digestive microflora

Bacterial samples

- Samples
  - Animals:
    - Ross PM3 broiler chickens
    - Males
    - 1 week and 6 weeks old

  - Localization:
    - crop, ileum, ceca
    - lumen and mucosa
    - 5 birds pools



DNA extraction methods (1)

- Studied methods:

  - A widely used one: QIAamp® DNA stool (Qiagen)

    With addition of lysozyme to improve lysis of gram+ bacteria

    (Guardia *et al.*, 2009)

  - A new developed one: G'NOME® kit (BIO 1O1)

    With addition of mechanical Lysis to improve wall rupture

    (Furet *et al.*, 2009)

DNA extraction methods

QIAamp

Chemical cell lysis
- ASL buffer
- Lysozyme

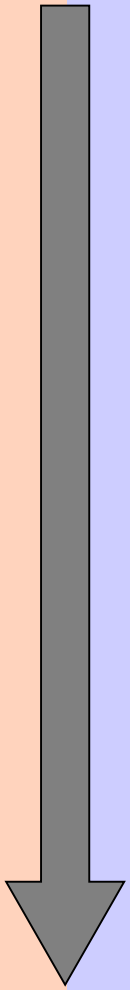
Inhibitors removal
- Inhibitex tablet

Protein hydrolysis
- Proteinase K
- AL buffer

DNA purification
- Silica membrane
- Ethanol
- AW1/AW2 buffer

DNA suspension
- AE buffer

Sample



DNA

G'NOME

Chemical cell lysis
- Cell lysis solution

Protein hydrolysis
- Proteinase mix

Mechanical cell lysis
- Bead beater

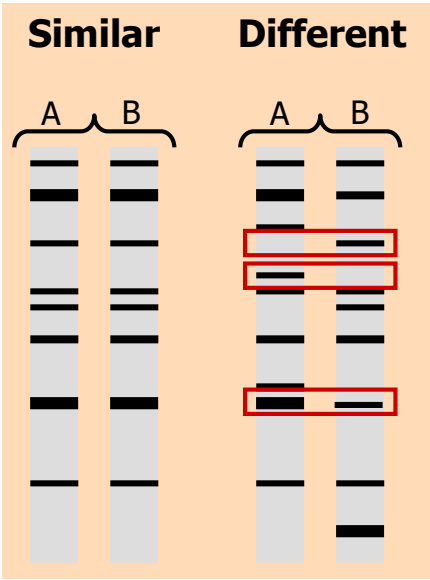
Inhibitors removal
- PVPP

DNA precipitation
- Isopropanol
- H_2O/salt out mix
- Ethanol

DNA suspension
- TE buffer

Comparison of DNA extraction methods

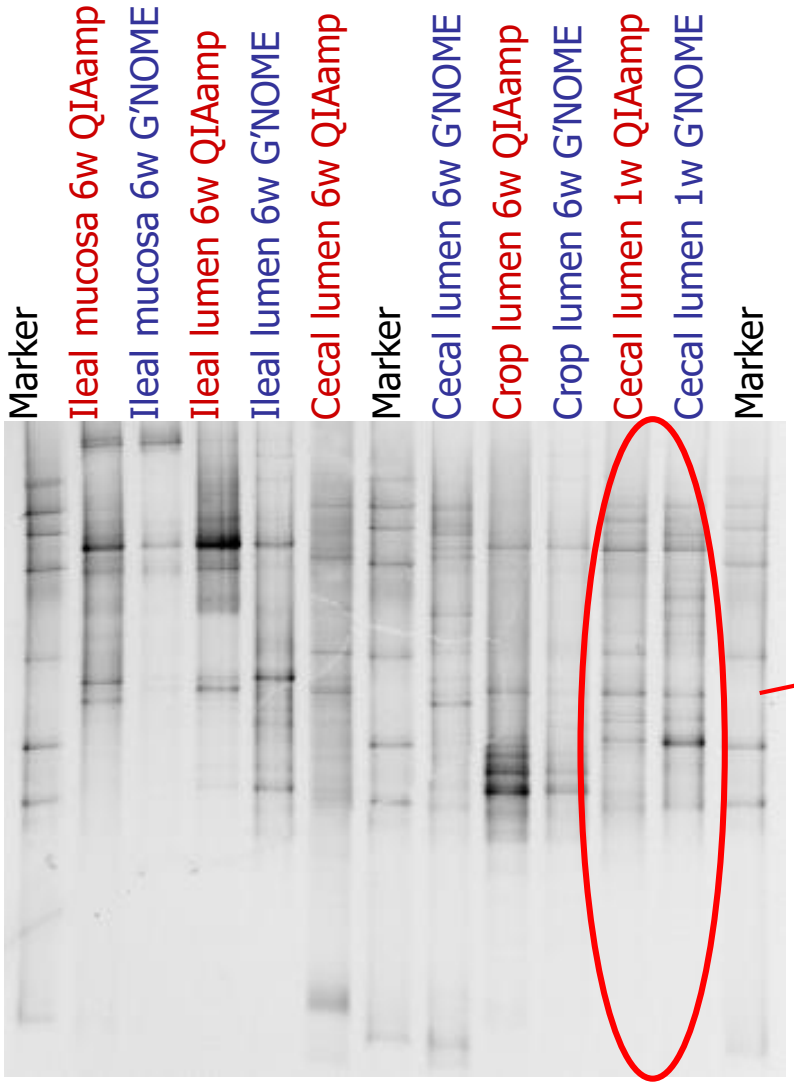
- **Qualitative analysis**
  - **T**emporal **T**emperature **G**radient gel **E**lectrophoresis (TTGE)
  - Amplification with *"all bacteria"* primers
  - Comparison of profiles



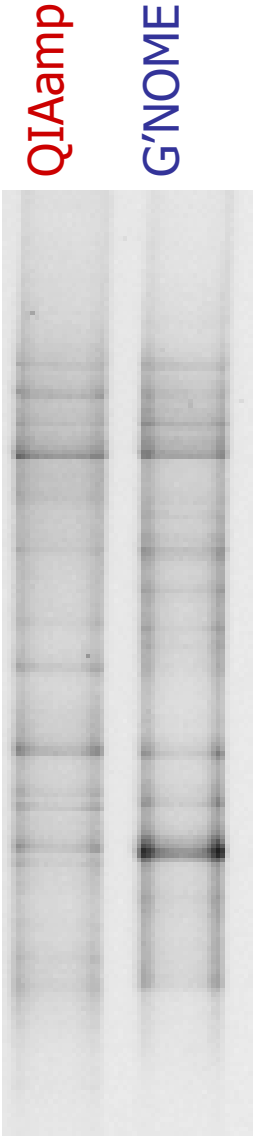
- **Quantitative analysis**
  - Real-time PCR
  - Amplification with *"all bacteria"* primers,

  And *C. leptum, C. coccoides, Bacteroides, Bifidobacterium, Lactobacillus, E. coli* group primers
  - Quantitative data

Qualitative analysis (1)

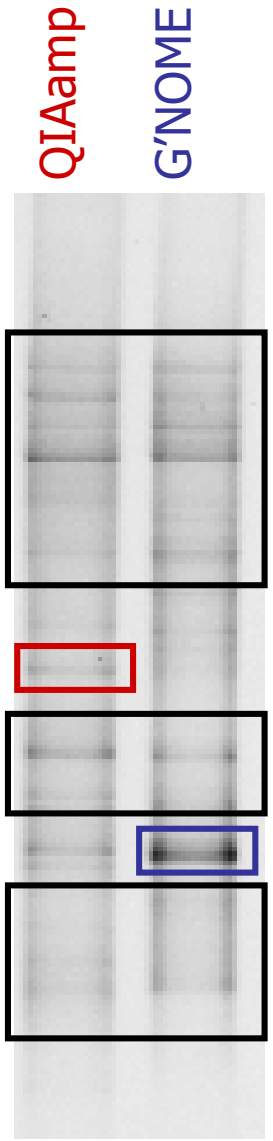


As an example



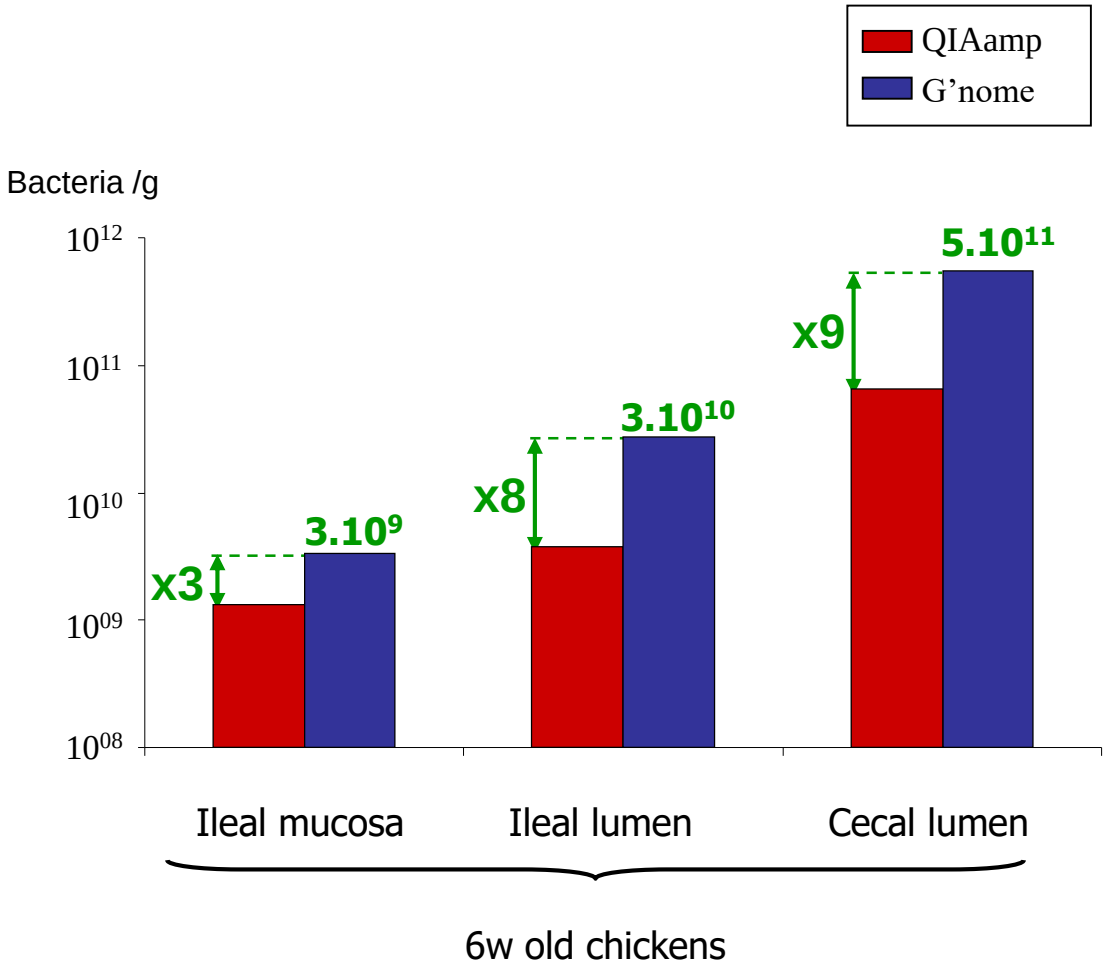
Qualitative analysis (2)

- Majority of bands are present with both methods
- Some amplicons are less intense or absent with the G'NOME one
- Some amplicons are less intense or absent with the QIAamp one



Quantitative analysis (1)

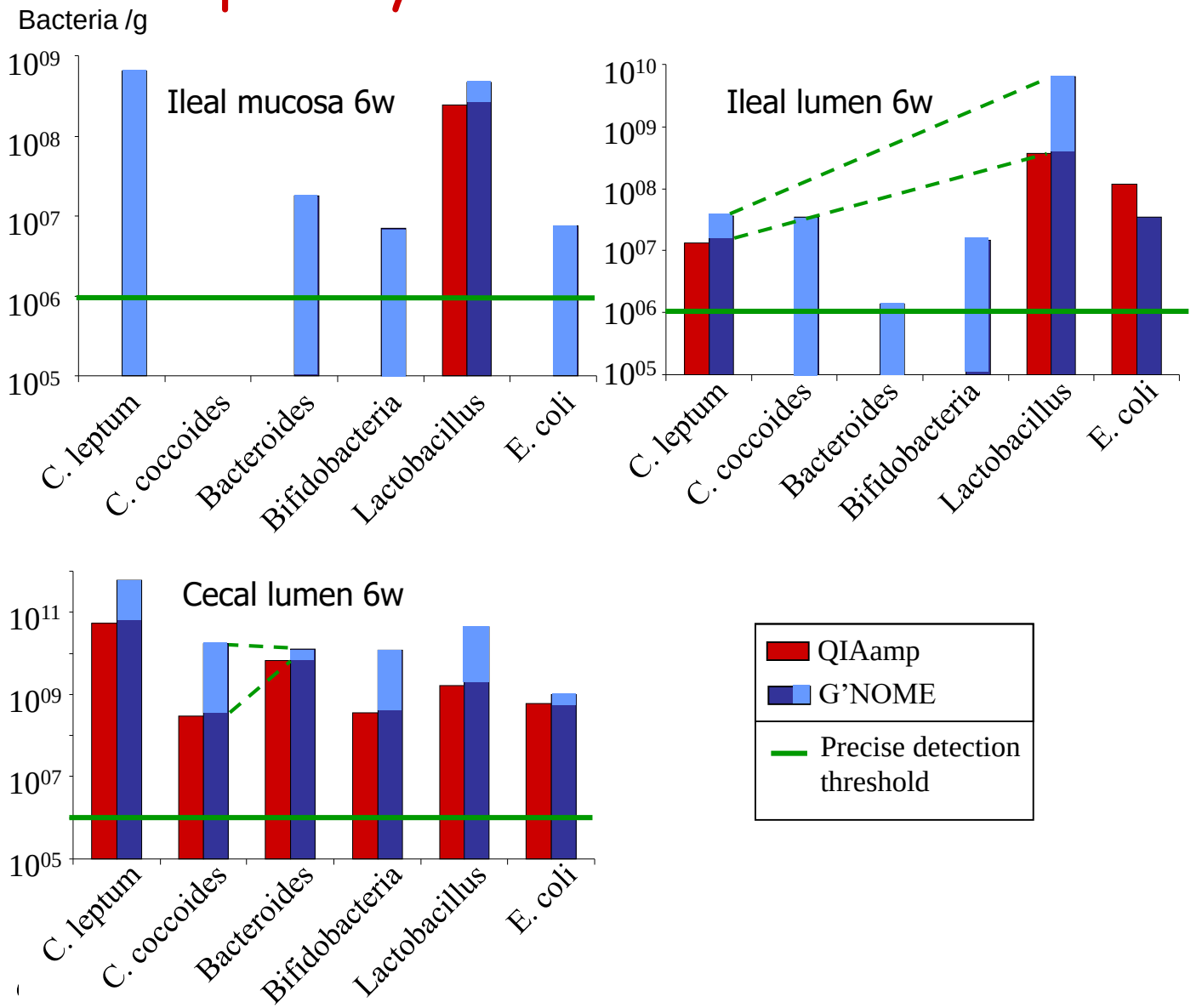
- *"All bacteria" analysis*



- Increased number of bacteria detected with the G'NOME method
- The larger the microbiota, the higher the difference (ileal mucosa vs cecal lumen)

Quantitative analysis (2)

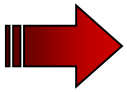
- Group analysis



- Increased number of bacteria detected with the G'NOME method for most groups
- Amplitude of differences between methods varies according to bacterial groups
- Groups, mainly anaerobes, are under detection threshold in ileum with QIAamp

How do they differ ?

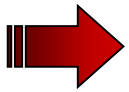
- Qualitative differences between methods
- Quantitative differences vary according to bacterial groups
  - Selective DNA extraction depending on bacterial species



Related on lysis resistance ?

Higher efficiency of mechanical lysis?

- Higher number of '*total bacteria*' is detected with G'NOME, particularly on the largest bacterial communities



Membrane overloading ?

Conclusion

G'NOME method is suitable for quantitative analysis of anaerobic digestive microflora of chicken, especially on upper digestive tract

However

DNA extraction efficiency depends on bacterial species

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Thanks !

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