

## Box 1 | Intestinal microorganisms

Bacteria are the main type of microorganism present in the mammalian intestine, although other types are also found, including protozoa and fungi. The stomach and small intestine have relatively low bacterial densities ( $10^3$ – $10^5$  organisms per gram or ml of luminal contents in mice, consisting mainly of acid-tolerant lactobacilli and streptococci). The distal portion of the small intestine, the ileum, is a transition zone with higher bacterial densities ( $10^8$  per gram) and species diversity, but the most dense colonization is in the colon ( $10^{10}$ – $10^{12}$  per gram), which hosts more than 400 bacterial species. In the lower intestine, anaerobes predominate, particularly the *Bacteroides*, bifidobacteria, fusobacteria and peptostreptococci (each group present at approximately  $10^9$  per gram); by contrast, aerobes and facultative aerobes, including enterobacteria and lactobacilli, are present at only moderate densities ( $10^6$ – $10^8$  per gram).

There are two main difficulties in understanding and measuring these complex flora. First, a comparison of two techniques used to assess faecal bacterial numbers — counting colonies of culturable bacteria and estimating numbers using smears — shows that less than 50% of intestinal bacteria can be cultured. This is because of the precise oxygen requirements of some species and their fastidious (and largely unknown) nutrient requirements. Second, although most measurements have been made using faecal bacteria, the intestine is not a homogeneous environment — groups of bacteria can also exist on the surface of the mucus layer or deep within it.

Fortunately, there are ways of overcoming the difficulties in culturing intestinal bacteria. The 1.5-kb gene encoding 16S ribosomal RNA is present in multiple copies in bacterial chromosomes, and it is highly polymorphic. Therefore, the nucleotide sequence of this gene (obtained after amplification by PCR) can be used to determine the species of each organism<sup>81</sup>, and the gene can serve as a target (in species-specific *in situ* hybridization) for studying the spatial arrangement of each bacterial group.