Microbial Detection and Imaging Tools

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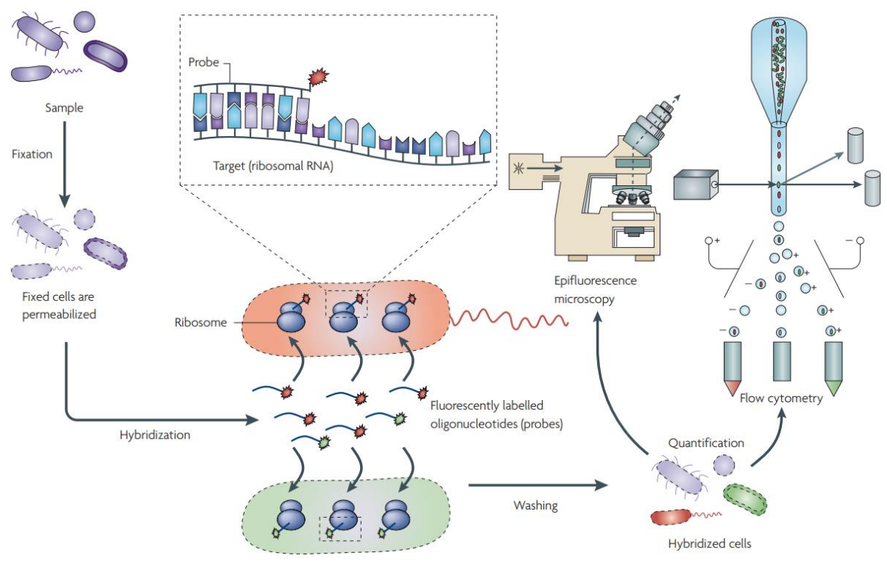
1a Fluorescent In Situ Hybridization (FISH) for Bacterial Detection



Amman R., et al, *Nature Rev.* 2008

Bacterial FISH probes are often primers for the 16s rRNA gene.

These small fluorescently labeled probes (16-20 nucleotides) penetrate the bacterial cell wall and hybridize with the complementary target sequence.

The bacterial sample is then ready for single-cell identification and quantification by either epifluorescence microscopy or flow cytometry.

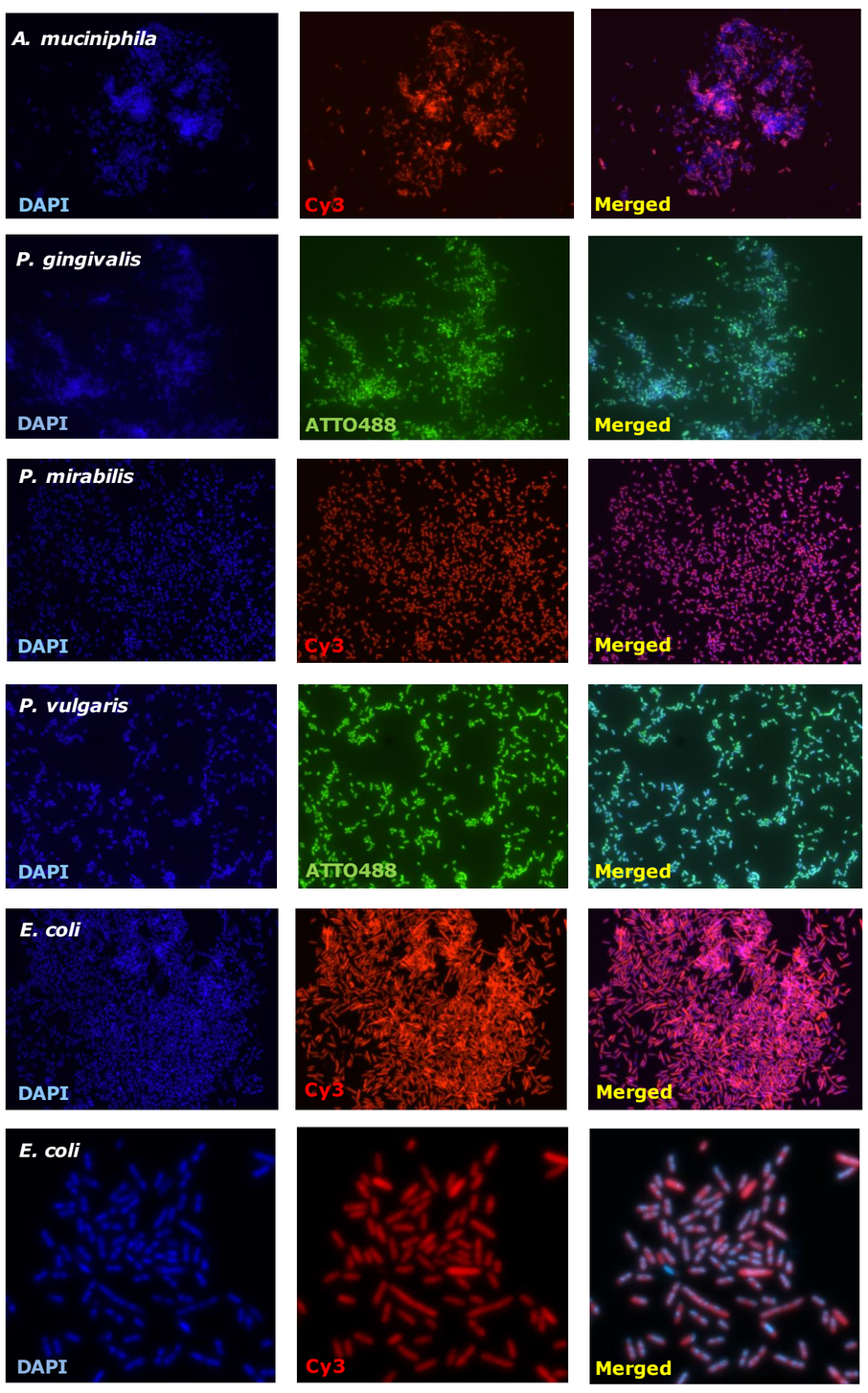
Microbiota is the multi-species community of microorganisms in a specific host habitat or ecosystem.
Microbiome refers to the genetic material of all the microbes reside within the human body (bacteria, viruses and fungi).
These microbe communities act as an essential organ that has extensive functions such as development of immunity, defense against pathogens, host nutrition, host energy metabolism and synthesis of vitamins.

Unraveling host-bacteria interaction and the ability to detect the microbiota is of high significance for promoting the human microbiome research.
Thus, the **FISH technique can serve as a powerful tool to observe native microbial populations including unculturable bacteria in diverse microbiome samples, for example:**

- **Human samples** (e.g. blood and tissue)
- **Microbial ecology** (e.g solid biofilms and aquatic environments)
- **Plants** (e.g root surface and Rhizosphere)

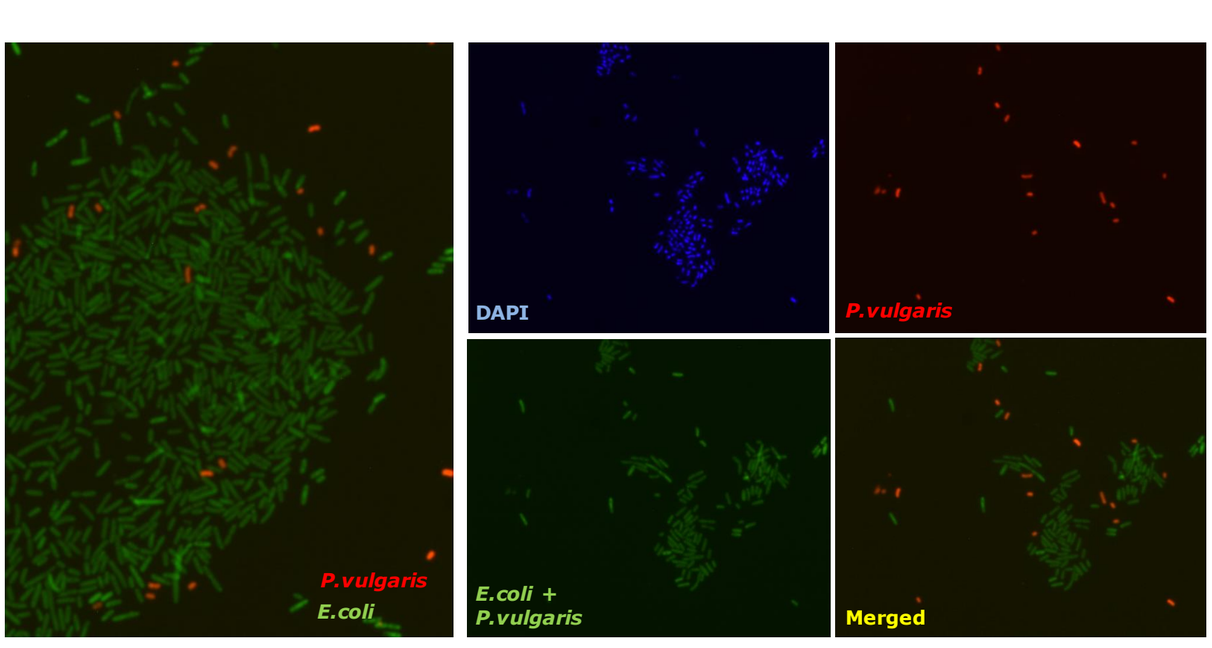
Various modifications of FISH technique are available as Single-molecule RNA-FISH, Card-FISH and Raman-FISH.

1b Bacterial FISH Detection



- ***A.muciniphila*** with Cy3 general bacterial FISH probe.
- ***P.gingivalis*** with ATTO488 *P.gingivalis* specific FISH probe.
- ***P.mirabilis*** with Cy3 *Proteus* species specific FISH probe.
- ***P.vulgaris*** with ATTO488 *Proteus* species specific FISH probe.
- ***E.coli*** with Cy3 general bacterial FISH probe.

FISH detection of *E.coli* using Cy3 general bacteria probe, visualized by confocal microscopy (X 200).

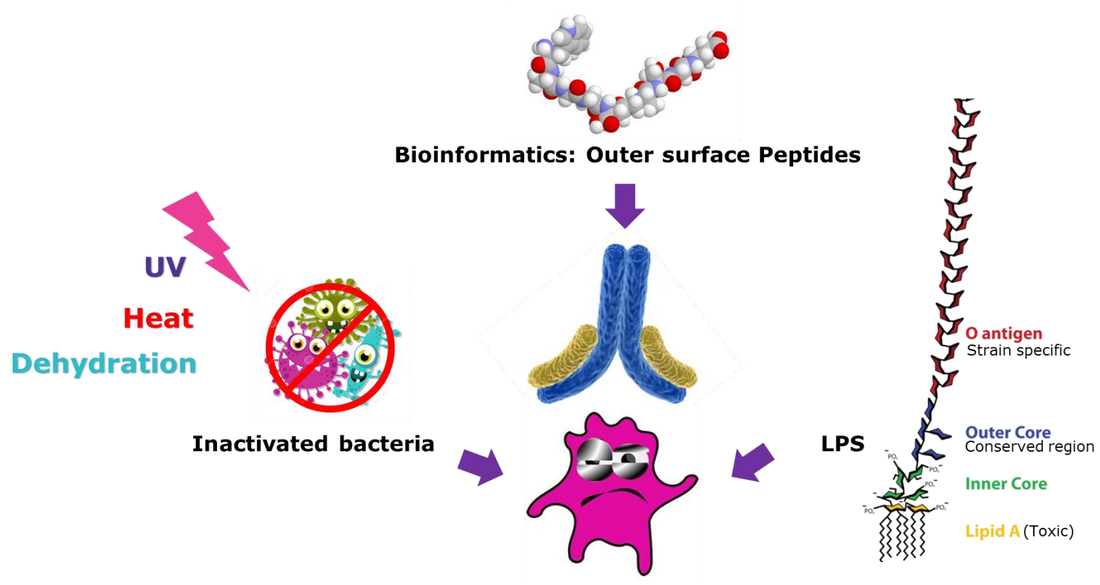


Mixed population of *E.coli and P.vulgaris* was submitted to FISH assay using ATTO488 general bacteria probe and Cy3 *Proteus* specific probe.
FISH Detection: ***E.coli*** *in Green*, ***P.vulgaris*** **in Red**, nuclear staining (**DAPI**) in Blue.

Bacteria were successfully detected with general and species specific FISH fluorescently labeled probes in both isolated and mixed bacterial samples.

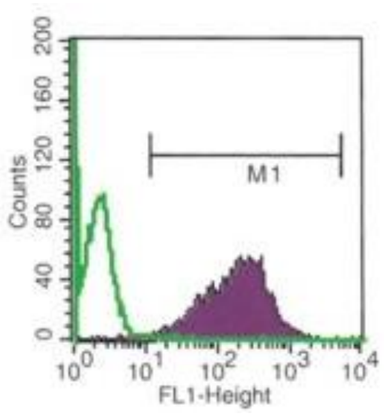
2a Anti-Bacterial Antibodies Development

Combined approaches using anti Bacteria & LPS Toxins as surface antigens for antibody development

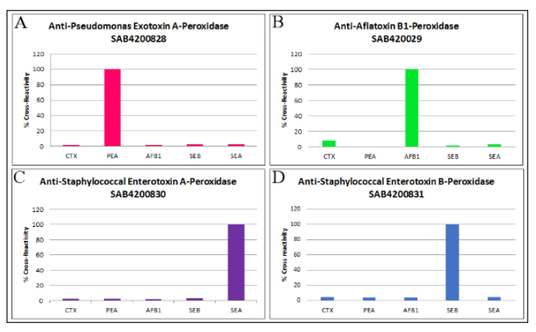


2b Detection and Identification of species-specific bacteria

WB Detection of *Proteus Vulgaris* Lipopolysaccharides (LPS; also known as lipoglycans and endotoxins) by Anti-*Proteus vulgaris* polyclonal Antibody (#**SAB4200817**)



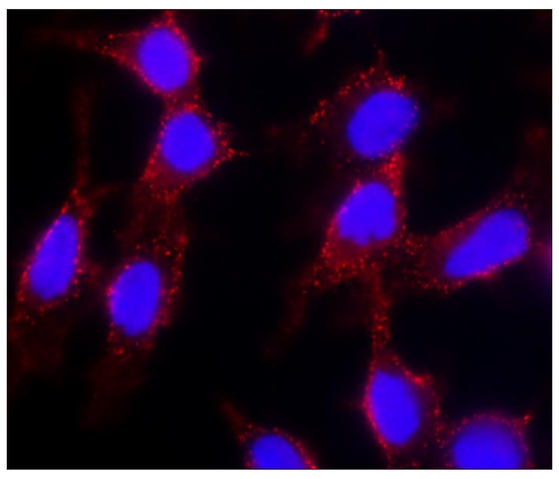
FACS Detection of Shiga Toxin 1, B subunit (STxB) (# **SML0562**) by Anti-Shiga Toxin 1, B Subunit (STxB) Monoclonal Antibody (#**SAB4200774**)



Endotoxins Elisa Detection using highly specific HRP-conjugated Antibodies against: *Pseudomonas* Exotoxin A (#**SAB4200828**) *Aspergillus* Aflatoxin B1 (#**SAB4200829**) *Staphylococal* Enterotoxin A (#**SAB4200830**) and Enterotoxin B (#**SAB4200831**)

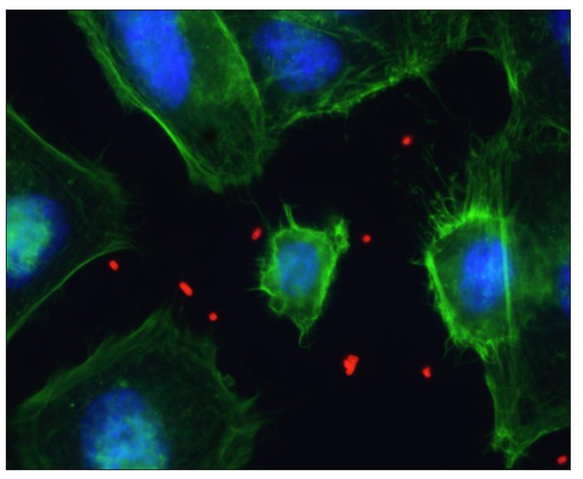
2c Bacterial Immunofluorescence (IF) Assays

Vibrio cholera **Toxin**



HeLa cells were treated with *Vibrio* cholera Toxin B subunit (CTxB) (#**SAE0069**) fixed, permeabilized and stained using Monoclonal Anti-Cholera Toxin B Subunit (CTxB) Antibody (# **SAB4200844**; In process). The antibody was developed using Goat Anti-Mouse IgG, Cy3™ conjugate (Red). Cells were counterstained with DAPI nuclear staining (Blue).

Proteus mirabilis **Infection**



HeLa cells were infected with *Proteus mirabilis* live bacteria, fixed, permeabilized and stained using Polyclonal Anti-Proteus mirabilis Antibody (#**SAB4200818**). The antibody was developed using Goat Anti-Mouse IgG, Cy3™ conjugate (Red). Cells were counterstained with Phalloidin actin filamentous (Green) and DAPI nuclear staining (Blue).

Summary:

The ability to detect and isolate specific bacteria is crucial in identifying key host-microbiome interactions and promoting the Microbiome research field. Herein, we show that our newly developed Fluorescent In Situ Hybridization (FISH) probes are specific and robust, allowing rapid identification of pathogenic bacteria (such as *Proteus* species and *Porphyromonas gingivalis*) in diverse samples including mixed bacterial population.

In addition, our microbiome antibodies portfolio is comprised of highly specific anti bacteria and anti bacterial components (e.g. toxins, unique proteins and Lipopolysaccharides) antibodies. These antibodies are suitable to be used in various applications including ELISA, WB, Bacterial Isolation and Imaging.

- **Look for " Microbiome Antibodies" in the Sigma-Millipore Catalog.**

Together these set of tools will allow both imaging, detection and isolation of specific bacteria which serve as a crucial step in the microbiome research field.