

Introduction

New insights in gut microbiota in health and disease have triggered an interest in its analysis in routine clinical diagnostics. Current investigational methods are however typically not well suited for this purpose due to high cost or labor intensiveness. We have developed a profiling technique, IS-pro, for high-throughput analysis of the human intestinal microbiota. This new technique combines species identification by 16S-23S interspace length with phylum identification by colour labelling and provides an instant overview of the *Bacteroidetes/Firmicutes* composition of samples. We validated IS-pro *in silico*, *in vitro* and *in vivo*, for colonic mucosal biopsies and feces.

In silico

The potential of IS-pro was evaluated *in silico* with a database containing sequences of 342 bacterial species. With this database we calculated that IS-pro can theoretically discriminate >50.000 bacterial species.

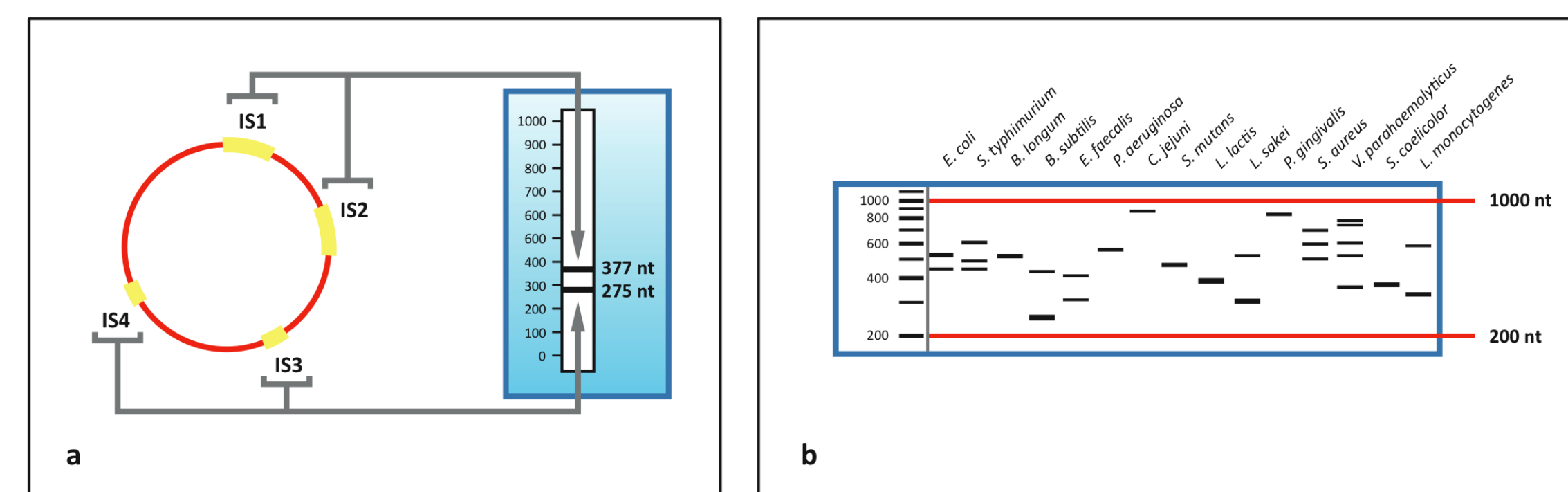


Figure 1a: A bacterial chromosome (red ring) can contain >1 IS region. These regions can have different lengths. *E. faecalis*, for example, has two regions of 377 nucleotides (nt) and two regions of 275nt. Amplification of these regions will result in a profile consisting of two distinct bands. **1b:** In silico profiling of a range of bacterial species illustrates diversity of fragment combinations. Note that fragment lengths are generally between 200 and 1000nt.

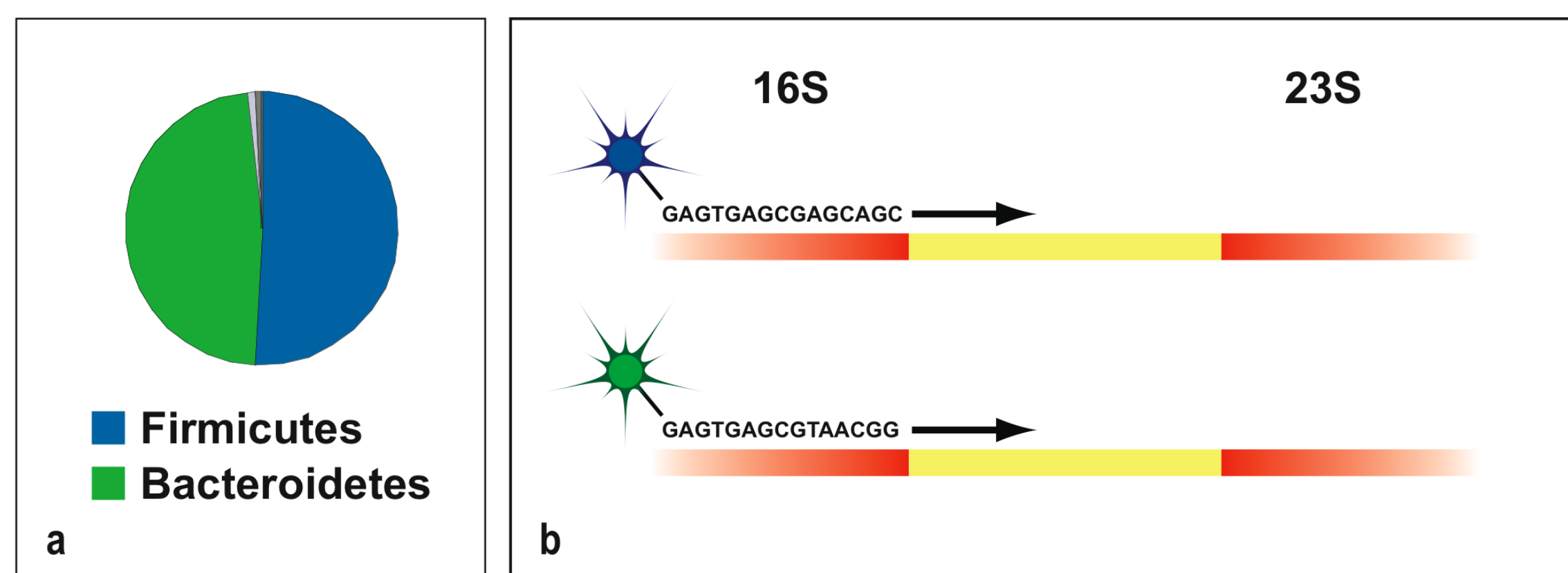


Figure 2a: *Firmicutes* and *Bacteroidetes* are the dominant phyla in the human gut (data shown from Eckburg et al). **2b:** Two different forward primers were designed, each specific for either *Firmicutes* (blue) or *Bacteroidetes* (green)

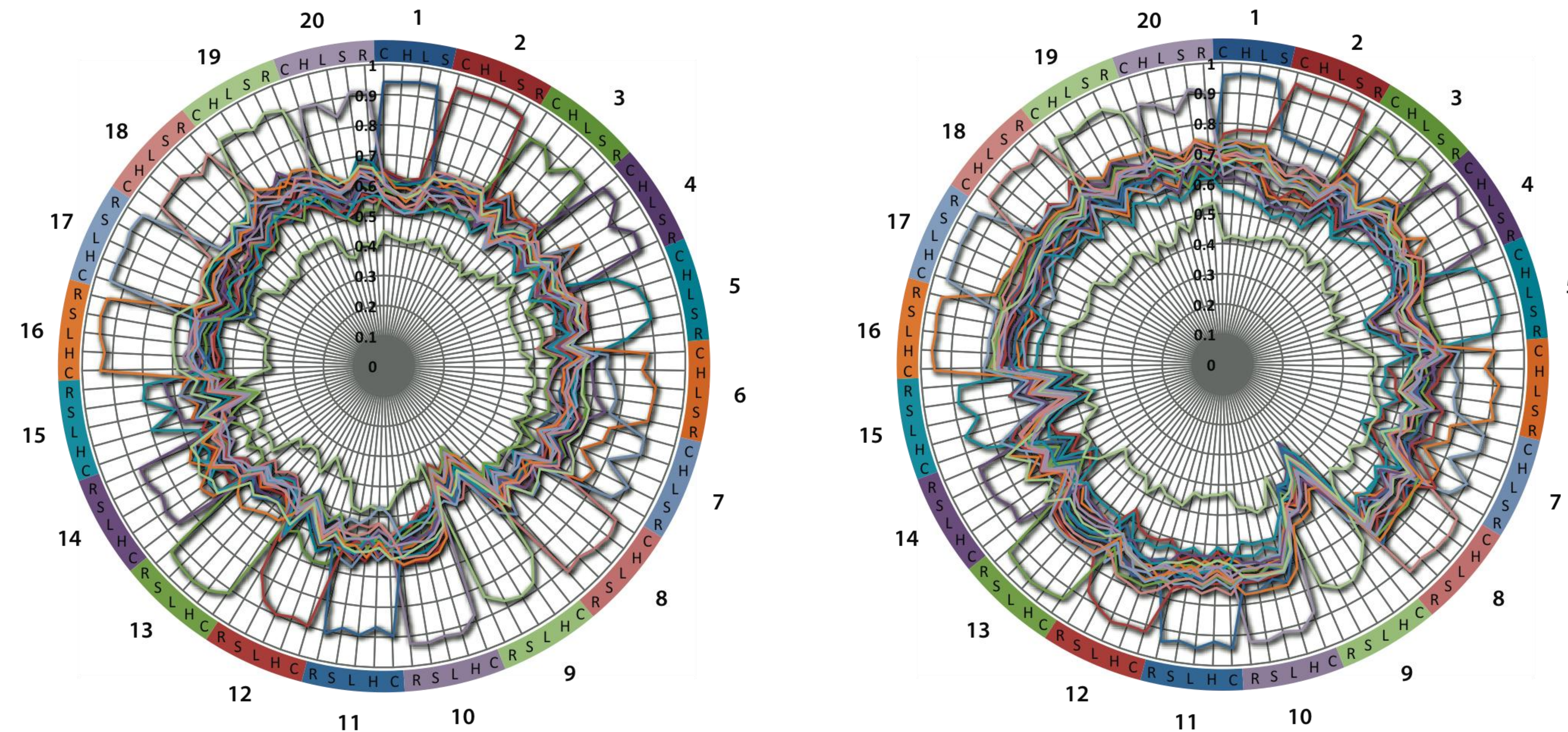


Figure 5: Correlation of profiles of *Firmicutes* (left) and *Bacteroidetes* (right) in colonic mucosal samples of 20 healthy subjects. Radial axis represents correlation. Colours and numbers on circular axis represent subjects, letters represent biopsy location. C=caecum, H=hepatic flexure, L=splenic flexure, S=sigmoid, R=rectum. Line colours correspond to subject colours. Each line represents average correlation of the profile obtained at the location indicated on the circular axis to all profiles of the subject represented on the circular axis. Profiles are subject specific and highly similar throughout the colon. Average intrasubject correlation of profiles is 90% (st.dev 5%) for *Firmicutes* and 92% (st.dev 4%) for *Bacteroidetes*. Average intersubject correlations are 58% (st.dev 8%) for *Firmicutes* and 66% (st.dev 9%) for *Bacteroidetes*. Clear outliers are subject 9, whose profiles correlate markedly less with other subjects, and subject 15, whose *Firmicutes* profiles are not stable along the colon.

In vitro

In vitro validation was performed with mono-cultures and mixes of cultured bacteria. Lower bacterial detection limit was 10 bacteria/μl. No interactions were found between species belonging to the same phylum, nor were interactions found between the phyla *Firmicutes* and *Bacteroidetes*.

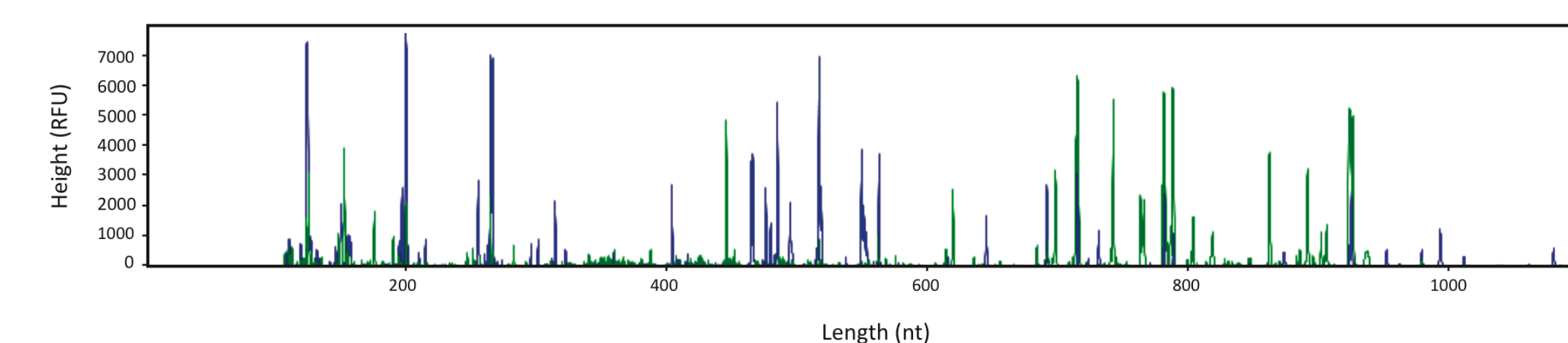


Figure 4: IS-profile of colonic mucosa. Peak length, expressed in nucleotides, corresponds to IS-fragment length. Peak height, expressed in RFU, reflects quantity of fragments. Blue peaks represent *Firmicutes*, green peaks represent *Bacteroidetes*.

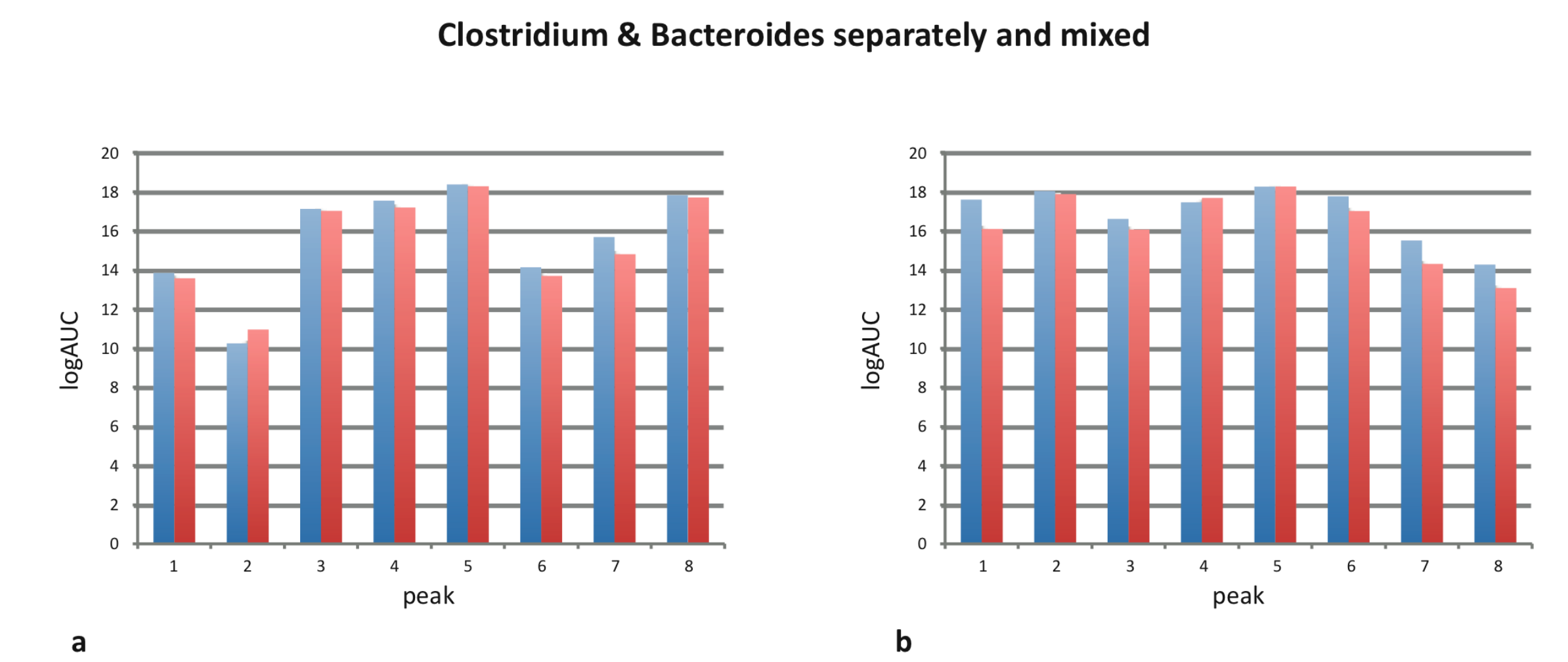


Figure 3a: Log2 transformed peak heights of 8 *Clostridium* peaks. Blue bars are peak heights as measured in a mixture of only *Clostridium* species, red bars represent heights if these same peaks as found in a mixture of *Clostridium* and *Bacteroides* species. **3b:** Same as 2A, now for *Bacteroides* peaks. Blue bars represent peak heights in a mixture of only *Bacteroides*, red bars in a mixture of both genera. No significant change is seen between the two series. This implies that the amplification of *Clostridium* and *Bacteroides* IS fragments is not influenced by the presence of species of the other genus.

In vivo

In vivo validation was performed with 100 colonic mucosal biopsies of 20 healthy individuals obtained from 5 locations throughout the colon: caecum, hepatic flexure, splenic flexure, sigmoid and rectum. Validation on duplicate samples showed excellent reproducibility of IS-pro. A high level of correlation of mucosal samples throughout the colon was identified by IS-pro, a low level of correlation was found between subjects. These data can be used as reference for further clinical analyses

Conclusion IS-pro has a high discriminatory potential
 IS-pro is highly reproducible
 IS-pro is easy to perform, enabling implementation in general microbiological laboratories
 IS-pro can make analysis of the human intestinal microbiota accessible to clinical practitioners.