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

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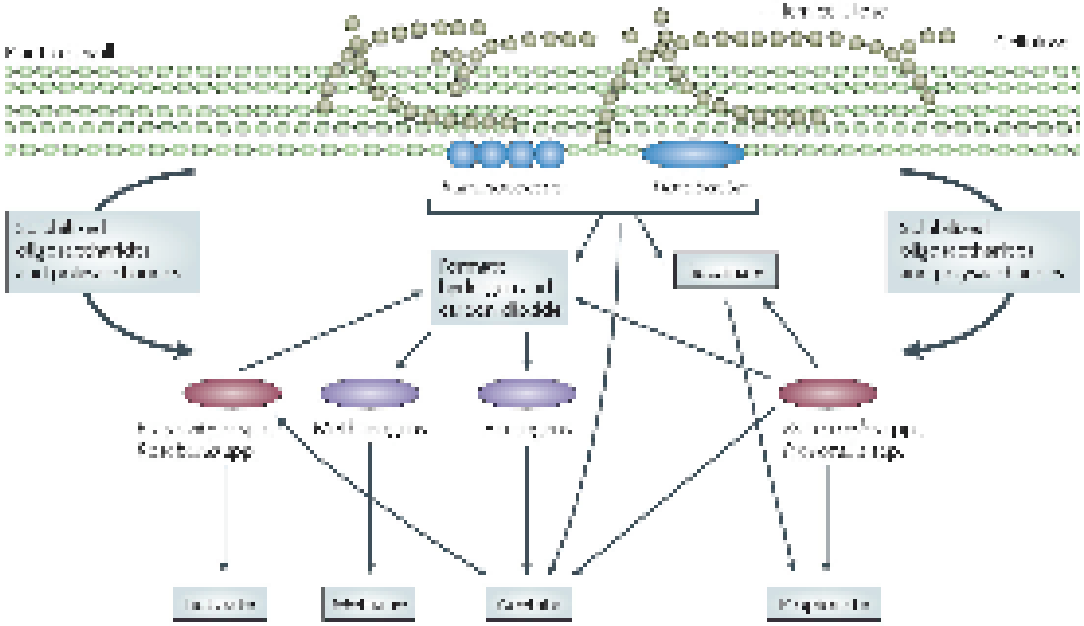
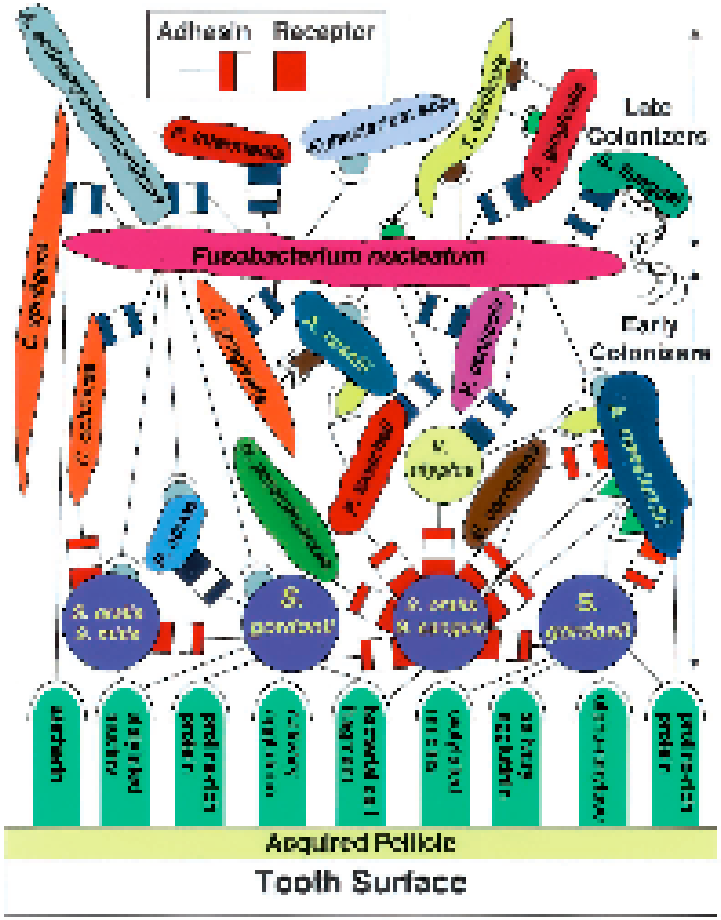
Microbial interactions in human microbiota



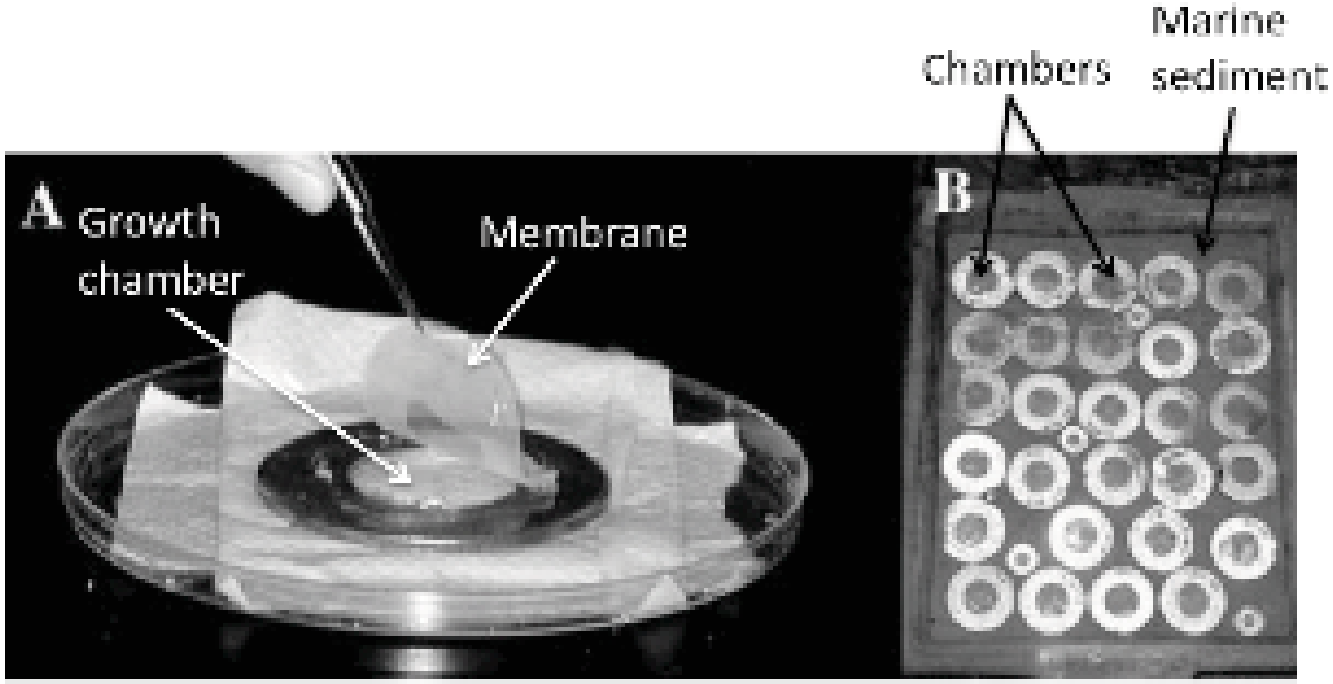
Flint et al. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol.* 6: 121-31 (2008).



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

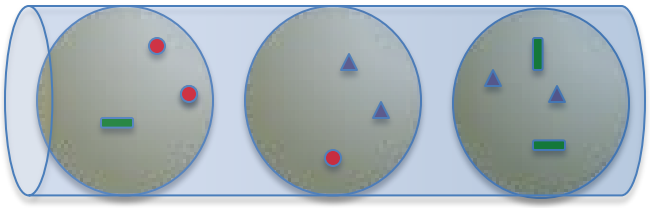
Implications for cultivation

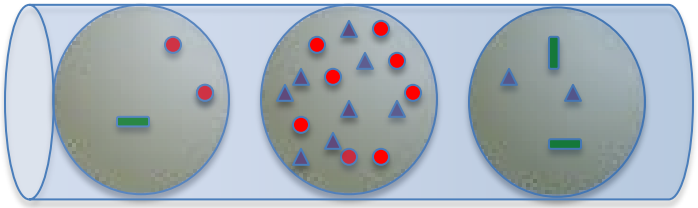
- Conventional cultivation: pure-culture based
- Fewer than 1% of all microbial species have been cultivated
- Co-cultivation: the way to go?

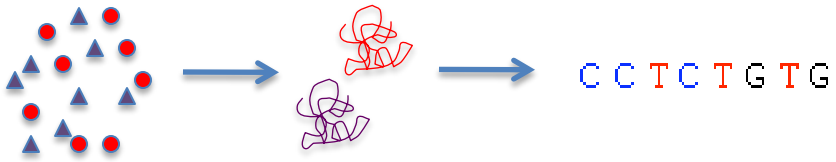


Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* 296(5570):1127 (2002).

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





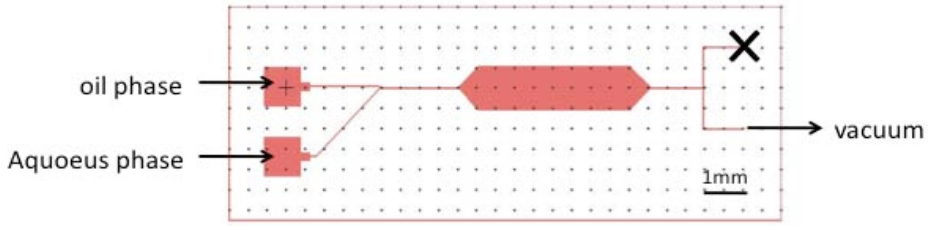


1. Encapsulation of subsets of microbiota - Multiple droplet generation by slanted T-junction geometry

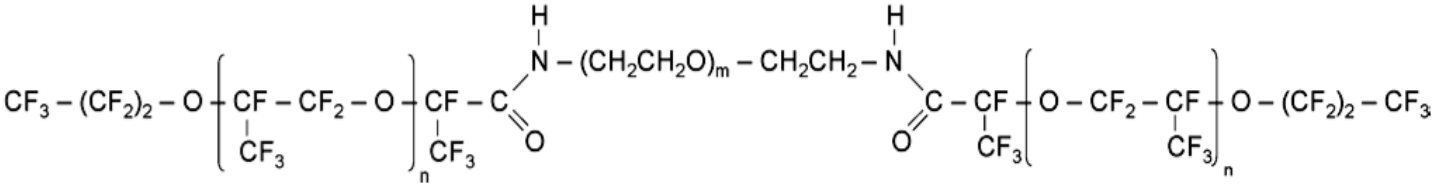
2. Cultivation of microbial subsets - Monitoring of cell growth by microscopy

1. Characterization of cultivated communities (e.g. terminal restriction fragments length polymorphism (TRFLP))

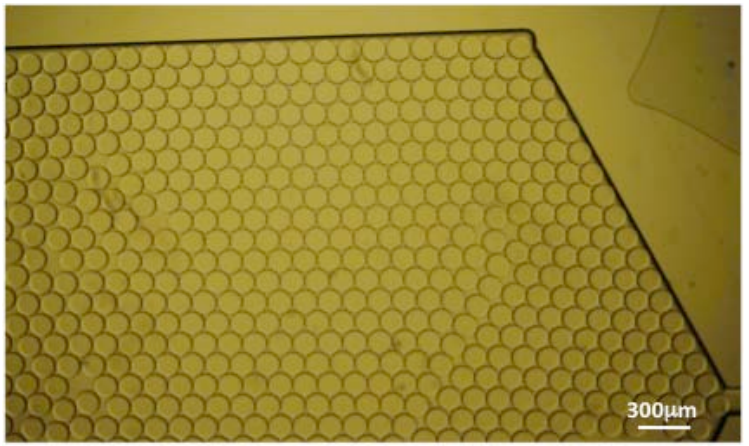
Generation of Monodispersed Droplets



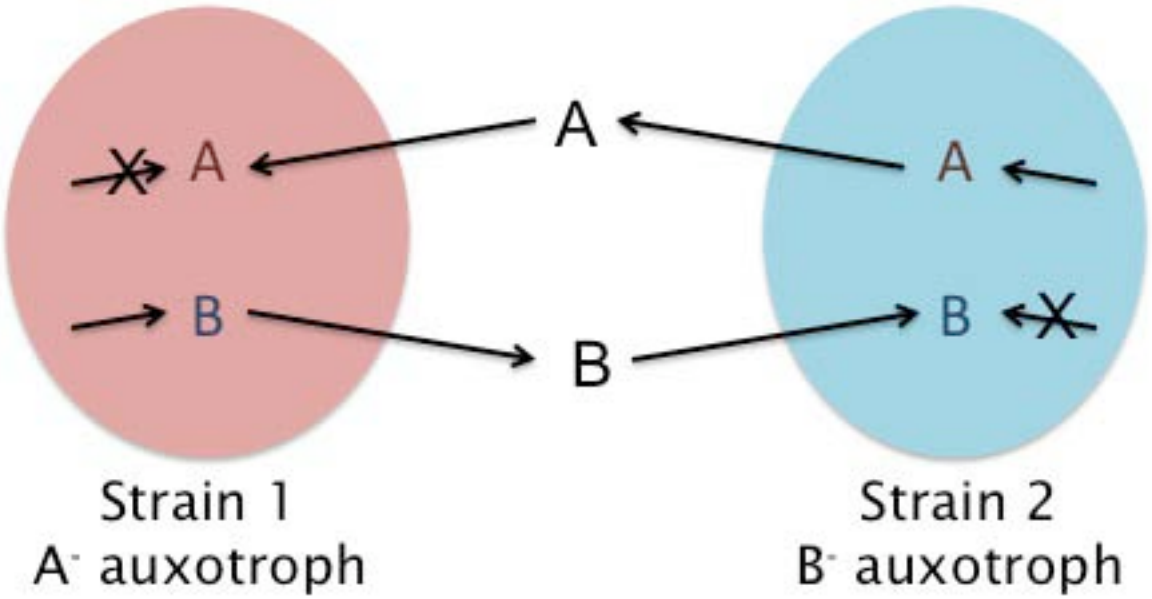
- simple and fast generation
- highly parallelized culture
  - PFPE-PEG surfactant in perfluorocarbon oil



Lab Chip 8 (2008) 1632

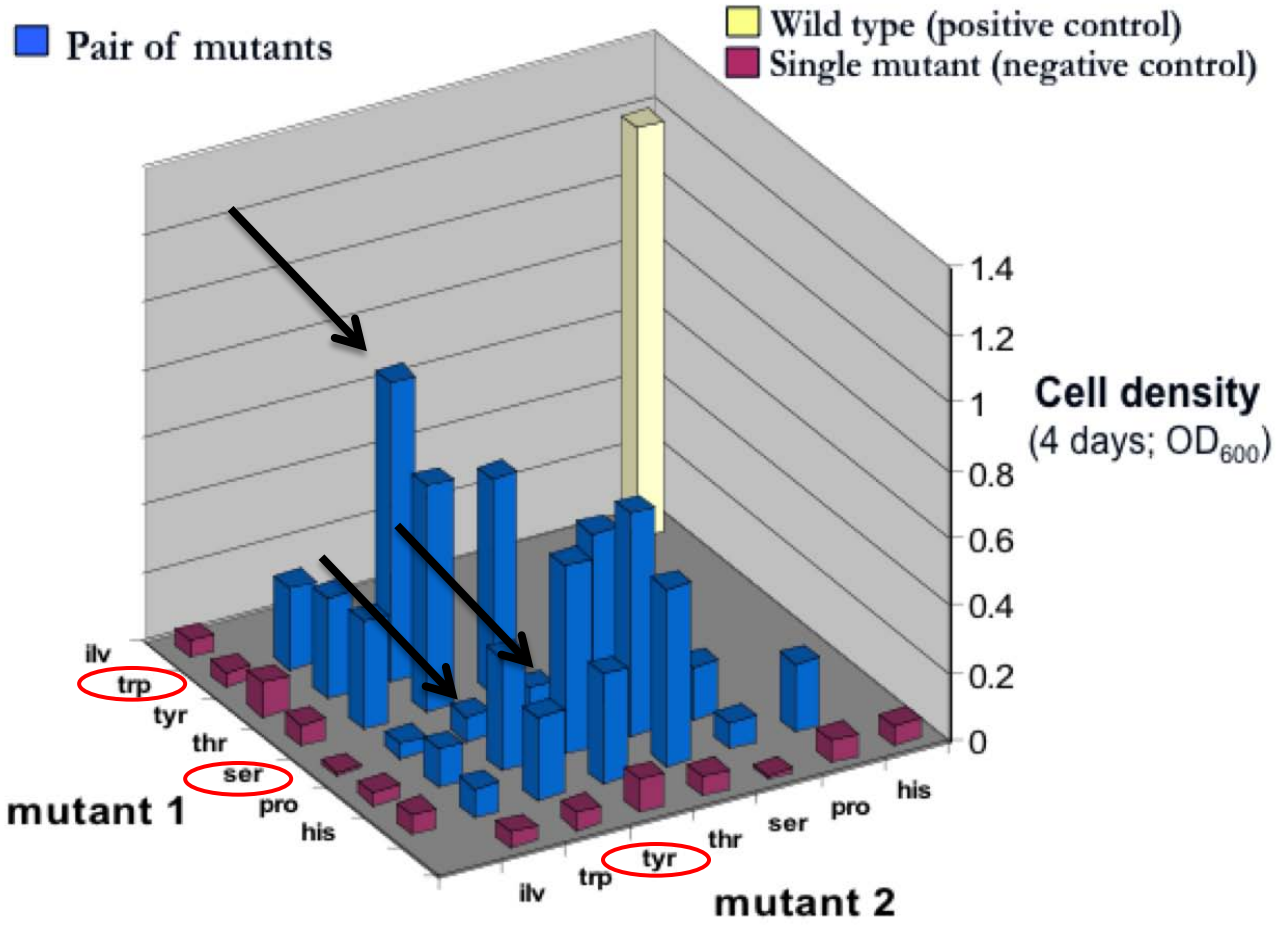


Model System: Synthetic *E.coli* Symbiosis

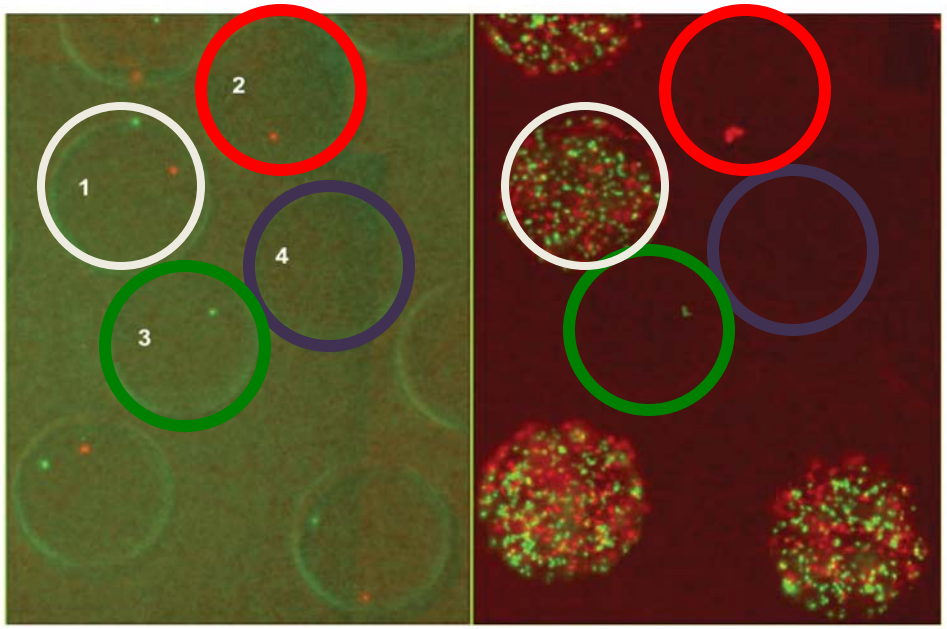


- Various pairs showing different growth rates
  depending on substrate A and B
- A prototype of microbial interactions in the human gut microbiota
- To screen highly symbiotic relationships from the community
  based on their growth in localized environment

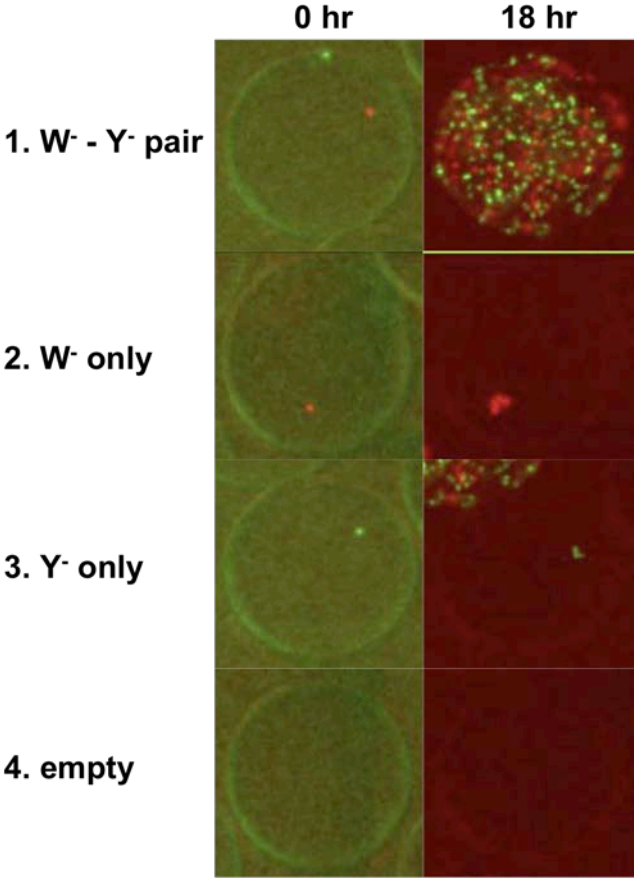
Model System: Synthetic *E.coli* Symbiosis

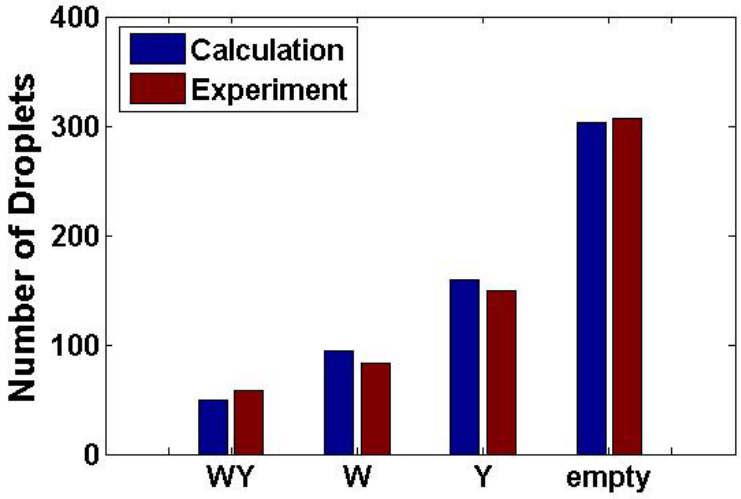


Model System: Synthetic *E.coli* Symbiosis - Pair



Before cultivation After cultivation

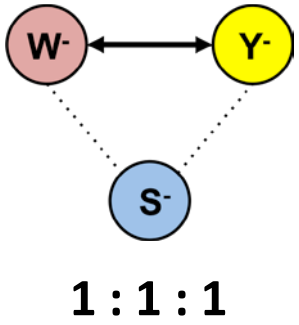




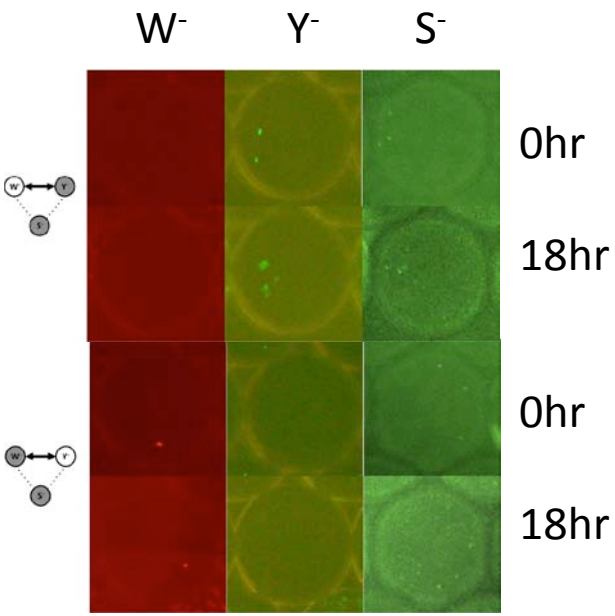
	empty	W⁻	Y⁻	W⁻Y⁻
Growing +	0	0	0	**56**
Growing -	317	83	150	2

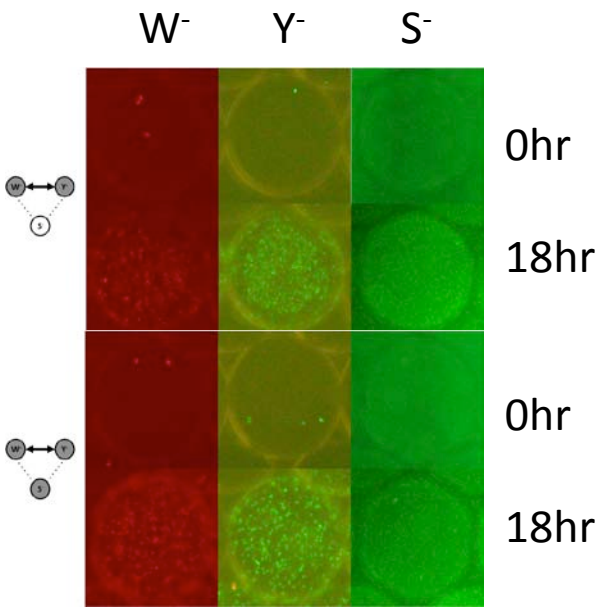
Park J, Kerner A, Burns MA, Lin XN (2011). *PLoS ONE* 6(2): e17019.

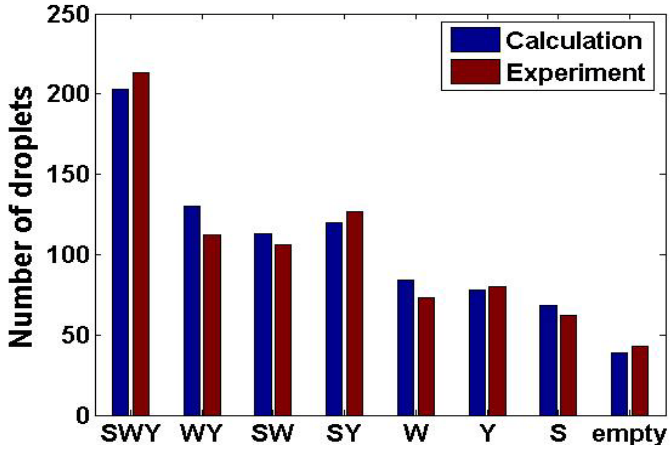
Model System: Synthetic *E.coli* Symbiosis – S^-:W^-:Y^- = 1:1:1



1 : 1 : 1

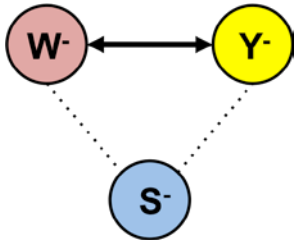






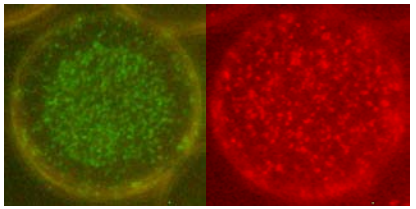
	empty	S^-	W^-	Y^-
Growing +	0	0	0	0
Growing -	43	62	73	80
	S^-W^-	S^-Y^-	W^-Y^-	$S^-W^-Y^-$
Growing +	0	0	**109**	**210**
Growing -	106	127	3	3

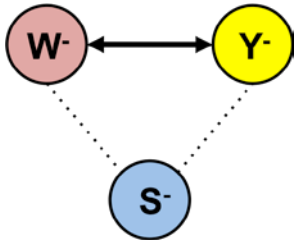
Model System: Synthetic *E.coli* Symbiosis – More Noise



1 : 1 : 30
977 drops
3% of W^- and Y^-

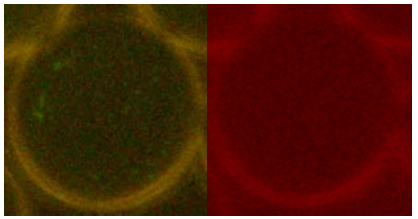
	empty	S^-	W^-	Y^-
Growing +	0	0	0	0
Growing -	32	798	2	1
	S^-W^-	S^-Y^-	W^-Y^-	$S^-W^-Y^-$
Growing +	0	0	**1**	**5**
Growing -	78	70	0	0

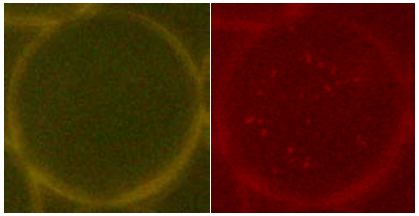




1 : 50 : 50
880 drops
1% of W^-

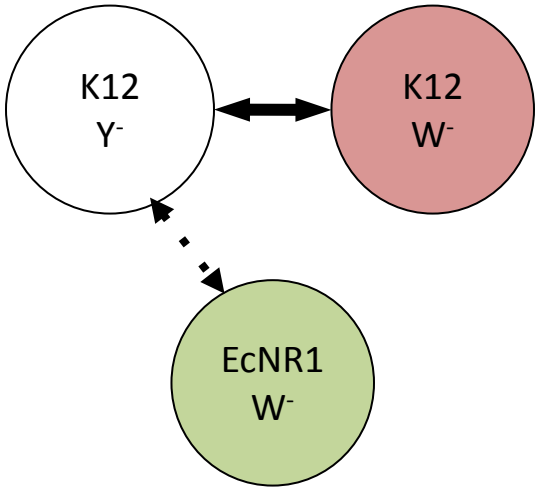
	empty	S^-	W^-	Y^-
Growing +	0	0	0	0
Growing -	40	153	138	2
	S^-W^-	S^-Y^-	W^-Y^-	$S^-W^-Y^-$
Growing +	0	0	**3**	**16**
Growing -	515	6	0	0



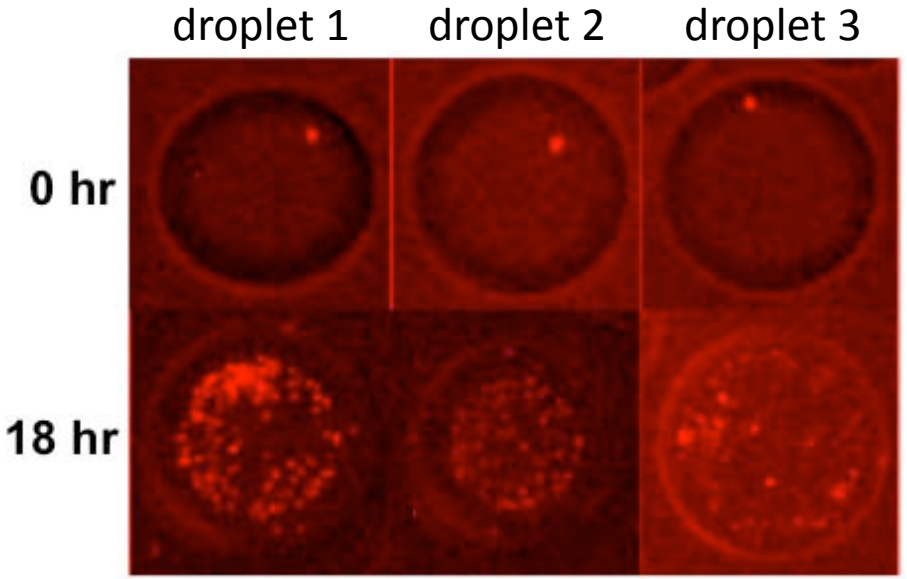


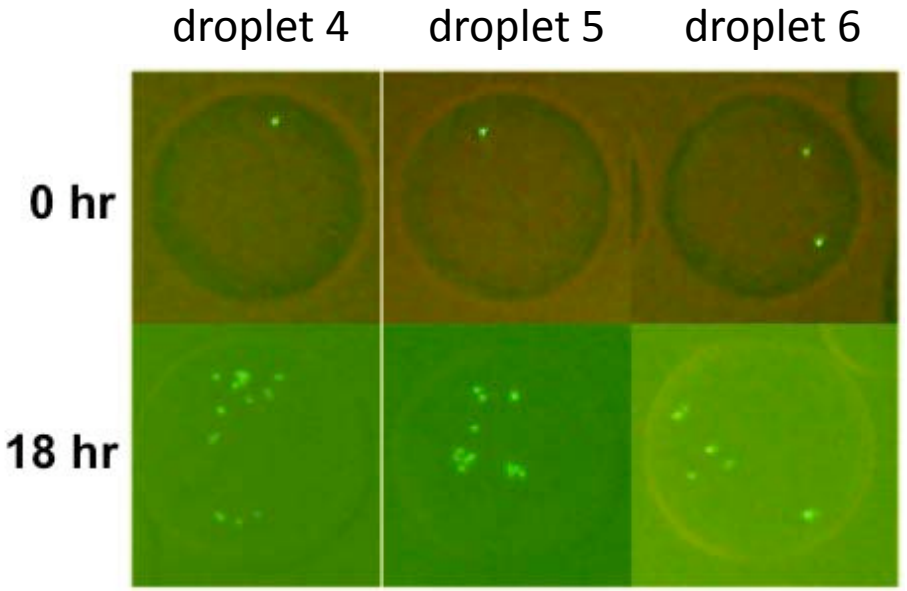
Model System: Strong Symbiosis vs. Weak Symbiosis

Stronger symbiosis
0.189±0.001/hr



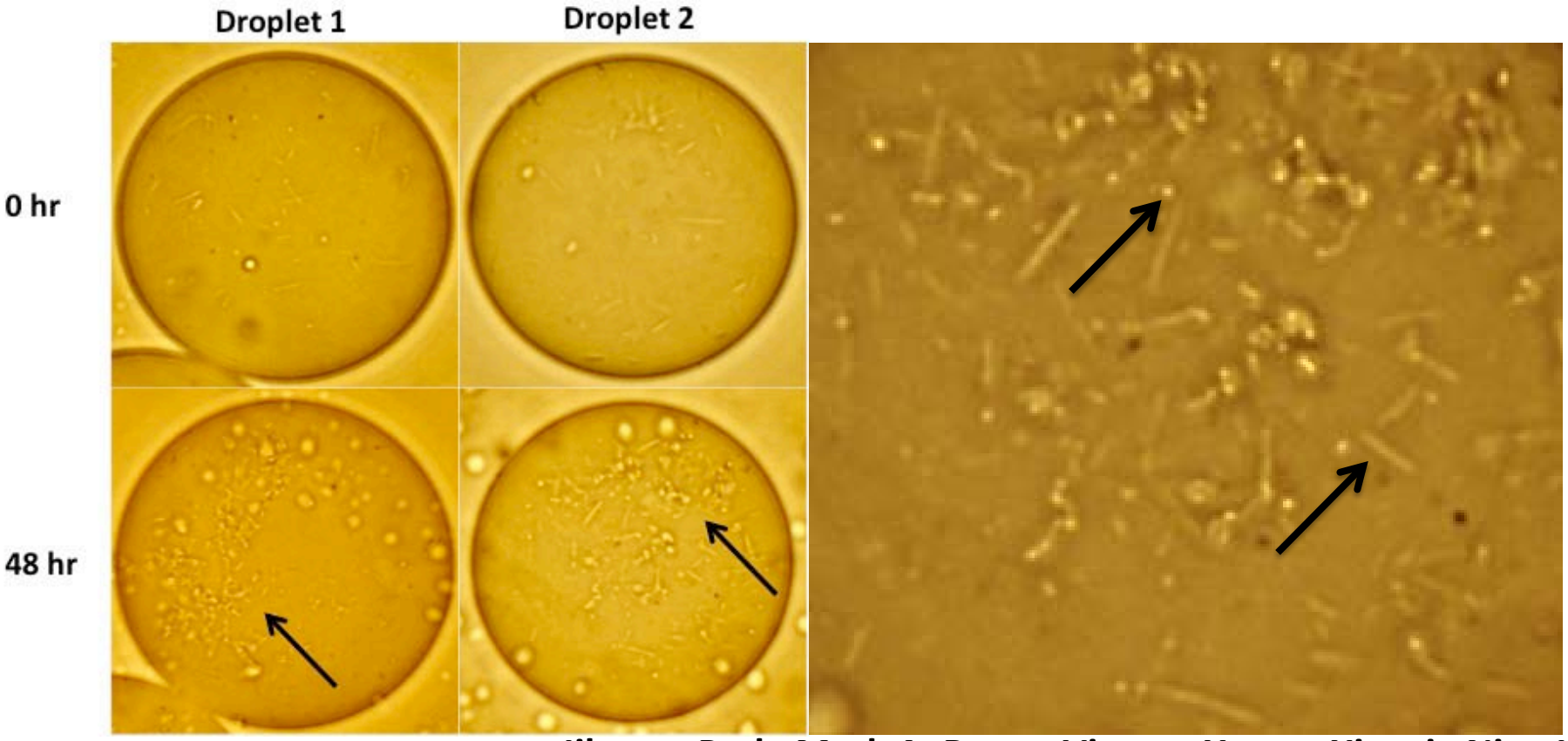
Weaker symbiosis
0.126±0.004/hr





On-chip Microbial Cultivation – Natural Microbiota

- 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



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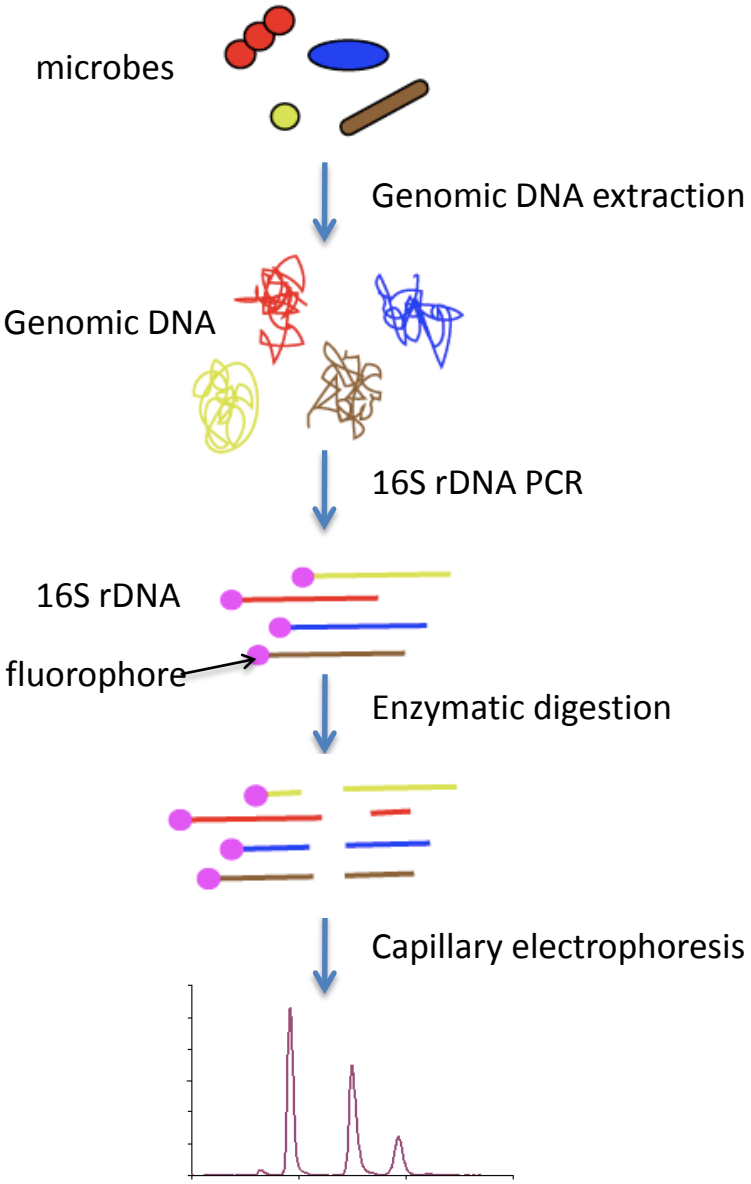
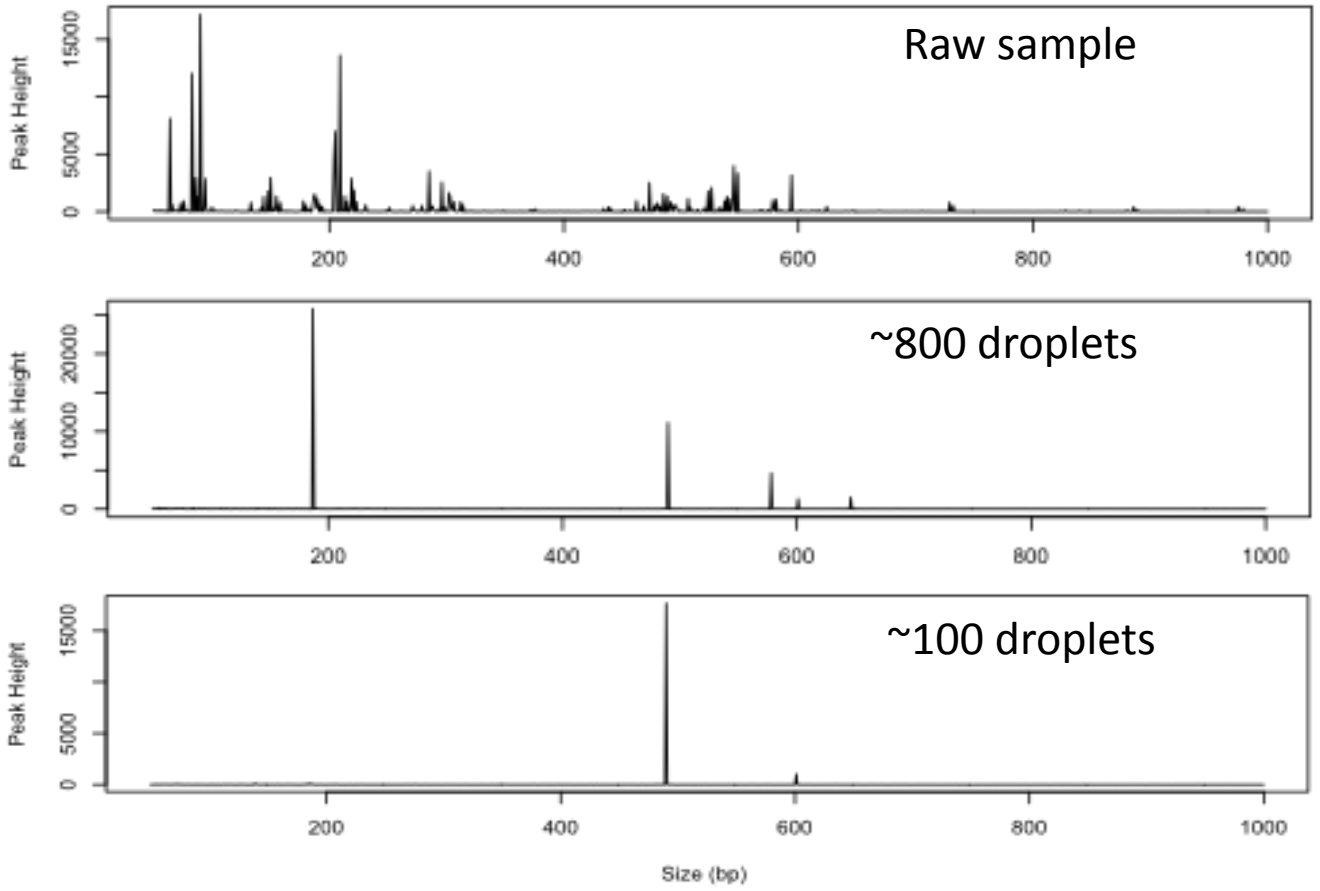
Off-chip Genetic Analysis - TRFLP



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

Result of TRFLP

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



Conclusion

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

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