Overview Microscale Applications Group

Mar 24, 2015
We need ng’s of DNA to sequence a microbial genome.
How do we study the uncultured majority?
Most microbes will not grow in the lab
Single-cell genomics bypasses need to culture
1. Microbes loaded into flow cytometer

2. Cells line up single file

3. Laser shines on cells

4. Cells identified by DNA fluorescence & size

5. Individual cells directed to wells of plate using electric charge
Multiple displacement amplification with Phi29 produces ~100ng of DNA from a single cell.
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Single Cell Genomics Pipeline

Sampling → Cell sorting → Genome amplification → Cell identification → Sequencing

Draft Genome → Annotation → Assembly → Data QC
Post-assembly QC identifies contaminant contigs
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Decontamination performed automatically - original and cleaned versions loaded to IMG

Manual decontamination protocol available
Most microbes will not grow in the lab
Single-cell genomics bypasses need to culture
User Requests for Single Cell Genomes Continues to Grow
New phylum revealed through metagenomics and single cell genomics
Emiley Eloe-Fadrosh’s talk on Wed @ 4:15pm

“Kryptonopia: A New Bacterial Candidate Phylum Discovered through Global Metagenomic Surveys”
Flow sorting enables metagenomic analysis of endophytes
Flow sorting enhances microbial signal in root metagenomes

Without sorting

![Bar chart showing the comparison of Billion Base Pairs between Plant and Bacteria across different Endophyte Samples. The chart illustrates a significant increase in microbial signal in root metagenomes after flow sorting.]
Flow sorting enhances microbial signal in root metagenomes

Sorted ~500,000 cells from homogenized roots
Flow sorting enhances microbial signal in root metagenomes

Would flow sorting help with your samples?
Sorting active bacteria with Raman-microfluidic device

Wagner Lab
Univ of Vienna

Stocker Lab
MIT
Sorting active bacteria with Raman-microfluidic device

Wagner Lab
Univ of Vienna
Stocker Lab
MIT
Raman active single cell sorting from mouse gut

Barry et al 2015
Bioorthogonal non-canonical amino acid tagging (BONCAT)

- L-Azidohomoalanine (AHA)
- L-Homopropargylglycine (HPG)

incorporated into new proteins instead of Met

Hatzenpichler et al., 2014     Hatzenpichler & Orphan, 2015
Bioorthogonal non-canonical amino acid tagging (BONCAT)

- **L-Methionine (Met)**
- **L-Azidohomoalanine (AHA)**
- **L-Homopropargylglycine (HPG)**

incorporated into new proteins instead of Met

Alkyne-labeled protein + Azide-modified dye → Triazole conjugate

Hatzenpichler et al., 2014  Hatzenpichler & Orphan, 2015
Identification of translationally active cells

Hatzenpichler et al., 2014
Sorting and genomic sequencing of translationally active microbial consortia

1. Incubate with HPG
2. BONCAT
3. Isolate via FACS
4. Cell lysis and WGA
5. Genome sequencing
6. Bioinformatics

16S rRNA gene tag screening

figure modified from Rinke et al., 2013
Questions?