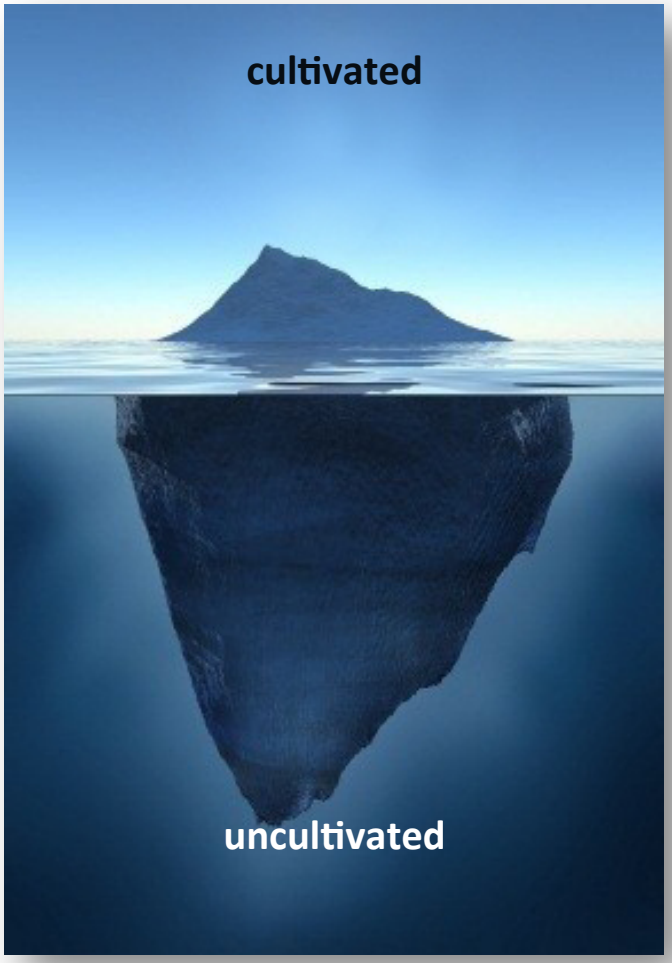
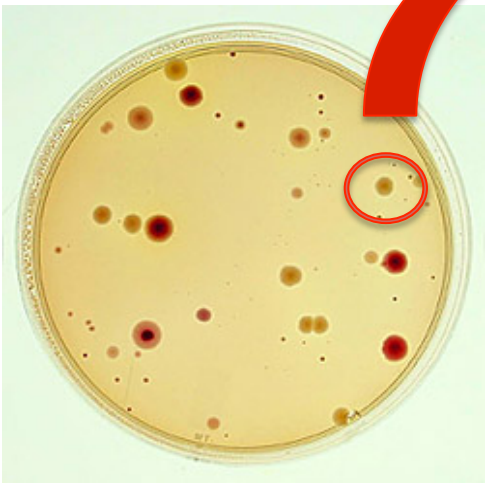
Overview Single Cell Genomics Pipeline

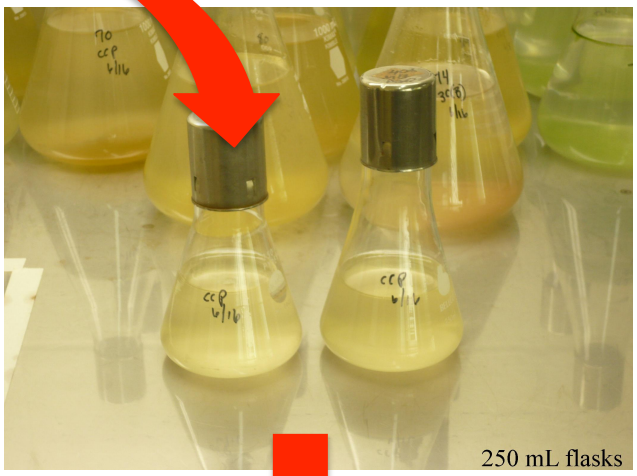
Mar 22, 2016

How do we study the uncultured microbial majority?

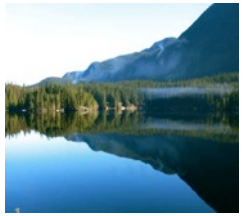


We need ng's of DNA to sequence a microbial genome

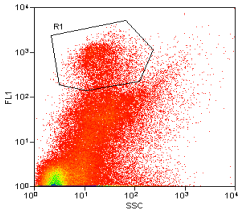




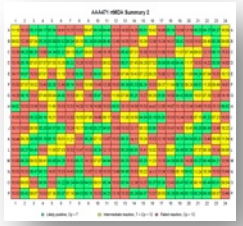
- Most microbes will not grow in the lab
- Single-cell genomics bypasses need to culture



Sampling



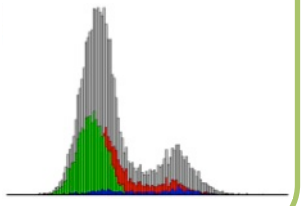
Cell sorting



Genome amplification

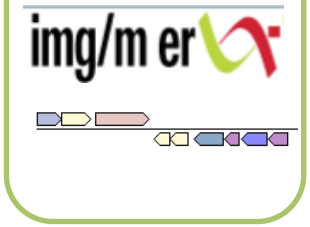
Cell identification

Sequencing



Data QC

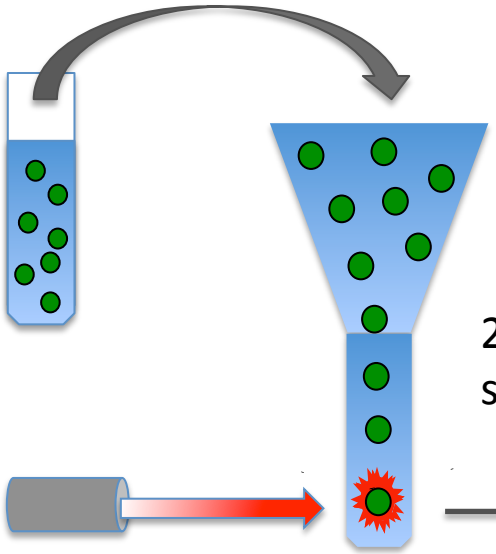
Assembly



Annotation

Draft Genome

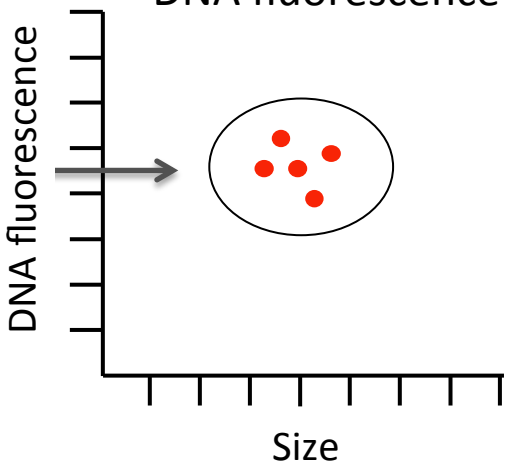
1. Microbes loaded into flow cytometer

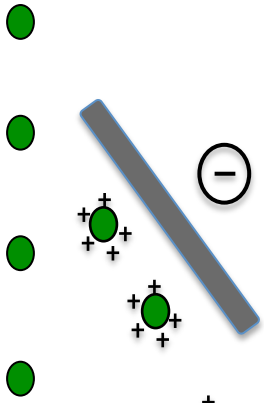


2. Cells line up single file

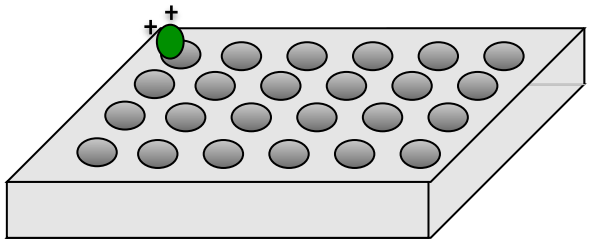
3. Laser shines on cells

4. Cells identified by DNA fluorescence & size

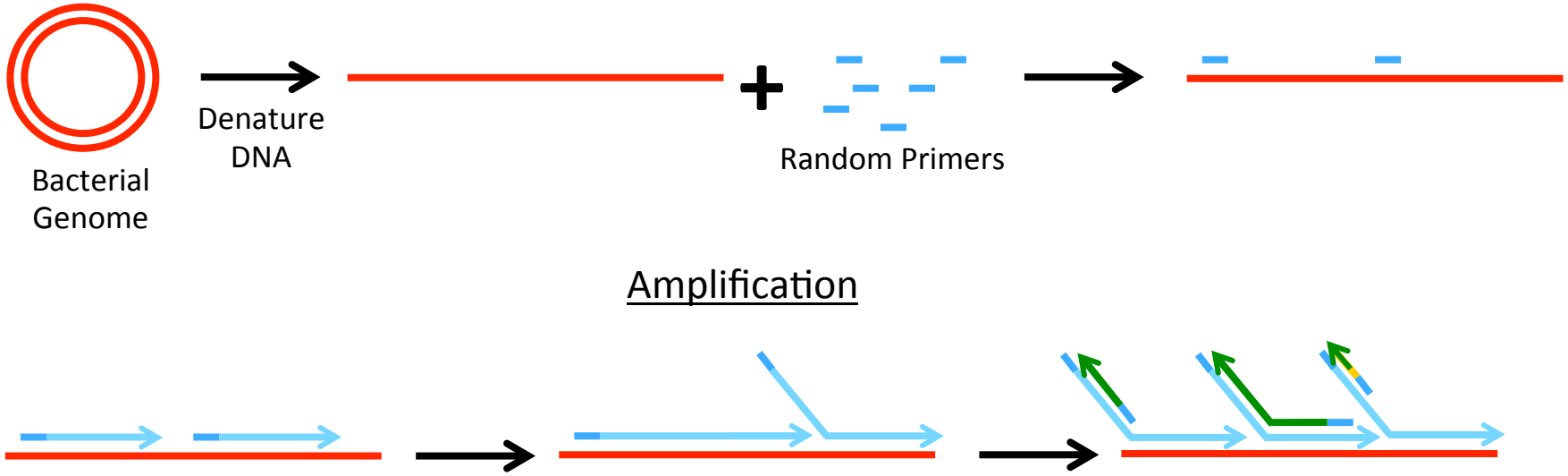




5. Individual cells directed to wells of plate using electric charge



Multiple displacement amplification with Phi29 produces ~100ng of DNA from a single cell



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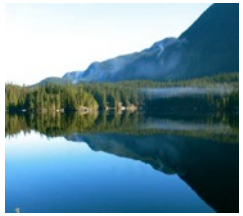

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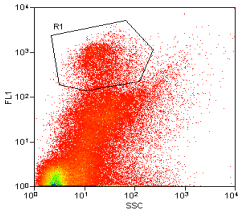
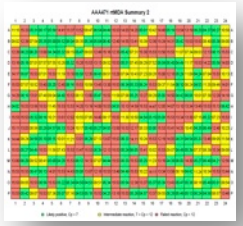


Cell sorting



Genome amplification

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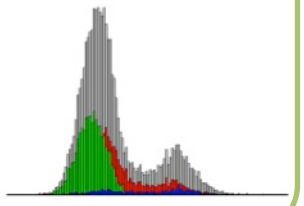

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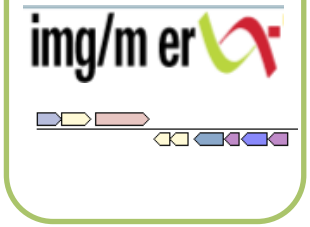
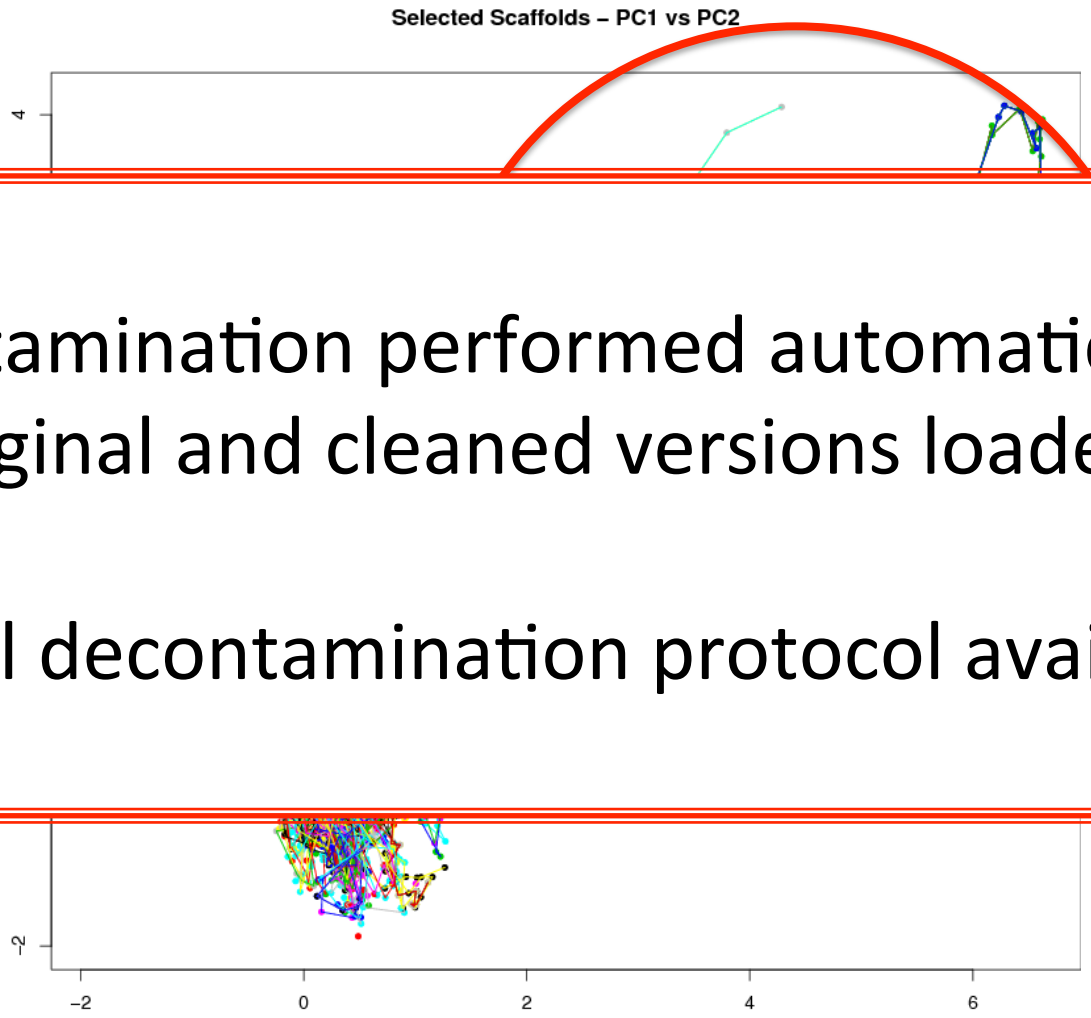


Annotation

Draft Genome

Post-assembly QC identifies contaminant contigs



Decontamination performed automatically
- original and cleaned versions loaded to IMG

Manual decontamination protocol available

nts

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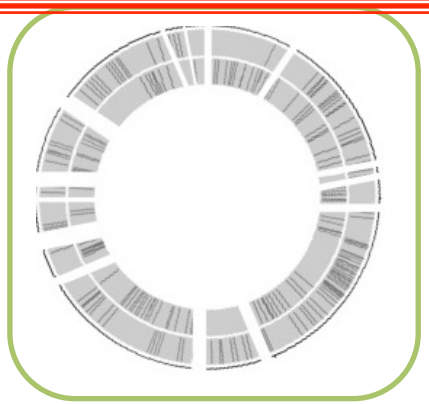
Service available for large and small scale CSP projects



Draft Genome

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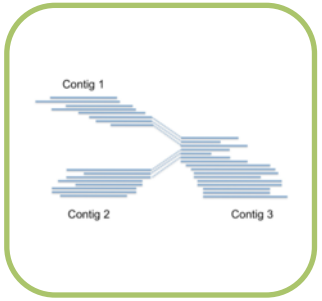

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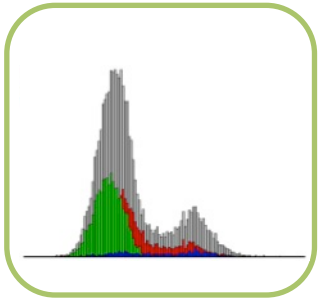
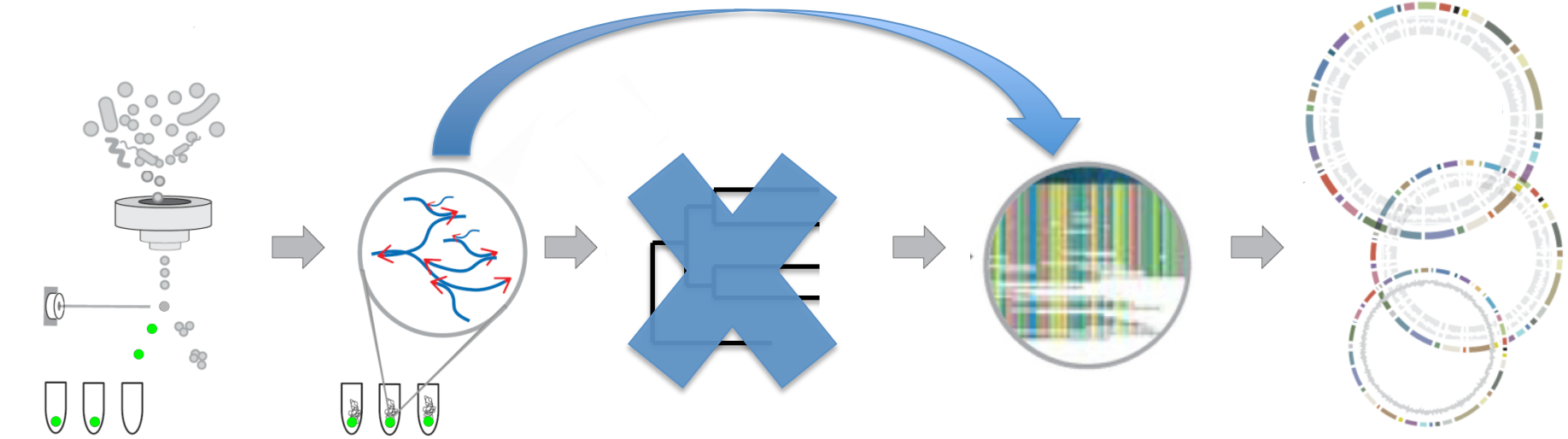


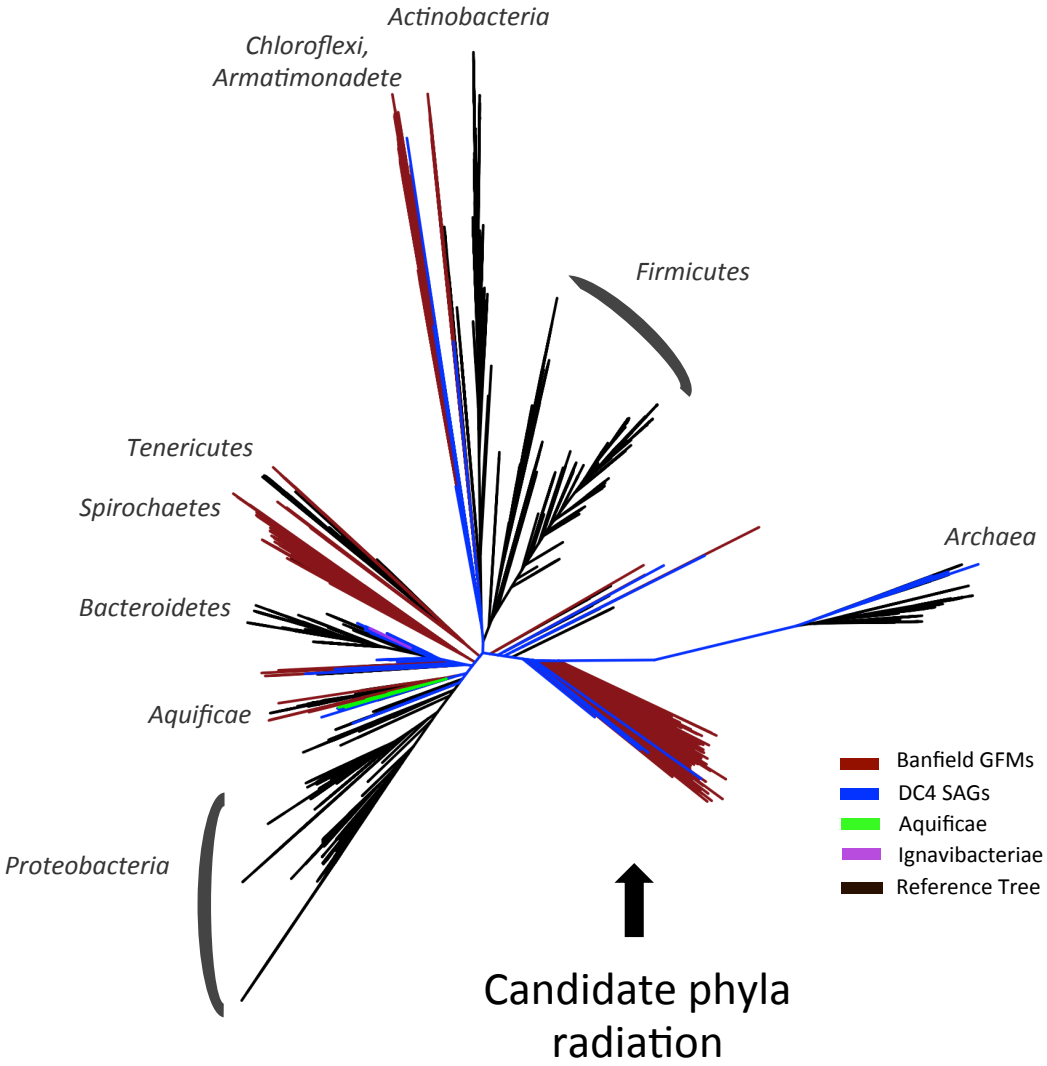
Data QC

Bypassing 16S screening prior to genome sequencing

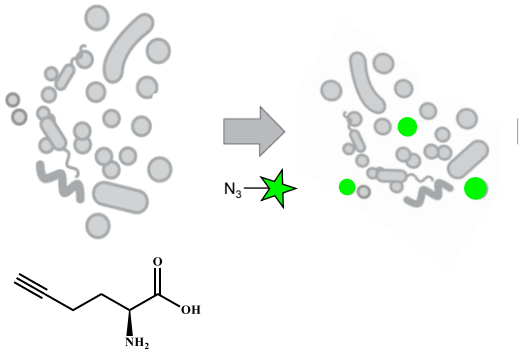


Recovering genomes from unusual microbes missed by “universal” 16S primers

300 SAGs from hot spring (Dewar Creek)



Sorting and genomic sequencing of translationally active microbes



Incubate with HPG

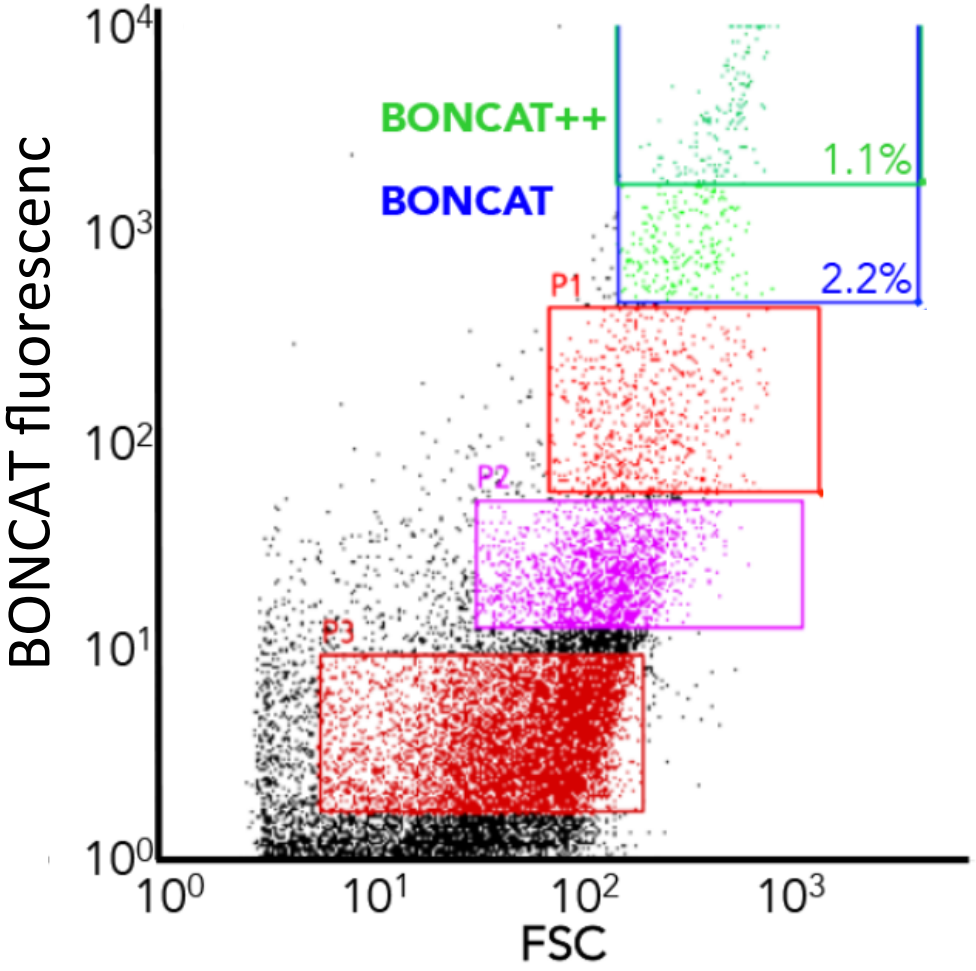
BONCAT

1) Incubate cells with non-canonical amino acid (HPG)

2) HPG incorporated into newly synthesize proteins

3) Fluorescent label added to HPG using click chemistry

Successful sorting and amplification of translationaly-active microbial aggregates



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Hatzenpichler et al (in review)

Questions?

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