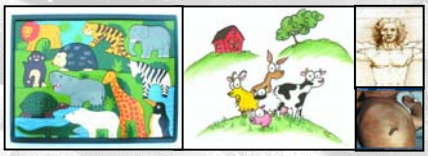
Laser Capture Microdissection



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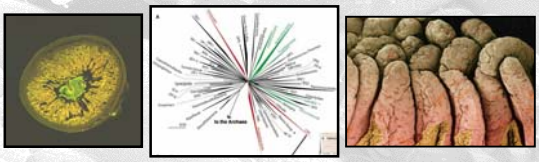
The gastro intestinal microbiota



- The GI tract contains approx. 10^{14} bacteria
- 1 g of intestinal content can inhabit 10^{11} bacteria
- The GI tract contains more than 500 different bacterial species.
- Most of the GI bacterial species are anaerobic and not yet culturable.

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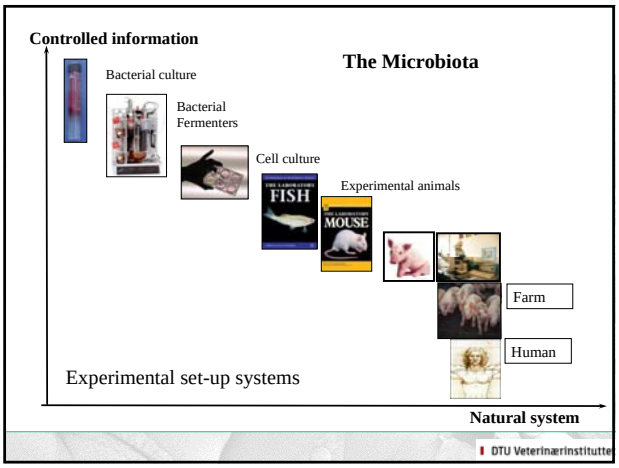

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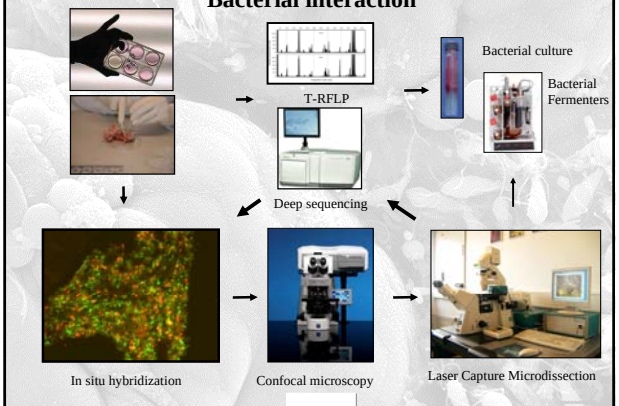
Bacterial interaction



P.A.L.M.

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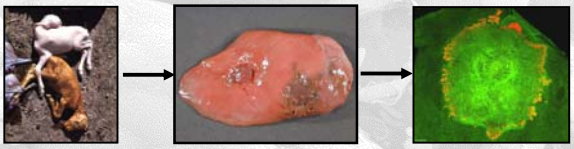
Bacterial identification

Abortion in sheep | Lung sample with lesions | In situ hybridization



16S rRNA gene = Fusobacterium necrophorum

Journal of Clinical Microbiology 2006 (44) 4537-4540

Molecular and Cellular Probes 2006 (20) 330-336

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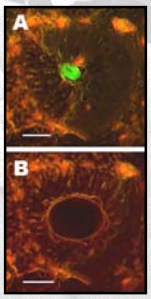
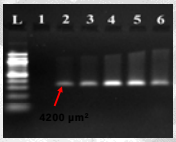
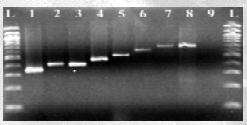
DNA extraction by LCM







324 bp to 670 bp in size from formalin fixated samples

BioTechniques 2006 (39) 864-868

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Bacterial interaction (gene expression)

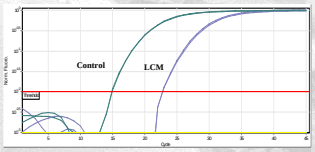
in vivo analyses of *Actinobacillus pleuropneumoniae* expression during an infection

Snap-frozen tissue section

Immunohistochemistry

LCM

qRT-PCR



Journal of Microbiological Methods 2007 (69) 414-416

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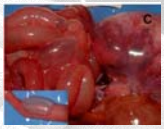
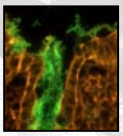
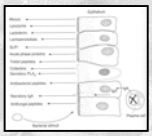
Bacterial-host interactions







- **Transcriptome analysis on epithelial cell layer from gnotobiotic or conventional preterm piglets**
- **mRNA in situ hybridization on host cell and isolation of potential pathogenic or probiotic bacteria**

Laser capture microdissection and microbial ecology

The DOs and the limitations on using microdissection

DOs

- fast and reliable harvesting of thousands of cells, "single cells" and subcellular structures from histological sections, cytospins etc.
- selectively capture single neurons, fetal cells, sperm cells and chromosomes
- no limitations for your **genetic and proteomic analysis**
- **DNA or RNA analysis** and **microarray technology** with non contact pure samples
- preparation from paraffin and frozen sections, also directly from glass slides
- Isolate and cultivate **living cells** from fresh tissue

Limitations

- Its not a FACS or a microtweezer
- You need bacterial microcolonies to get enough material (RNA and DNA)
- LCM only makes sense if you have a solid or embedded material with substructures
- **Most known applications are for tissue sections and cell cultures!**