

# **Micro-Droplet Enabled Parallel Co-Cultivation of Symbiotic Microbial Communities**

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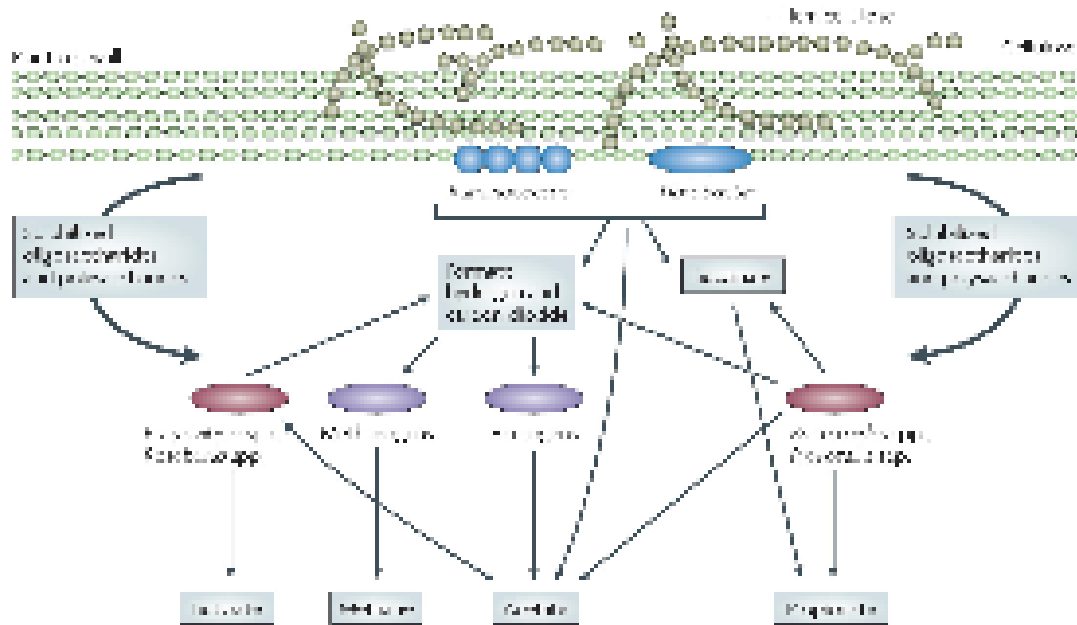
**University of Michigan**

**Department of Chemical Engineering**

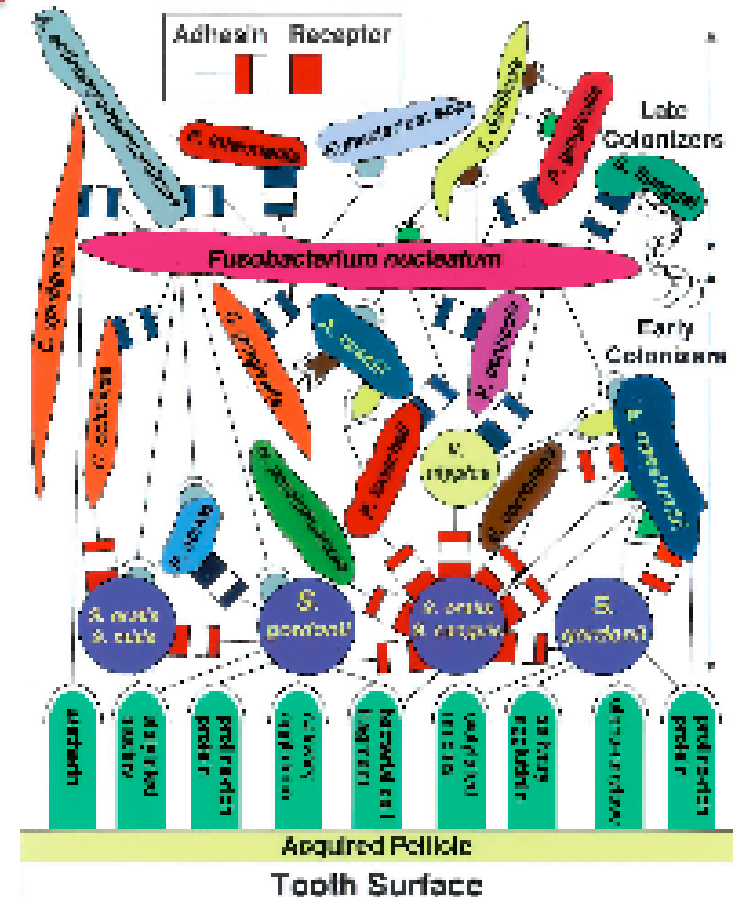
**International Human Microbiome Congress**

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# Microbial interactions in human microbiota



Flint et al. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol.* 6: 121-31 (2008).

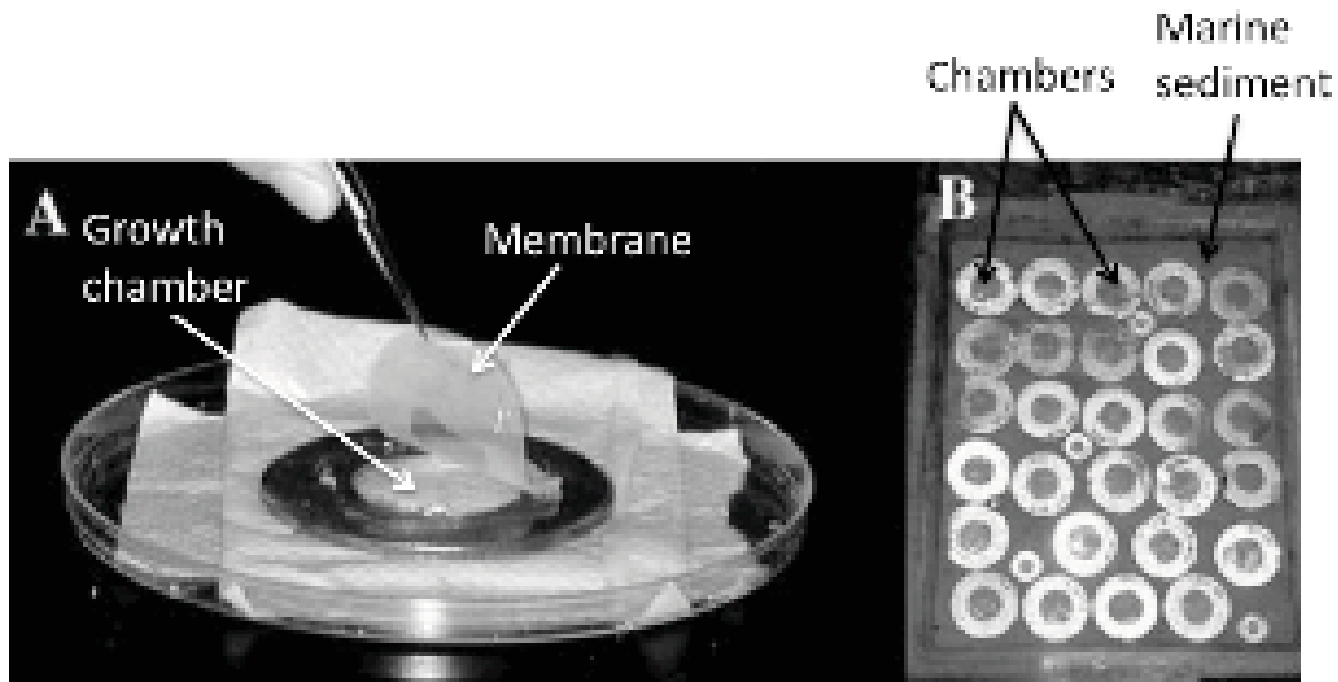


Kolenbrander et al. Bacterial interactions and successions during plaque development. *Periodontol* 2000 42: 47 (2006).

## Implications for cultivation

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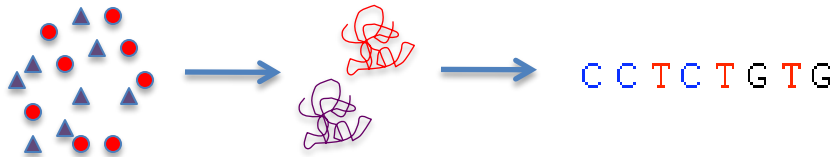
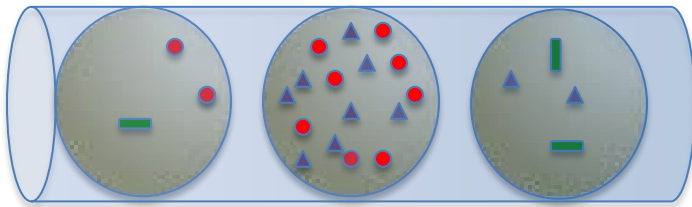
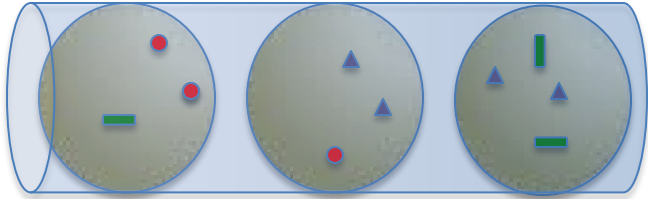
- Conventional cultivation: pure-culture based
- Fewer than 1% of all microbial species have been cultivated
- Co-cultivation: the way to go?



Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* 296(5570):1127 (2002).

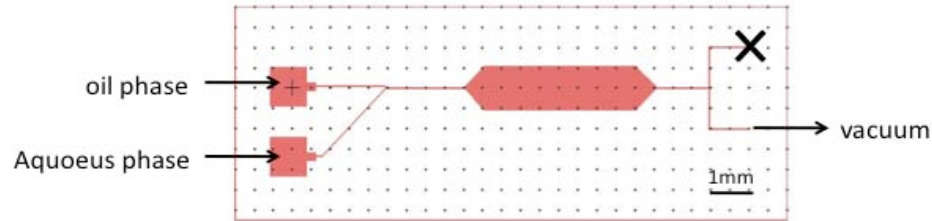
# Our idea: droplet-based parallel co-cultivation of microbes

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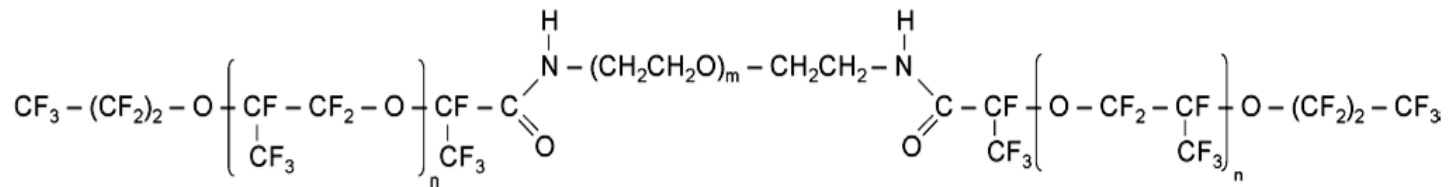


1. Encapsulation of subsets of microbiota - Multiple droplet generation by slanted T-junction geometry
2. Cultivation of microbial subsets - Monitoring of cell growth by microscopy
1. Characterization of cultivated communities (e.g. terminal restriction fragments length polymorphism (TRFLP))

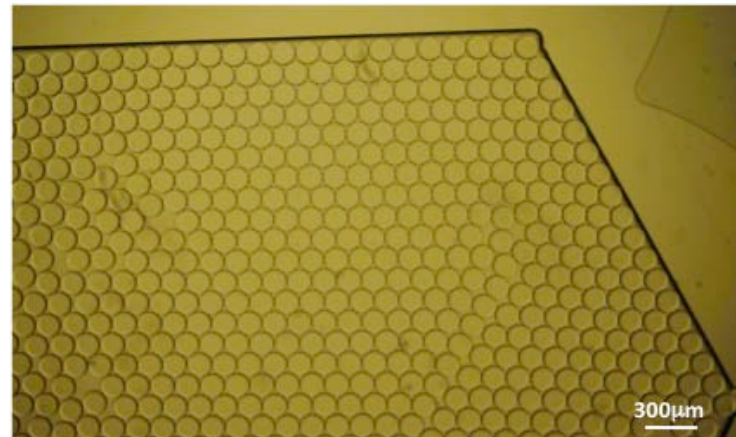
# Generation of Monodispersed Droplets



- simple and fast generation
- highly parallelized culture
  - PFPE-PEG surfactant in perfluorocarbon oil

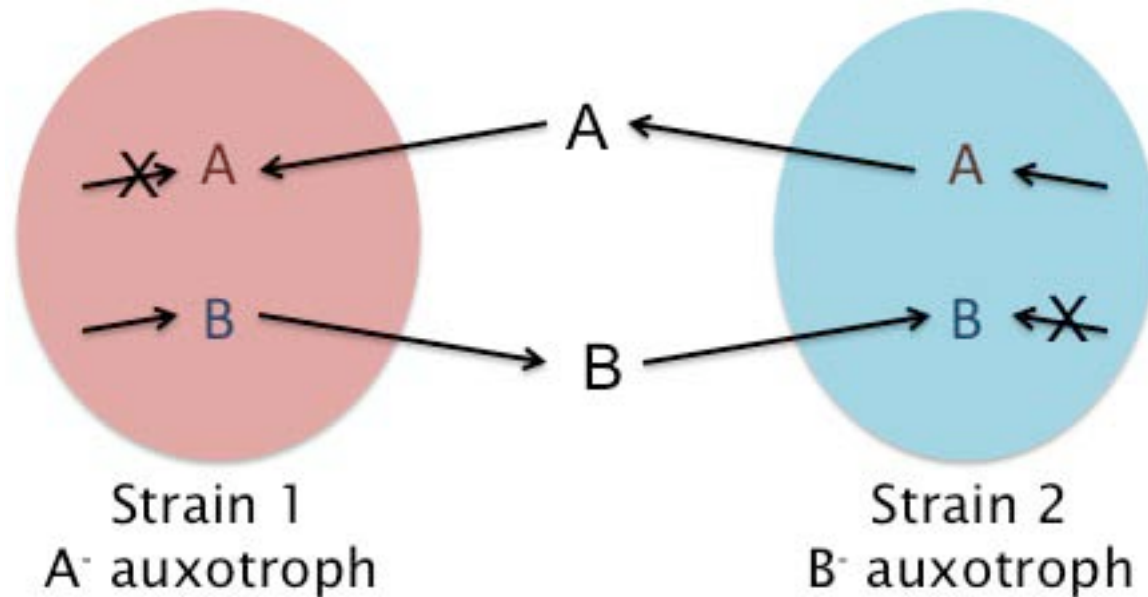


Lab Chip 8 (2008) 1632



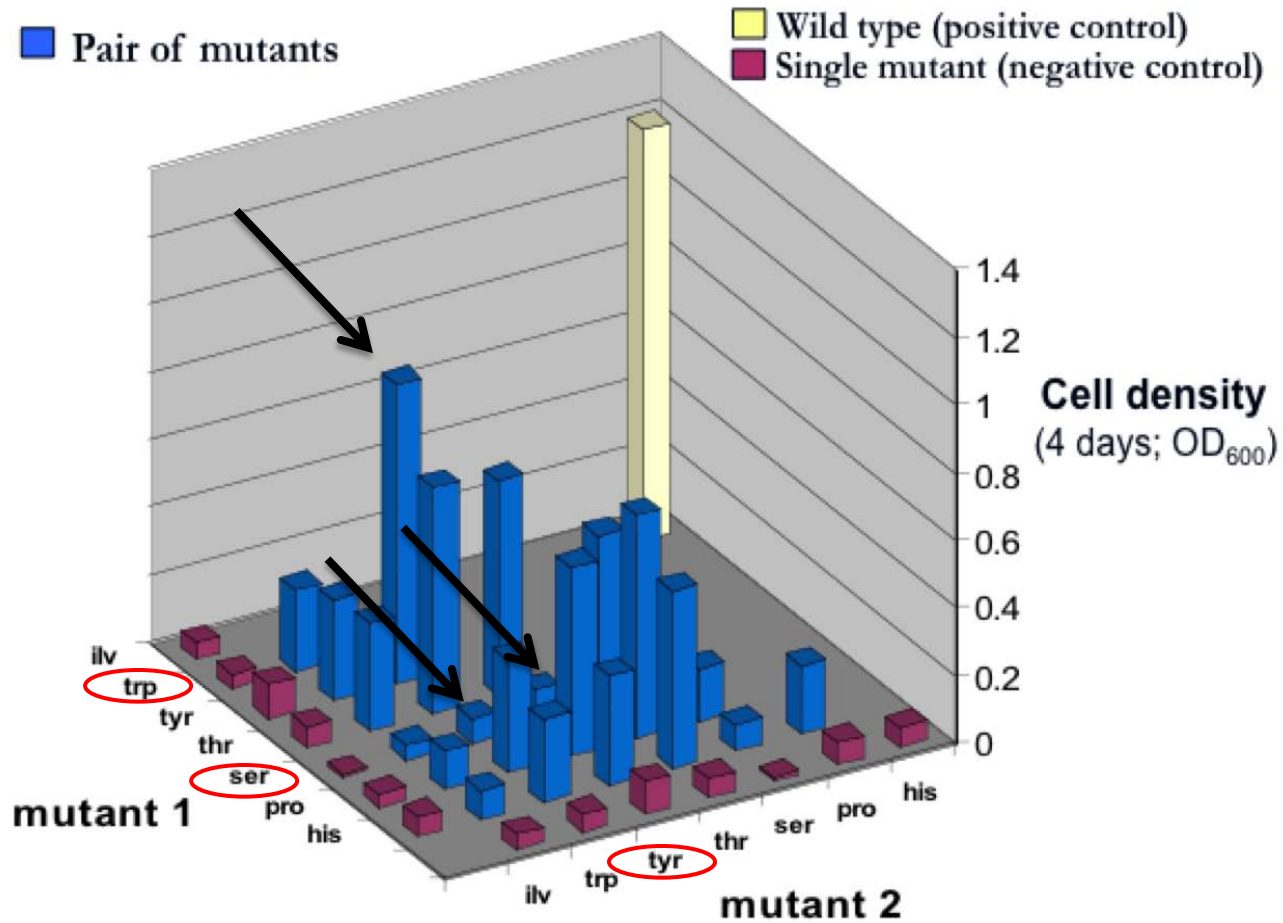
# Model System: Synthetic *E.coli* Symbiosis

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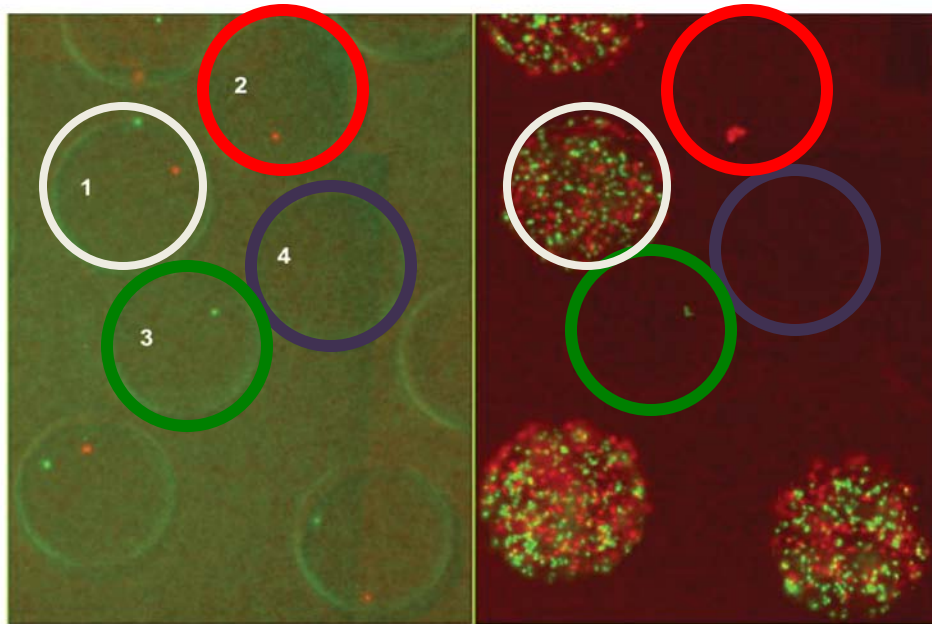


- Various pairs showing different growth rates  
depending on substrate A and B
- A prototype of microbial interactions in the human gut microbiota
- To screen highly symbiotic relationships from the community  
based on their growth in localized environment

# Model System: Synthetic *E.coli* Symbiosis



# Model System: Synthetic *E.coli* Symbiosis - Pair



Before cultivation

After cultivation

1. W<sup>-</sup> - Y<sup>-</sup> pair

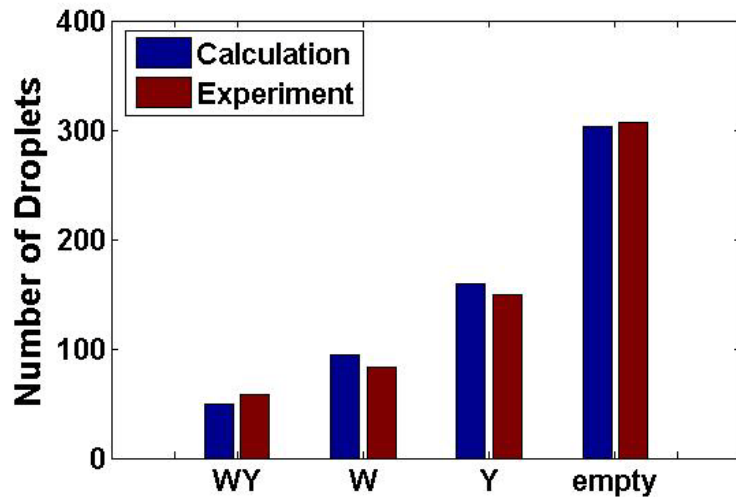
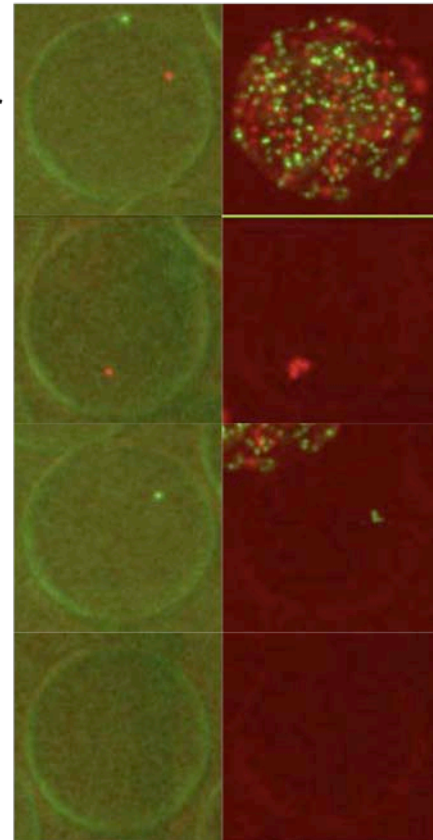
2. W<sup>-</sup> only

3. Y<sup>-</sup> only

4. empty

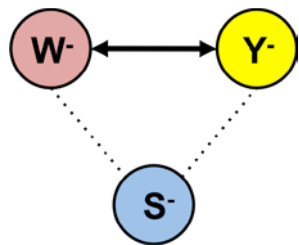
0 hr

18 hr

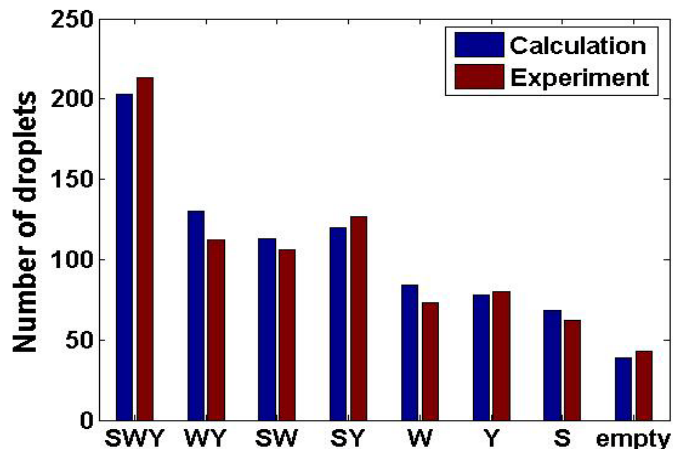
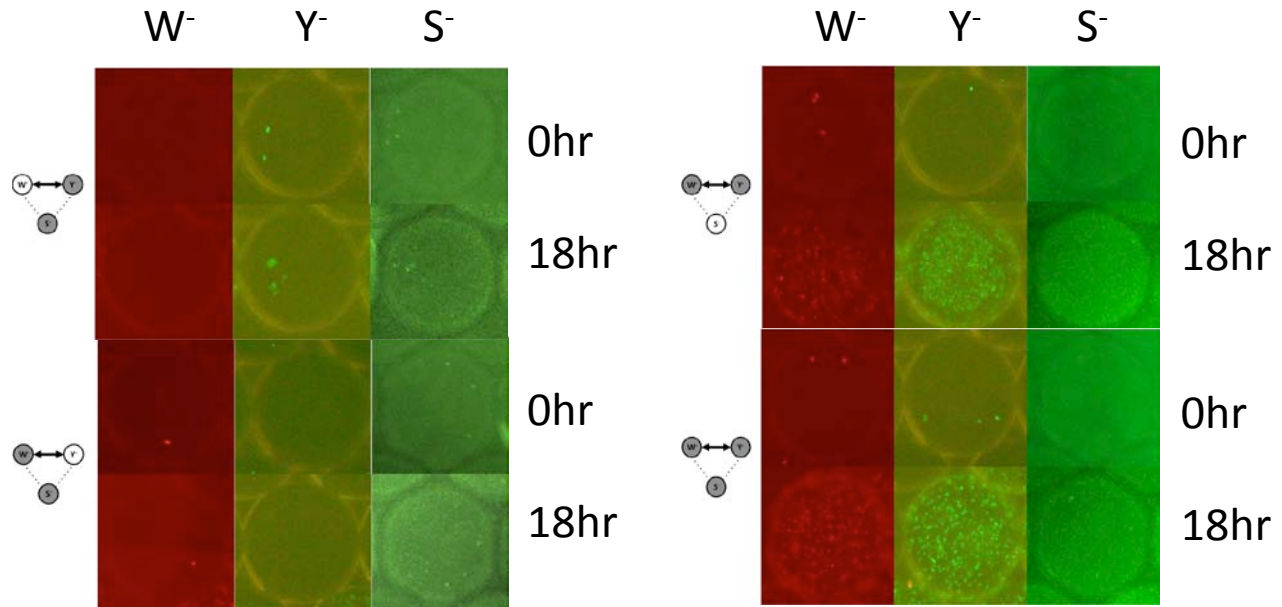


|           | empty | W <sup>-</sup> | Y <sup>-</sup> | W-Y <sup>-</sup> |
|-----------|-------|----------------|----------------|------------------|
| Growing + | 0     | 0              | 0              | 56               |
| Growing - | 317   | 83             | 150            | 2                |

# Model System: Synthetic *E.coli* Symbiosis – $S^-:W^-:Y^- = 1:1:1$

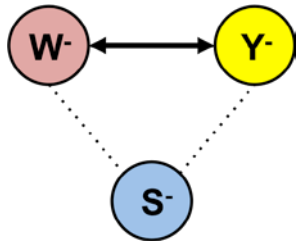


1 : 1 : 1



|                  | empty    | $S^-$    | $W^-$    | $Y^-$       |
|------------------|----------|----------|----------|-------------|
| <b>Growing +</b> | 0        | 0        | 0        | 0           |
| <b>Growing -</b> | 43       | 62       | 73       | 80          |
|                  | $S^-W^-$ | $S^-Y^-$ | $W^-Y^-$ | $S^-W^-Y^-$ |
| <b>Growing +</b> | 0        | 0        | 109      | 210         |
| <b>Growing -</b> | 106      | 127      | 3        | 3           |

# Model System: Synthetic *E.coli* Symbiosis – More Noise

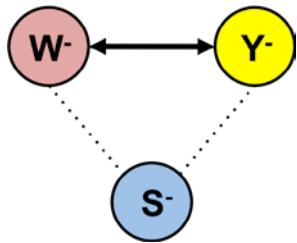
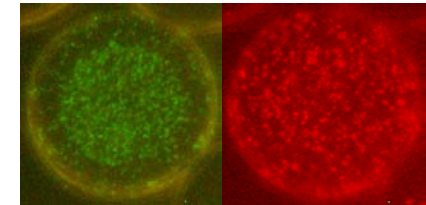


**1 : 1 : 30**

**977 drops**

**3% of  $W^-$  and  $Y^-$**

|           | empty    | $S^-$    | $W^-$    | $Y^-$       |
|-----------|----------|----------|----------|-------------|
| Growing + | 0        | 0        | 0        | 0           |
| Growing - | 32       | 798      | 2        | 1           |
|           | $S^-W^-$ | $S^-Y^-$ | $W^-Y^-$ | $S^-W^-Y^-$ |
| Growing + | 0        | 0        | 1        | 5           |
| Growing - | 78       | 70       | 0        | 0           |

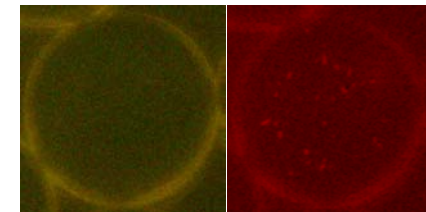
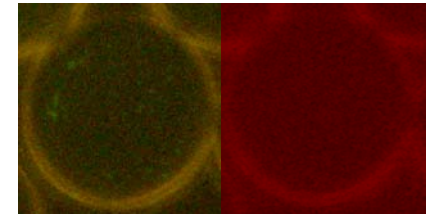


**1 : 50 : 50**

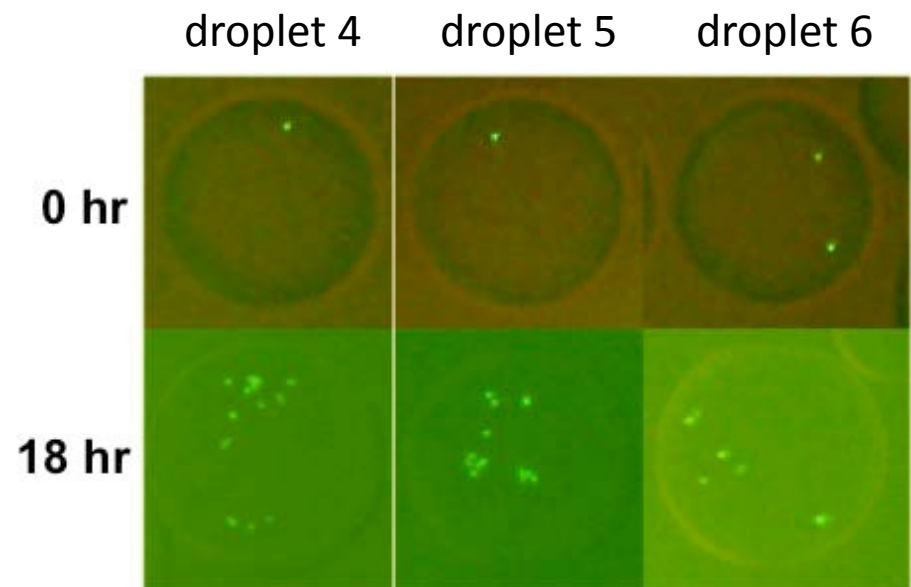
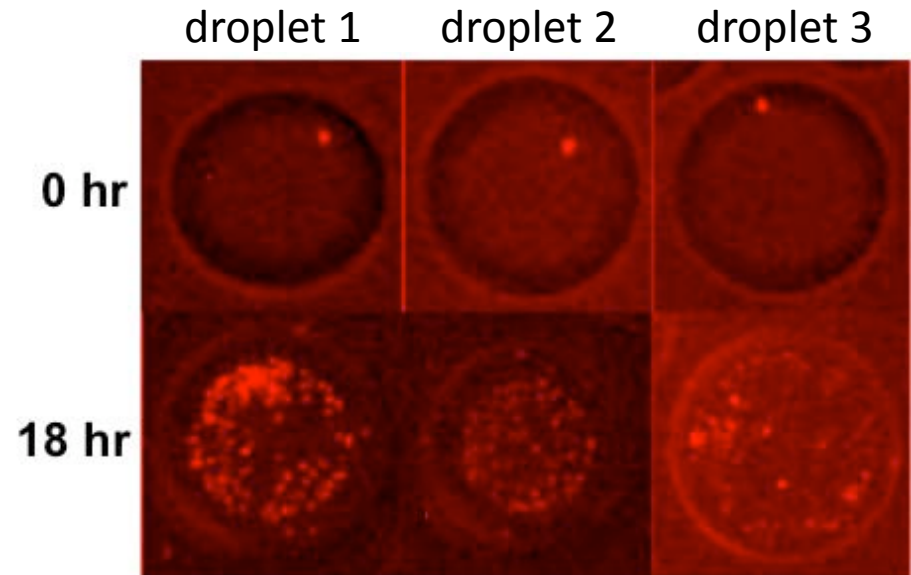
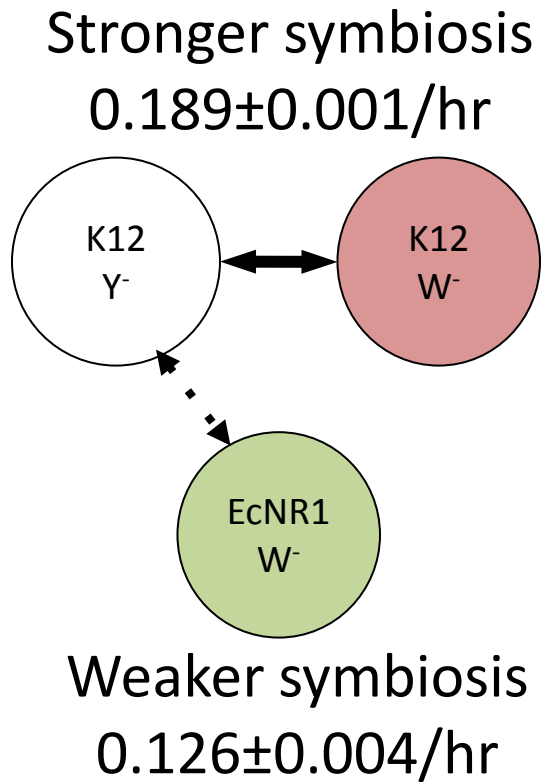
**880 drops**

**1% of  $W^-$**

|           | empty    | $S^-$    | $W^-$    | $Y^-$       |
|-----------|----------|----------|----------|-------------|
| Growing + | 0        | 0        | 0        | 0           |
| Growing - | 40       | 153      | 138      | 2           |
|           | $S^-W^-$ | $S^-Y^-$ | $W^-Y^-$ | $S^-W^-Y^-$ |
| Growing + | 0        | 0        | 3        | 16          |
| Growing - | 515      | 6        | 0        | 0           |



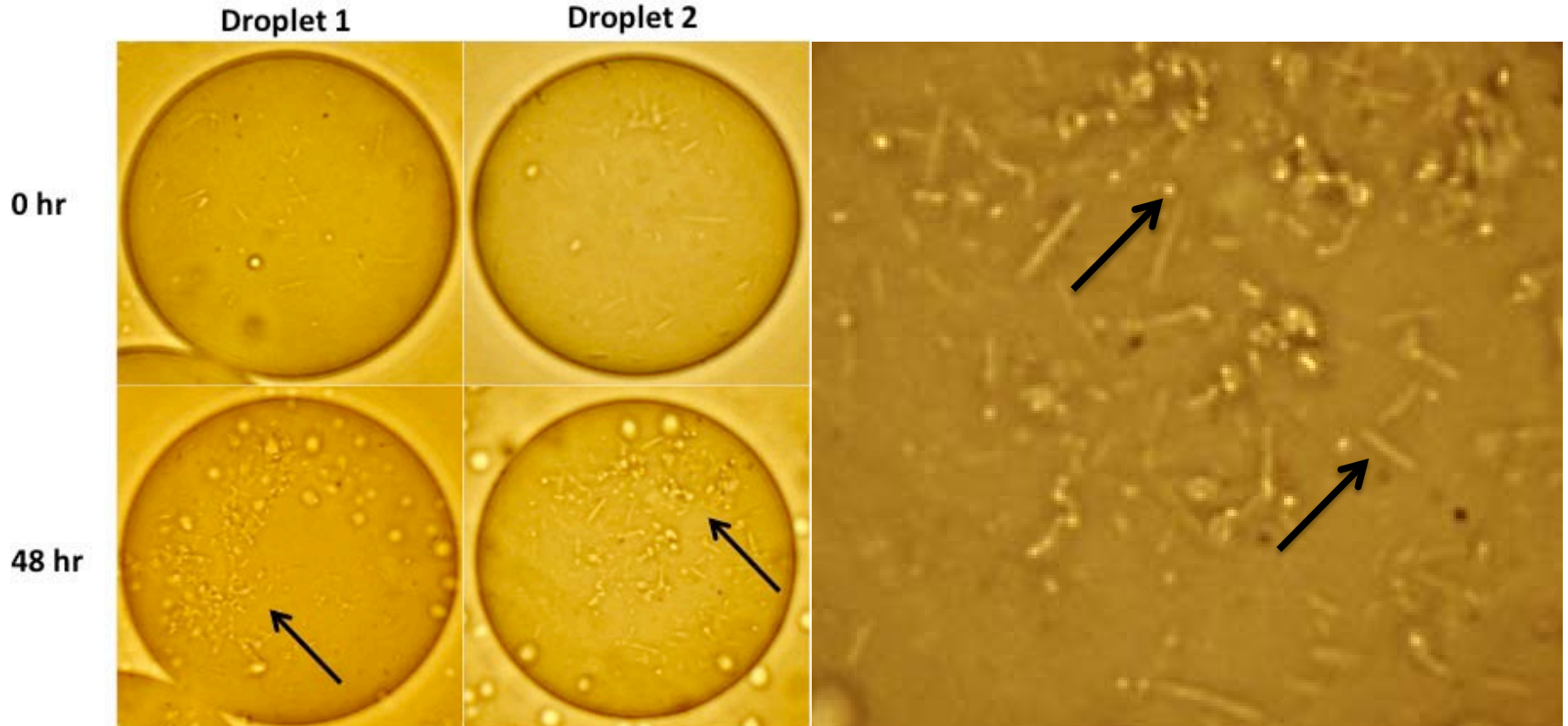
# Model System: Strong Symbiosis vs. Weak Symbiosis



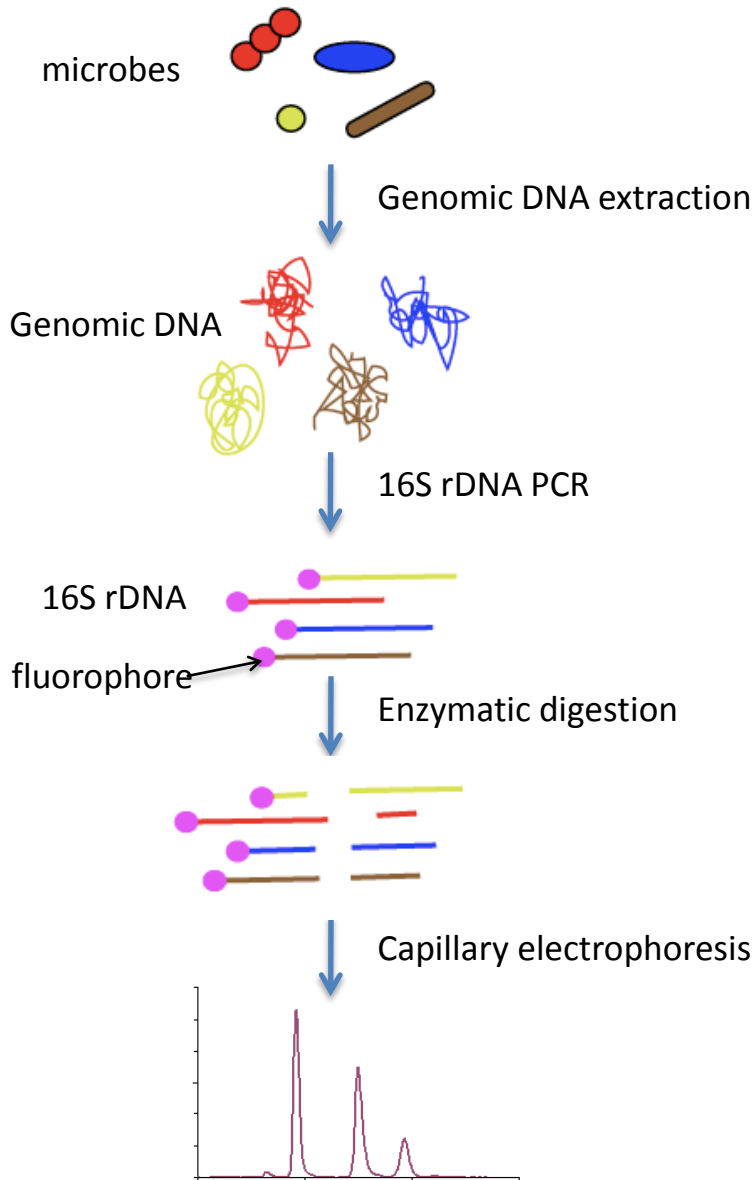
# On-chip Microbial Cultivation – Natural Microbiota

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- Murine fecal microbiota
- Aerobic culture in TSB media for 48 hours at 37°C
- Cell clusters containing morphologically different cells
- # clusters in droplets – depending on the dilution ratio



# Off-chip Genetic Analysis - TRFLP

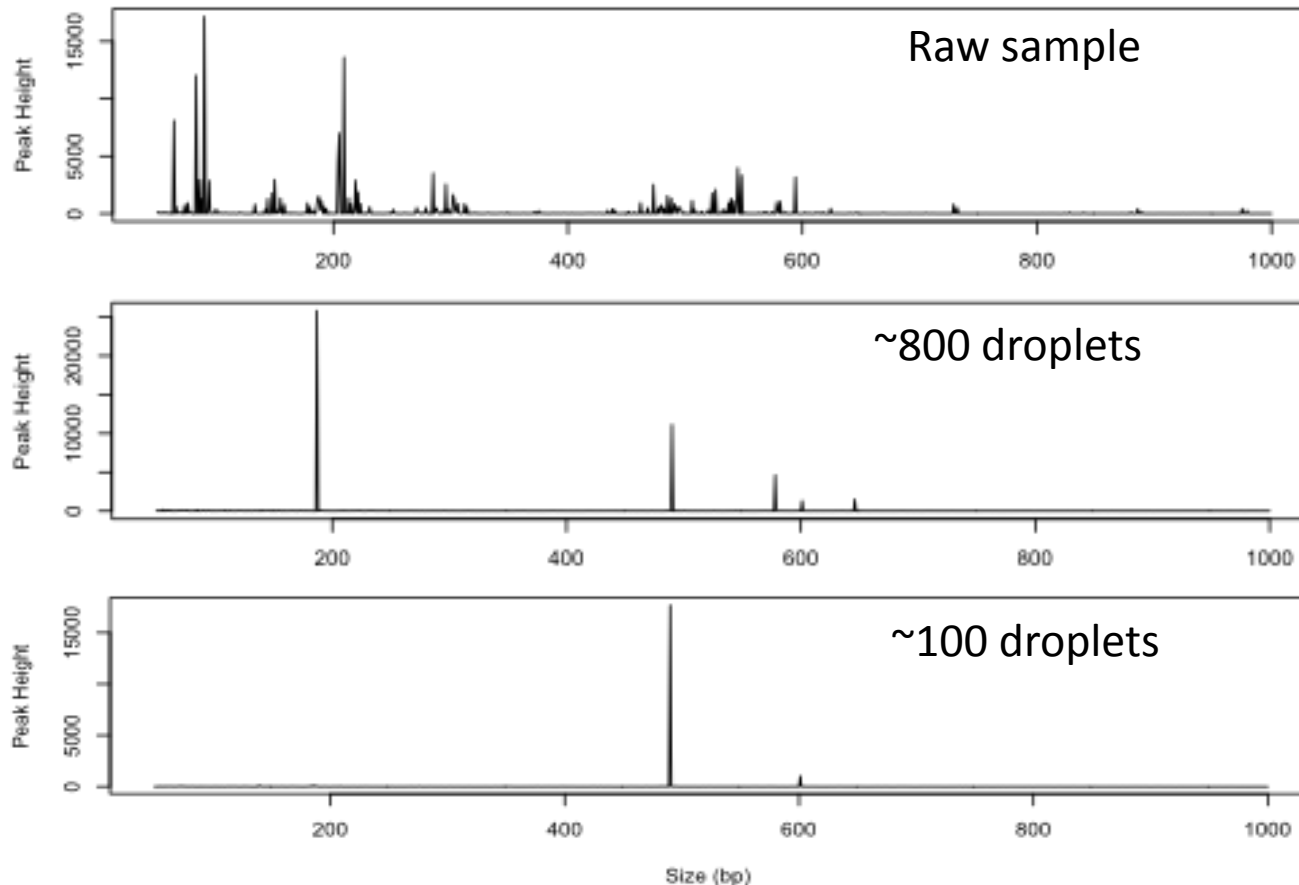


- 16S rDNA analysis of microbial communities
- Length polymorphism of terminal fragment
  - each peak mostly representing one species

# Result of TRFLP

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- Change in the composition of microbiota after on-chip cultivation
- Further investigation is needed for identification



# Conclusion

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1. Monodispersed multiple droplets were used for highly parallelized microbial culture.
2. Complicated microbiota can be effectively compartmentalized and cultivated in localized environment using microfluidic devices.
3. Droplet-enabled co-cultivation can be applied to various artificial or natural microbiota for high-throughput screening.
4. On-chip cultivation and off-chip analysis were coupled for elucidating microbial interactions in complex microbial communities.

# Future Work

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## Short-term:

- Device design for generating oxygen gradient
  - Diverse culture environment
- Device design for automated droplet sorting
- Characterization of droplet derived co-cultures
  - 16s cloning and sequencing
  - Shotgun metagenomic sequencing

## Mid-term:

- Reconstruction of community-wide metabolic networks
- Development of integrated devices for on-chip culture and analyses

# Oxygen Gradient Generating Device

