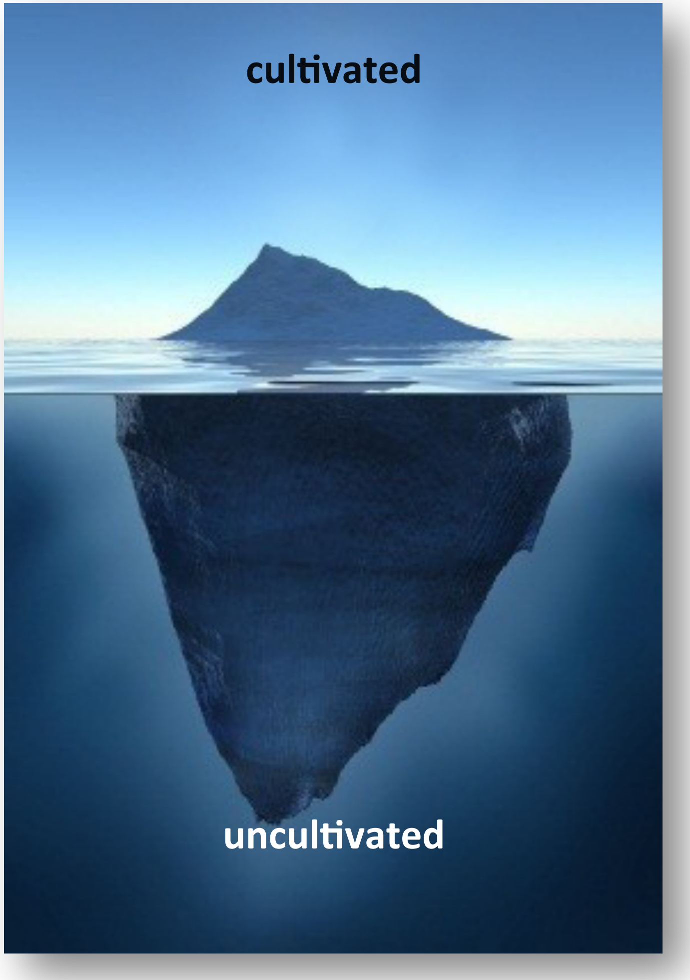
Overview Microscale Applications Group

Mar 24, 2015

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

How do we study the uncultured majority?



Single Cell Genomics Pipeline

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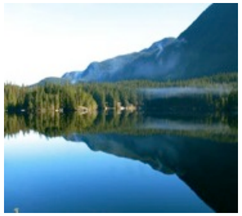
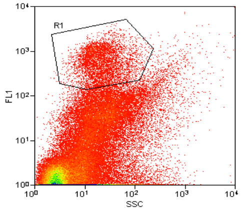
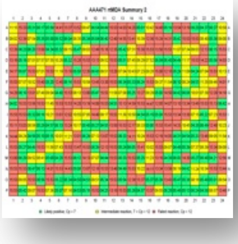
Single-cell genomics bypasses need to culture



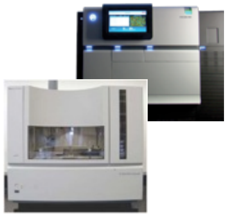
Sampling



Cell sorting

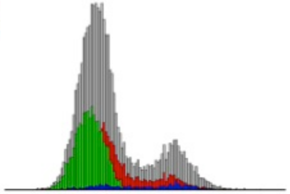


Genome amplification

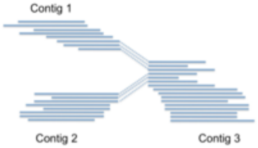


Cell identification

Sequencing



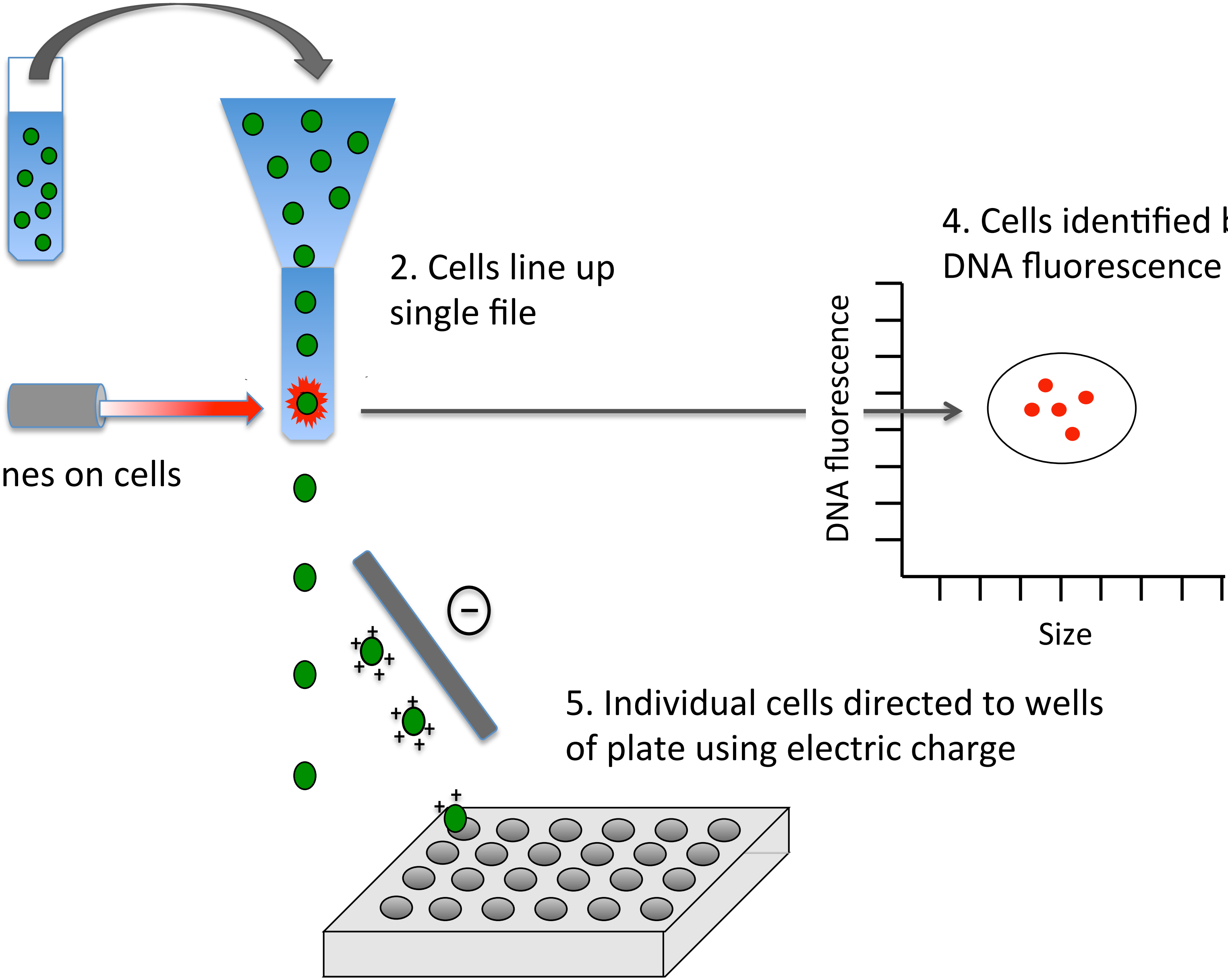
Data QC



Assembly

Annotation

Draft Genome



1. Microbes loaded into flow cytometer

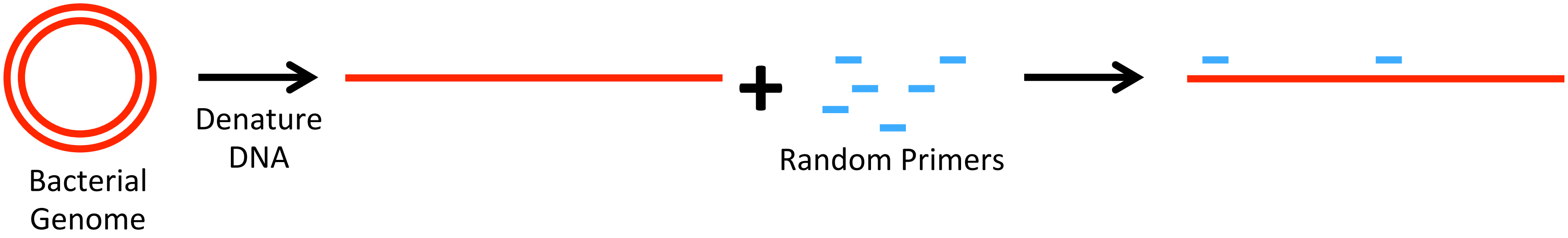
2. Cells line up single file

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

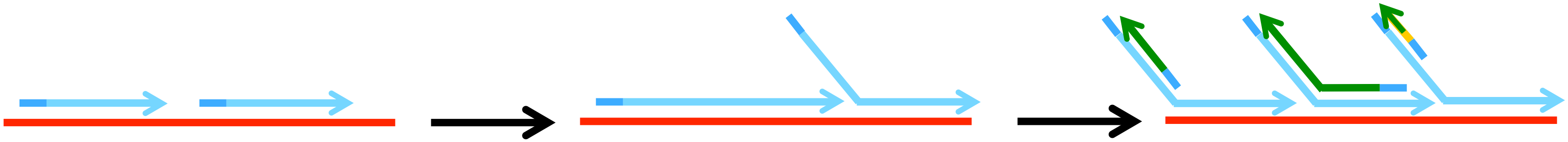


Bacterial Genome

Denature DNA

Random Primers

Amplification



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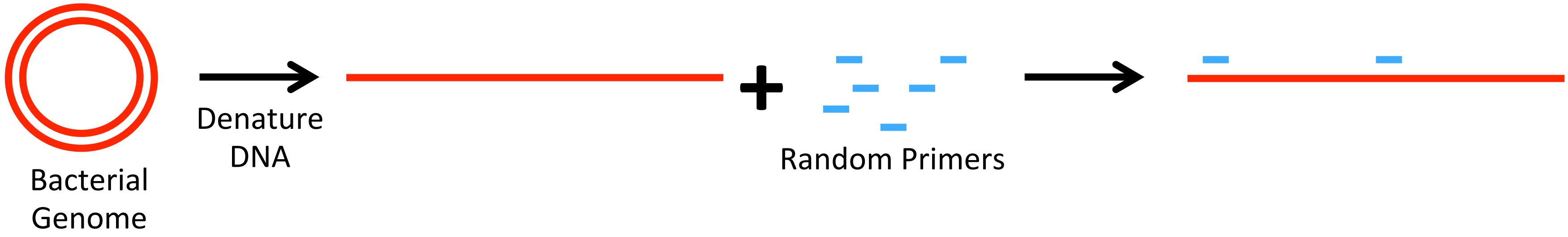

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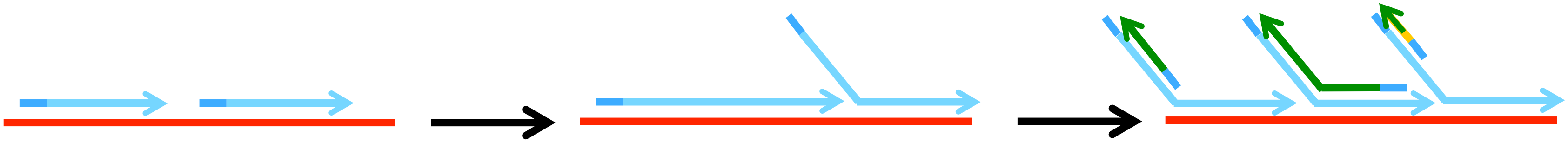

Lots of Copies!

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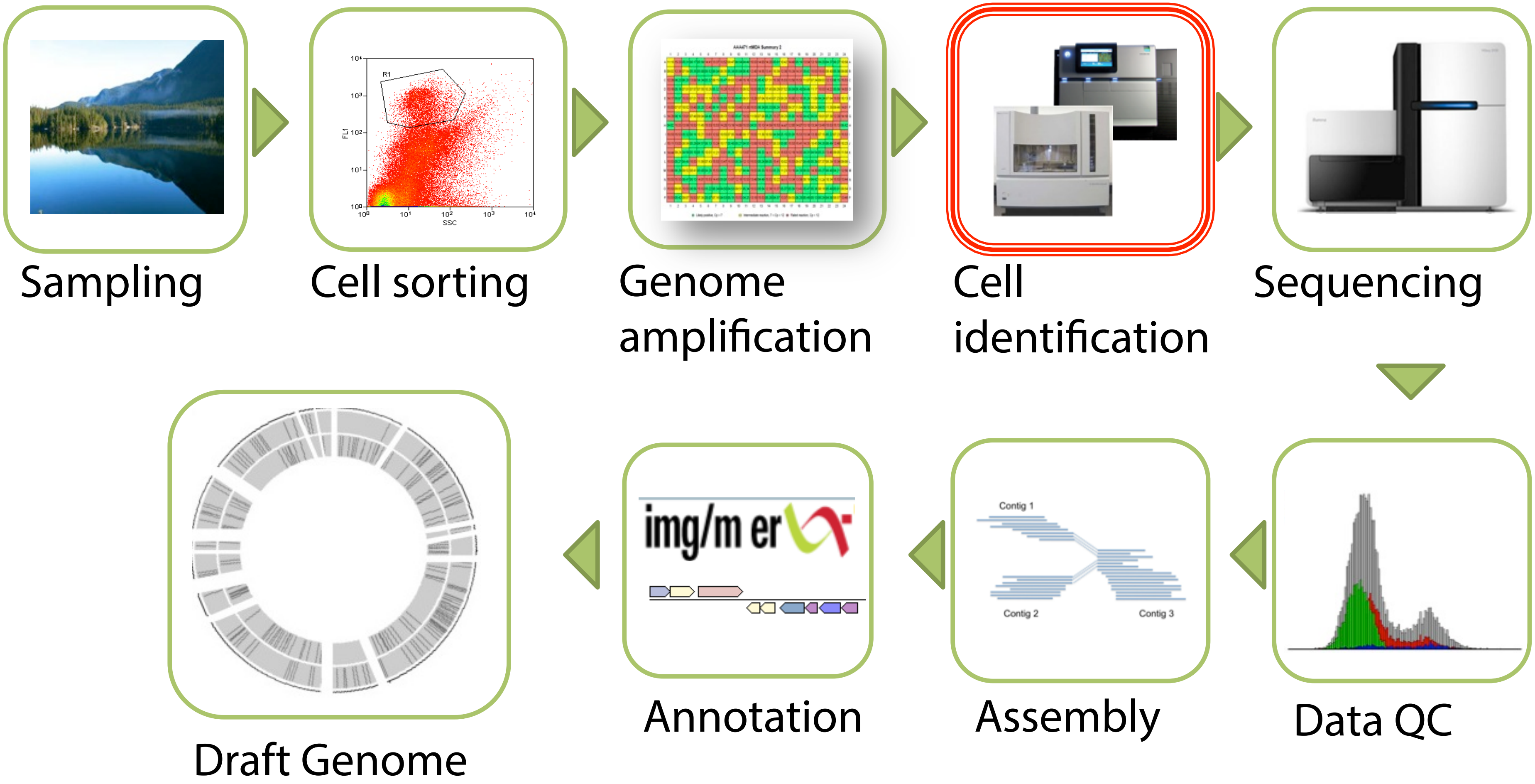


Sampling

Cell sorting

Genome amplification

Cell identification

Sequencing

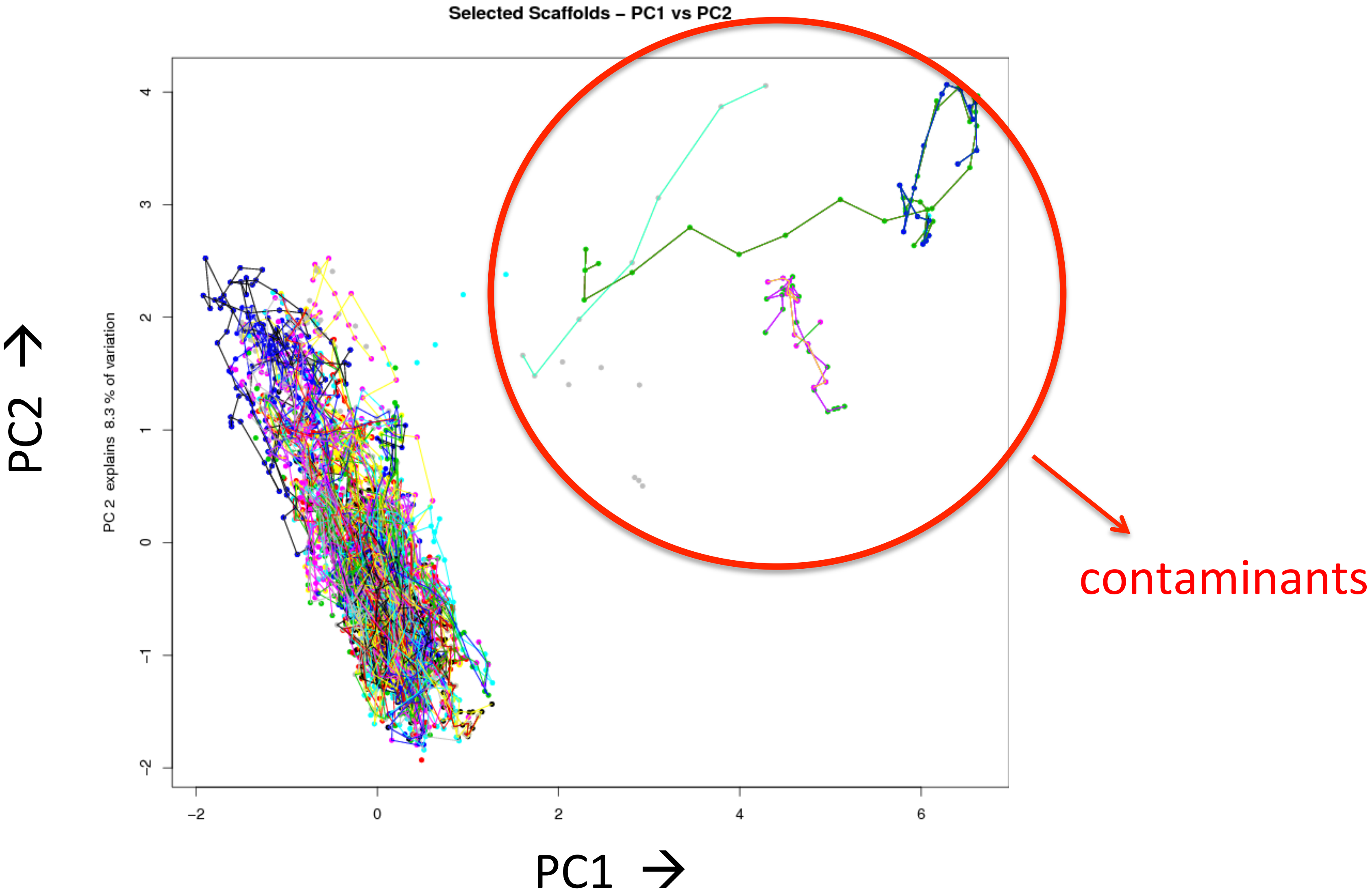
Data QC

Assembly

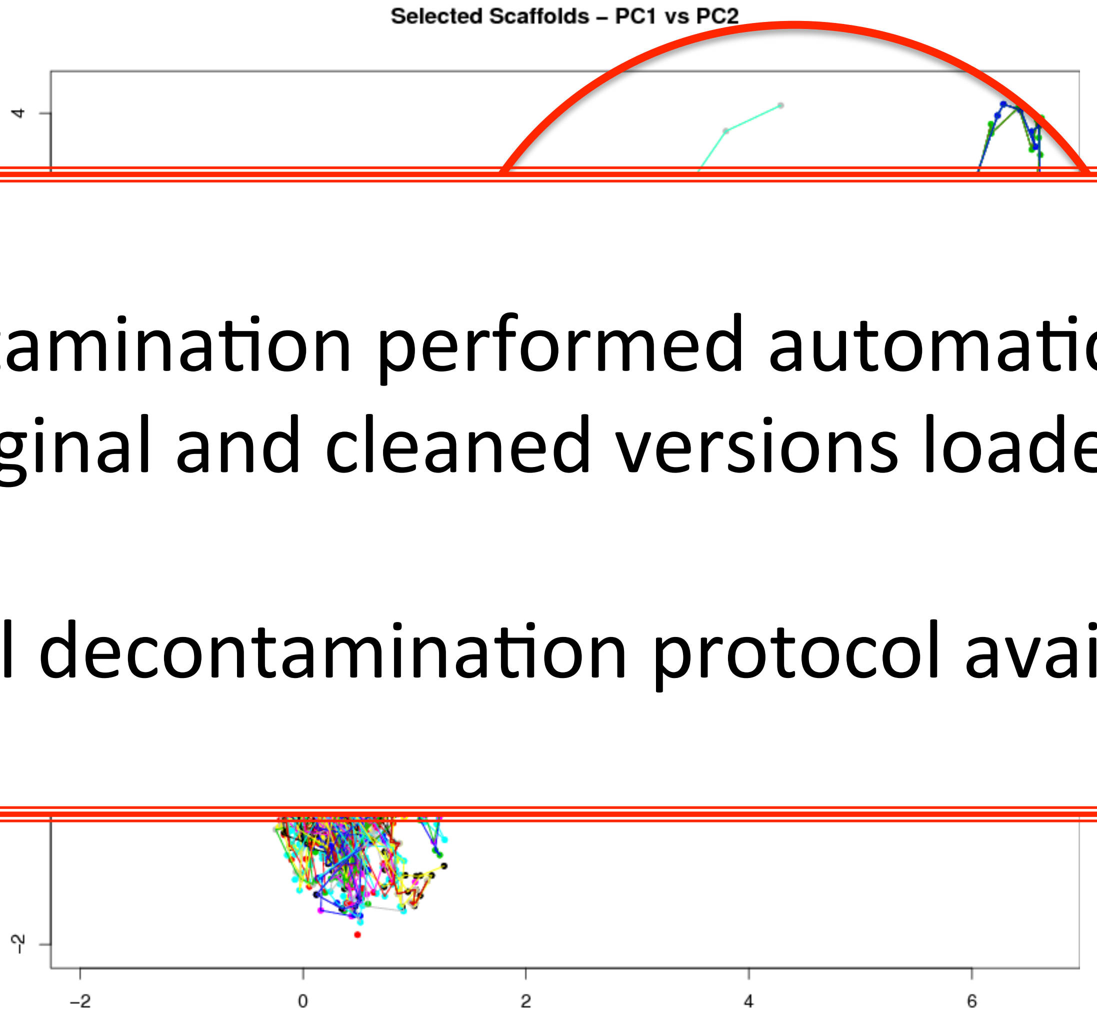
Annotation

Draft Genome

Post-assembly QC identifies contaminant contigs



Post-assembly QC identifies contaminant contigs



Decontamination performed automatically

- original and cleaned versions loaded to IMG

Manual decontamination protocol available

nts

PC1 →

Single Cell Genomics Pipeline

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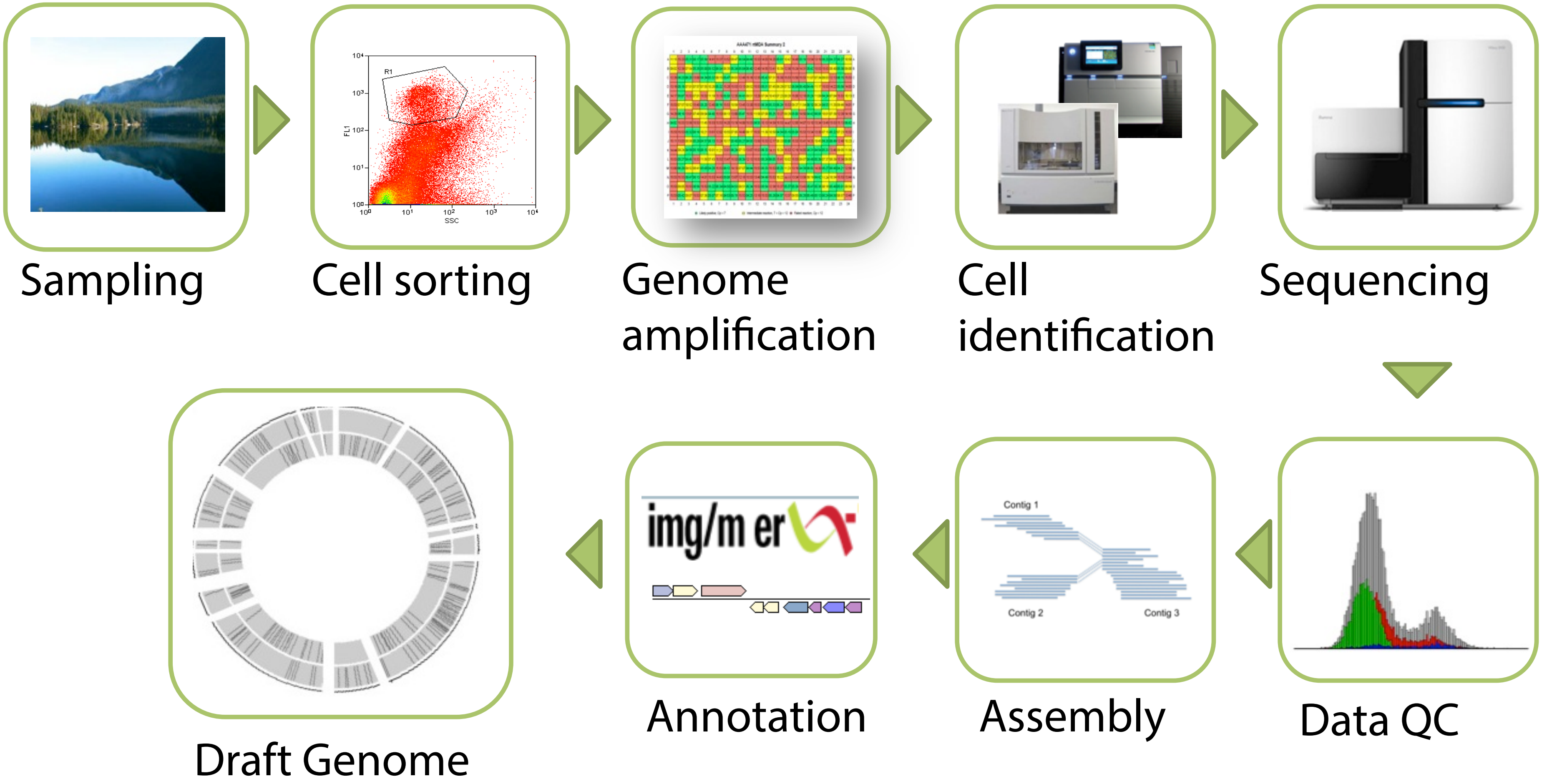

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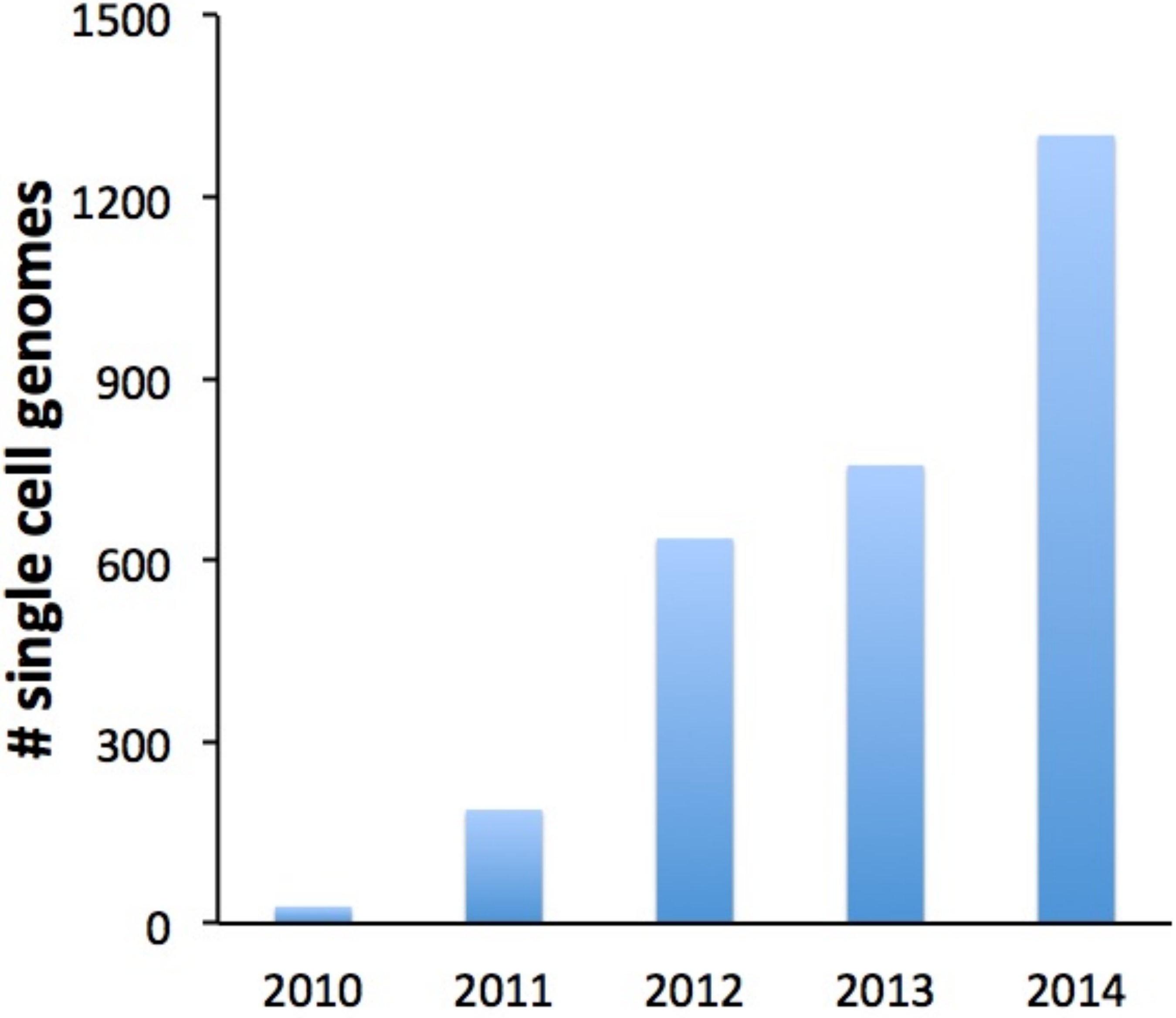
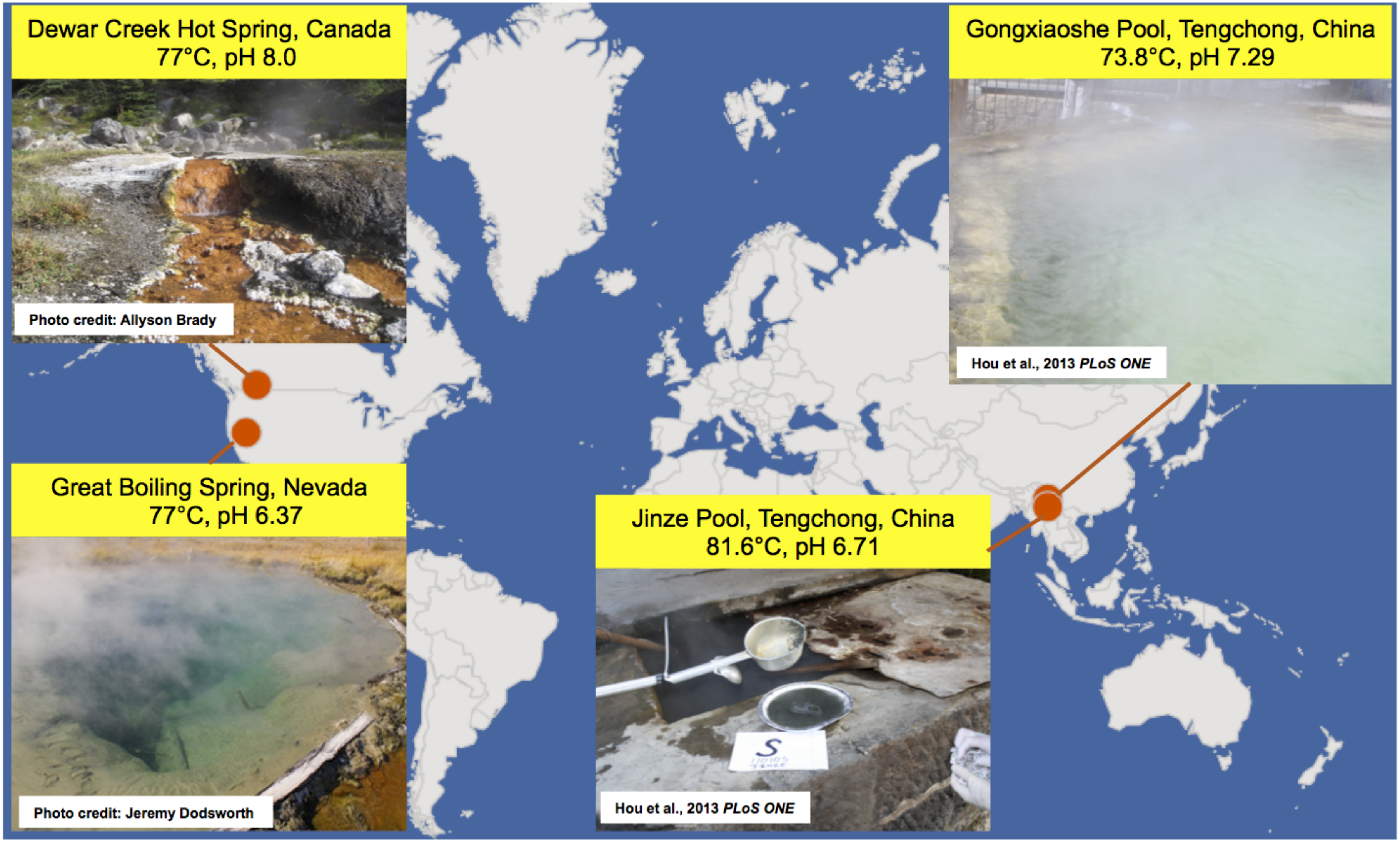
User Requests for Single Cell Genomes Continues to Grow



New phylum revealed through metagenomics and single cell genomics



Dewar Creek Hot Spring, Canada
77°C, pH 8.0

Photo credit: Allyson Brady

Great Boiling Spring, Nevada
77°C, pH 6.37

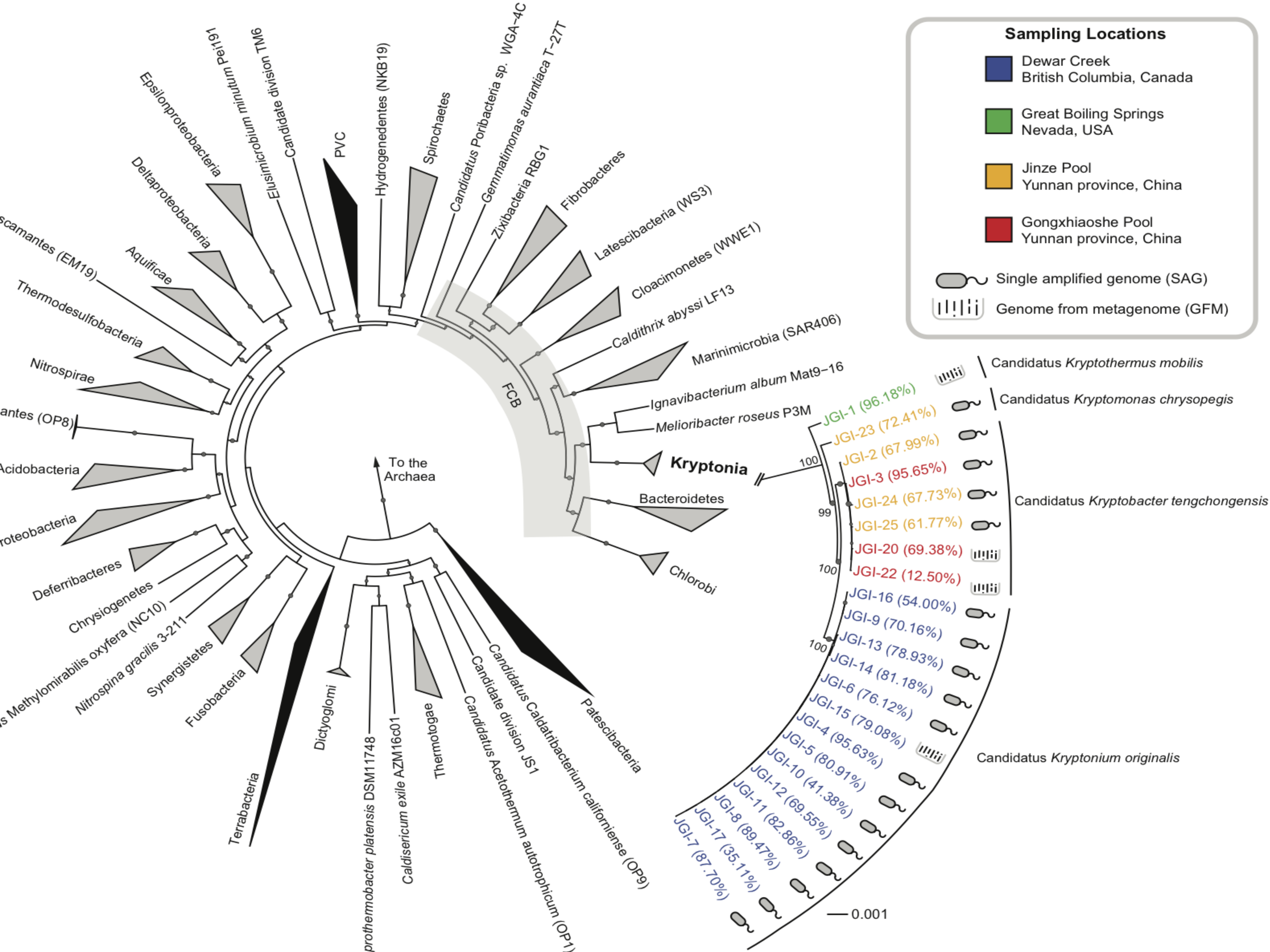
Photo credit: Jeremy Dodsworth

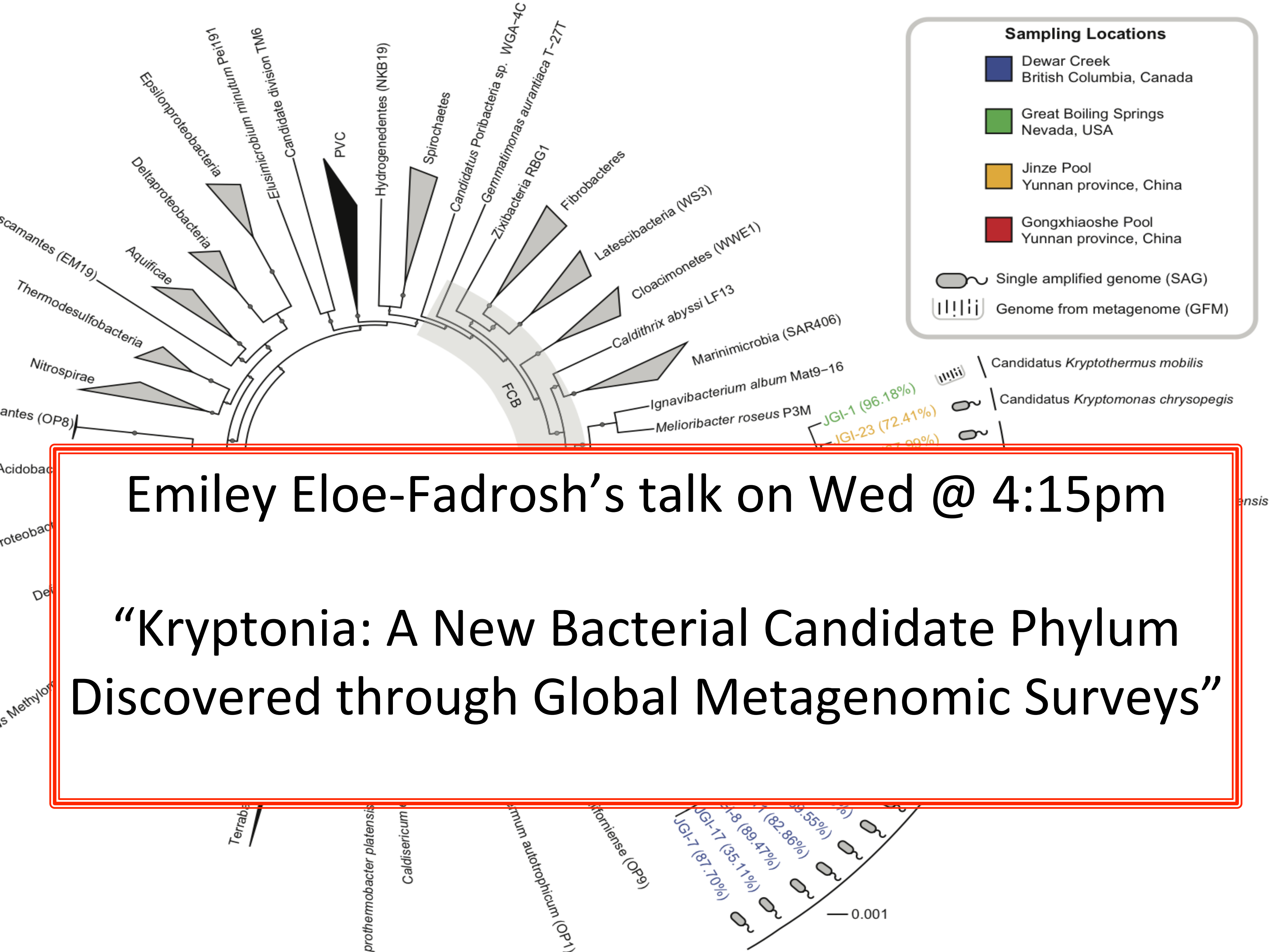
Gongxiaoshe Pool, Tengchong, China
73.8°C, pH 7.29

Hou et al., 2013 *PLoS ONE*

Jinze Pool, Tengchong, China
81.6°C, pH 6.71

Hou et al., 2013 *PLoS ONE*

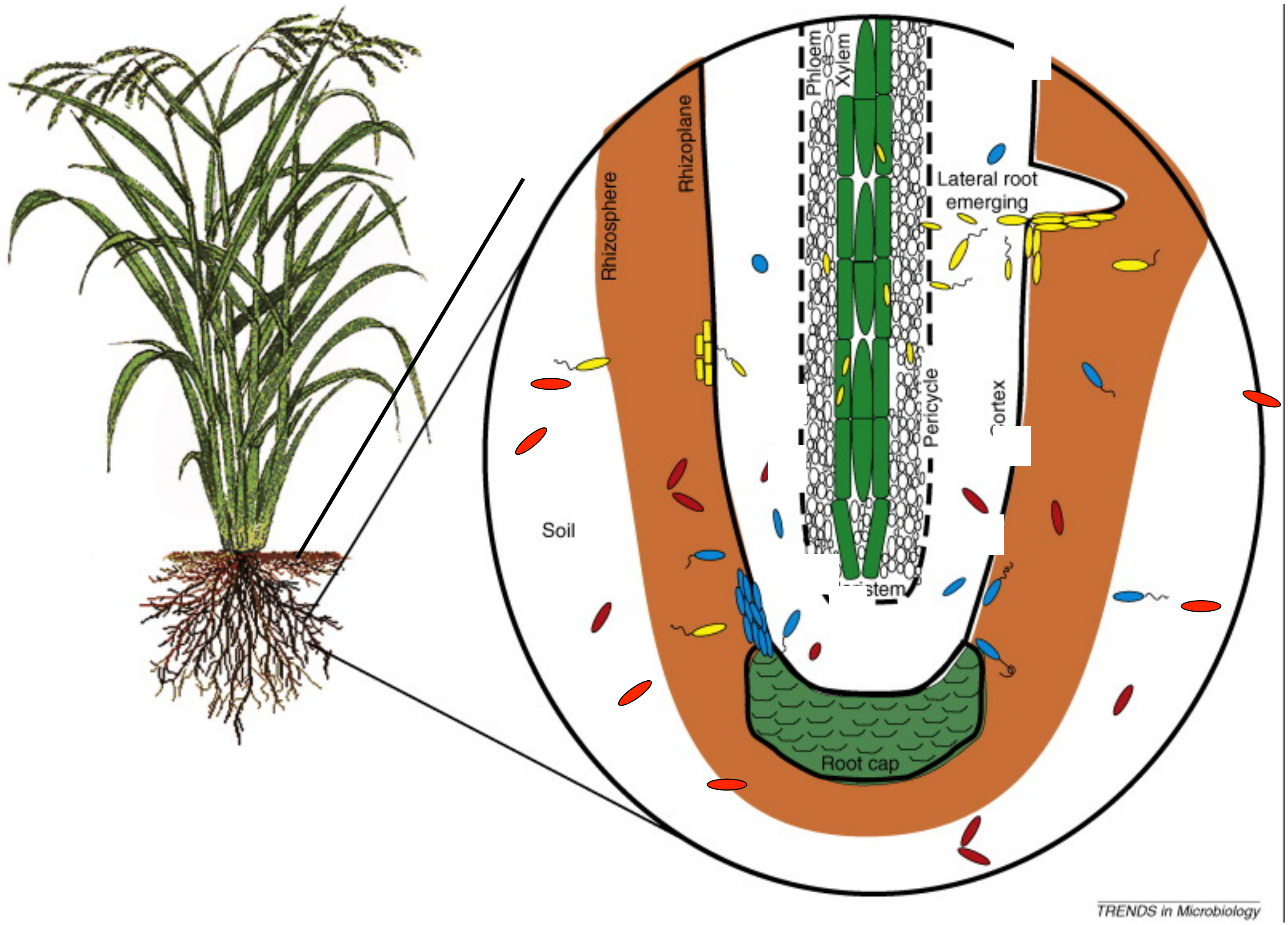

Sampling Locations
🟦 Dewar Creek
British Columbia, Canada
🟩 Great Boiling Springs
Nevada, USA
🟨 Jinze Pool
Yunnan province, China
🟥 Gongxhiaoshe Pool
Yunnan province, China
Single amplified genome (SAG)
Genome from metagenome (GFM)
scamantes (EM19)
Thermodesulfobacteria
Nitrospirae
antes (OP8)
Acidobacteria
roteobacteria
Deferribacteres
Chrysiogenetes
s Methylomirabilis oxyfera (NC10)
Nitrospina gracilis 3-211
Synergistetes
Fusobacteria
Terrabacteria
Dictyoglomi
prothermobacter platensis DSM11748
Caldisericum exile AZM16c01
Thermotogae
Candidatus Acetothermum autotrophicum (OP1
Candidate division JS1
Candidatus Caldatribacterium californiense (OP9)
Patescibacteria
Aquificae
Deltaproteobacteria
Epsilonproteobacteria
Elusimicrobium minutum Pei191
Candidate division TM6
PVC
Hydrogenedentes (NKB19)
Spirochaetes
Candidatus Poribacteria sp. WGA-4C
Gemmatimonas aurantiaca T-27T
Zixibacteria RBG1
Fibrobacteres
Latescibacteria (WS3)
Cloacimonetes (WWE1)
Caldithrix abyssi LF13
Marinimicrobia (SAR406)
Ignavibacterium album Mat9-16
Melioribacter roseus P3M
Kryptonia
Bacteroidetes
Chlorobi
FCB
To the Archaea
100
99
100
100
JGI-1 (96.18%)
JGI-23 (72.41%)
JGI-2 (67.99%)
JGI-3 (95.65%)
JGI-24 (67.73%)
JGI-25 (61.77%)
JGI-20 (69.38%)
JGI-22 (12.50%)
JGI-16 (54.00%)
JGI-9 (70.16%)
JGI-13 (78.93%)
JGI-14 (81.18%)
JGI-6 (76.12%)
JGI-15 (79.08%)
JGI-4 (95.63%)
JGI-5 (80.91%)
JGI-10 (41.38%)
JGI-12 (69.55%)
JGI-11 (82.86%)
JGI-8 (89.47%)
JGI-17 (35.11%)
JGI-7 (87.70%)
Candidatus Kryptothermus mobilis
Candidatus Kryptomonas chrysopegis
Candidatus Kryptobacter tengchongensis
Candidatus Kryptonium originalis
0.001



Emiley Eloe-Fadrosh’s talk on Wed @ 4:15pm

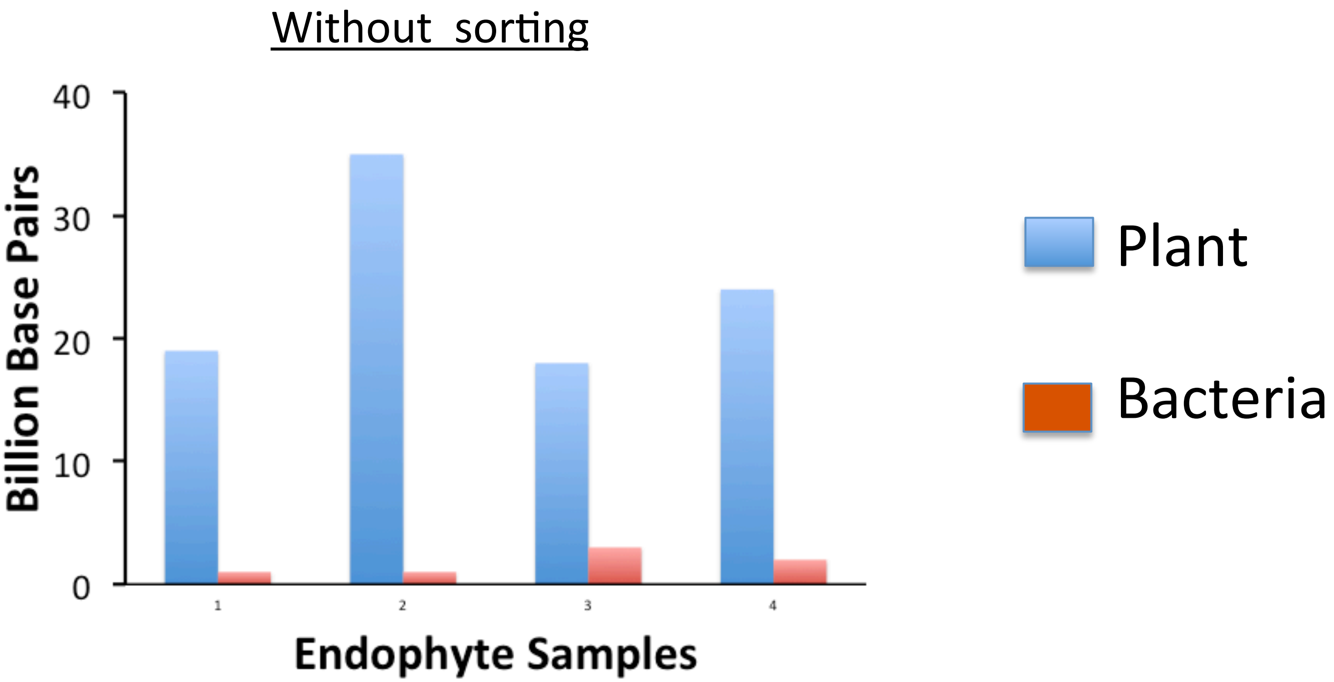
“Kryptonia: A New Bacterial Candidate Phylum Discovered through Global Metagenomic Surveys”

Flow sorting enables metagenomic analysis of endophytes

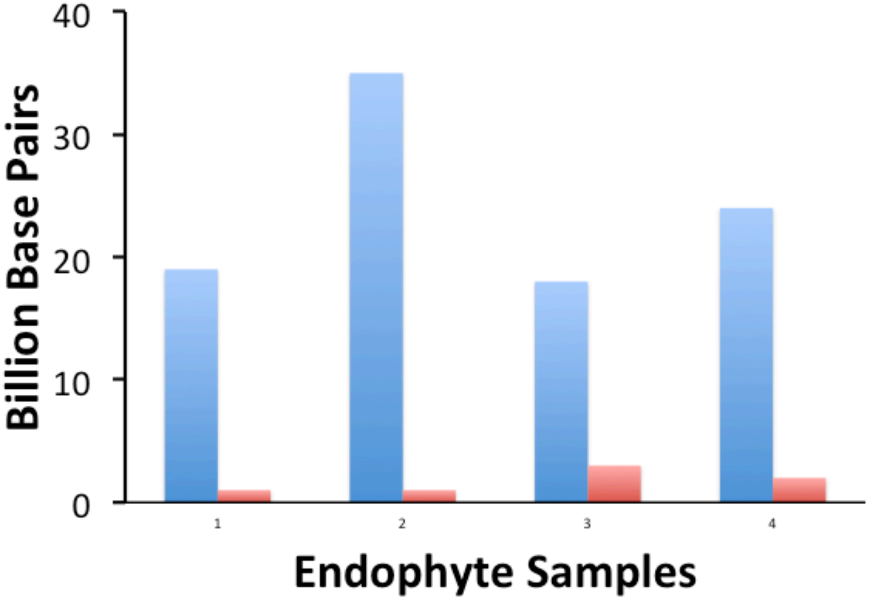


TRENDS in Microbiology

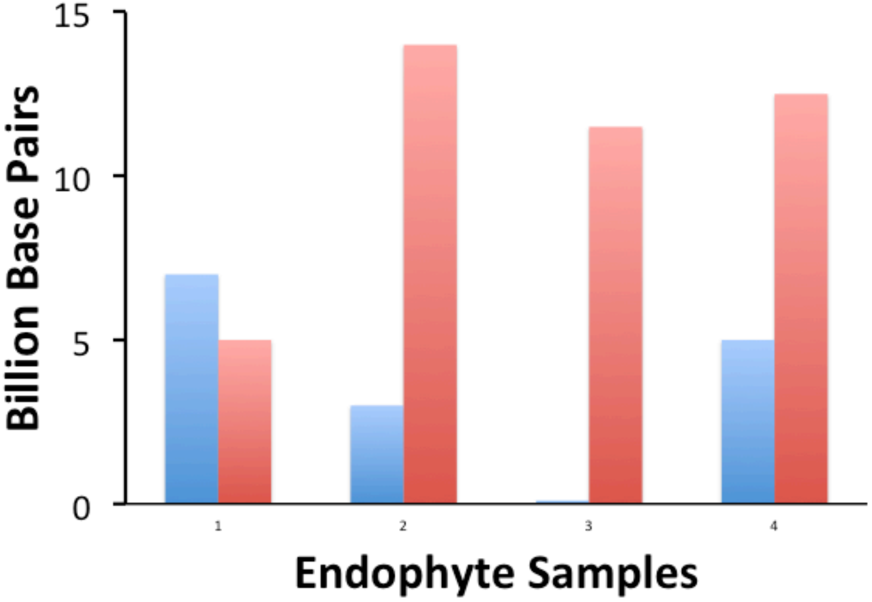
Flow sorting enhances microbial signal in root metagenomes


Without sorting

Flow sorting enhances microbial signal in root metagenomes



Without sorting

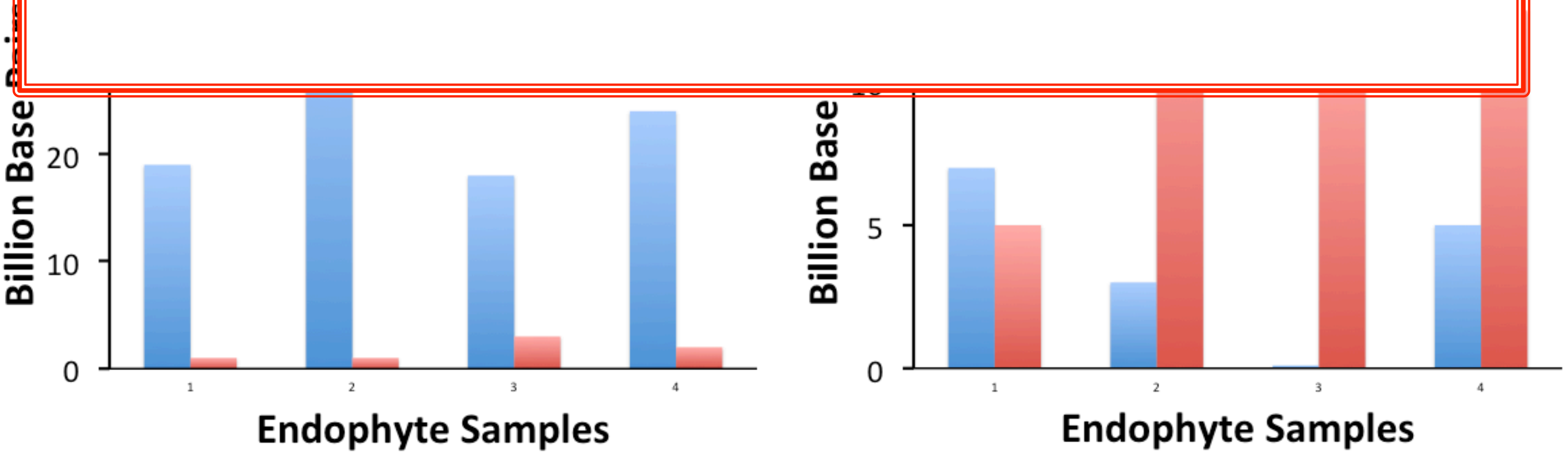


With sorting

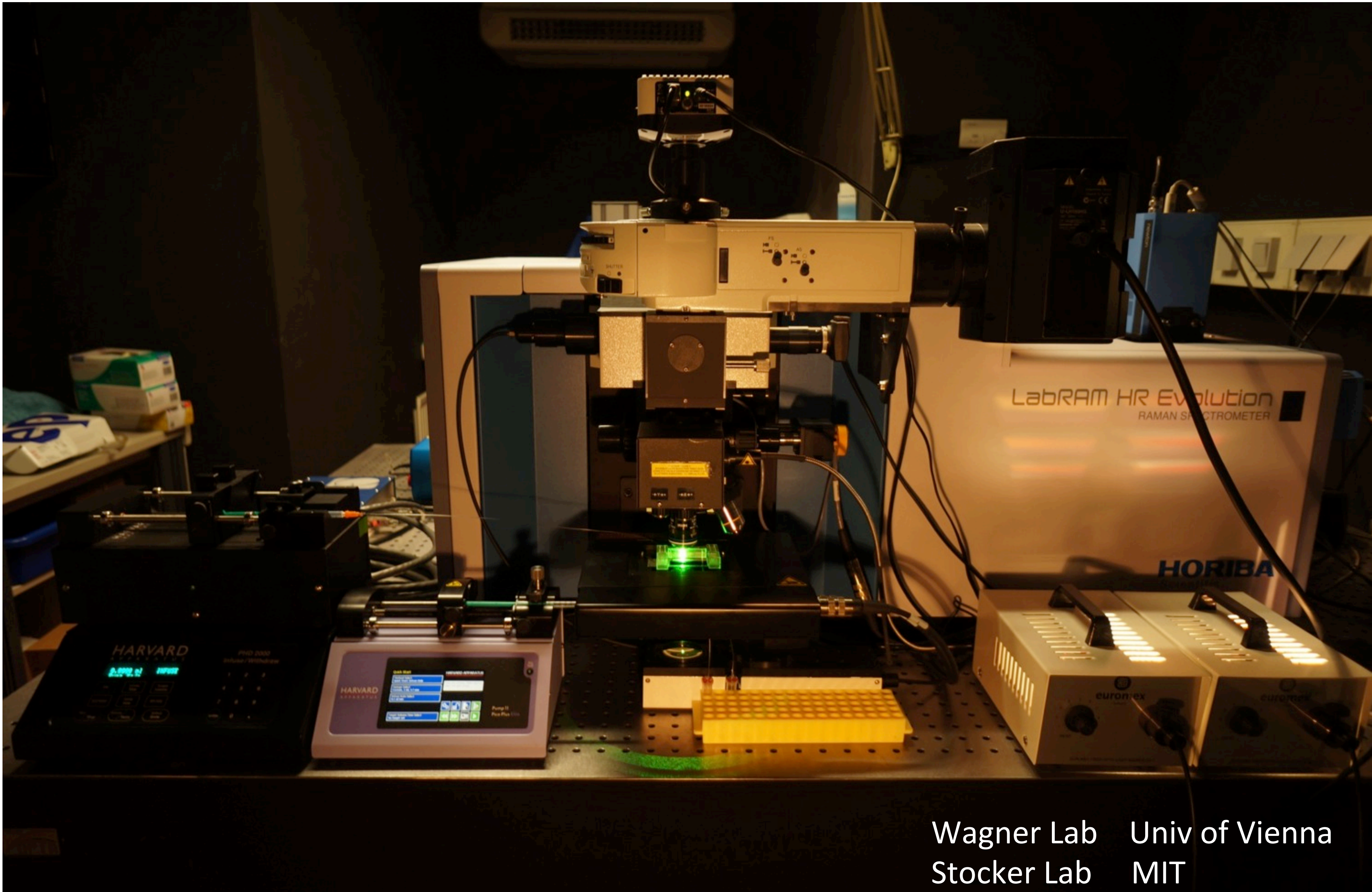
Sorted ~500,000 cells from homogenized roots

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

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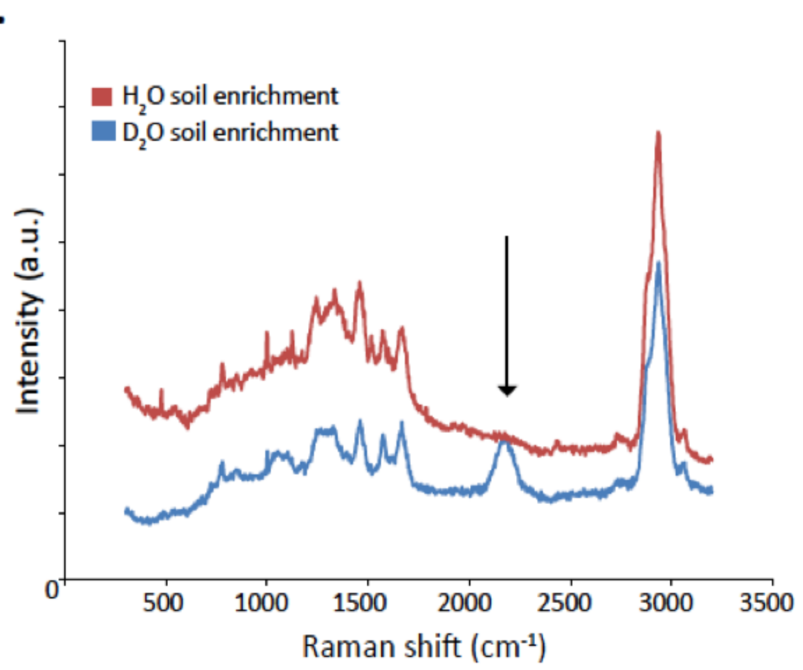

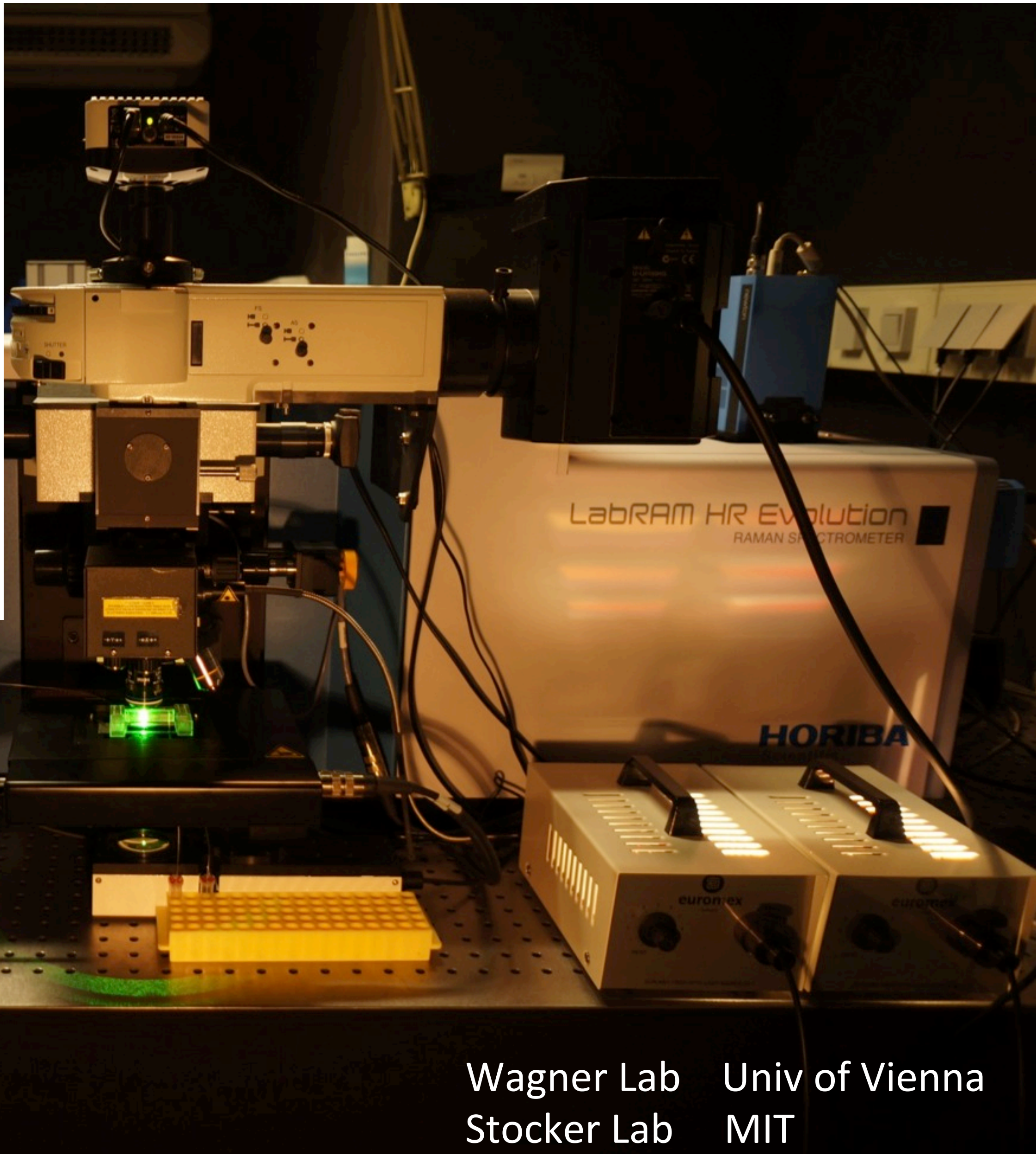

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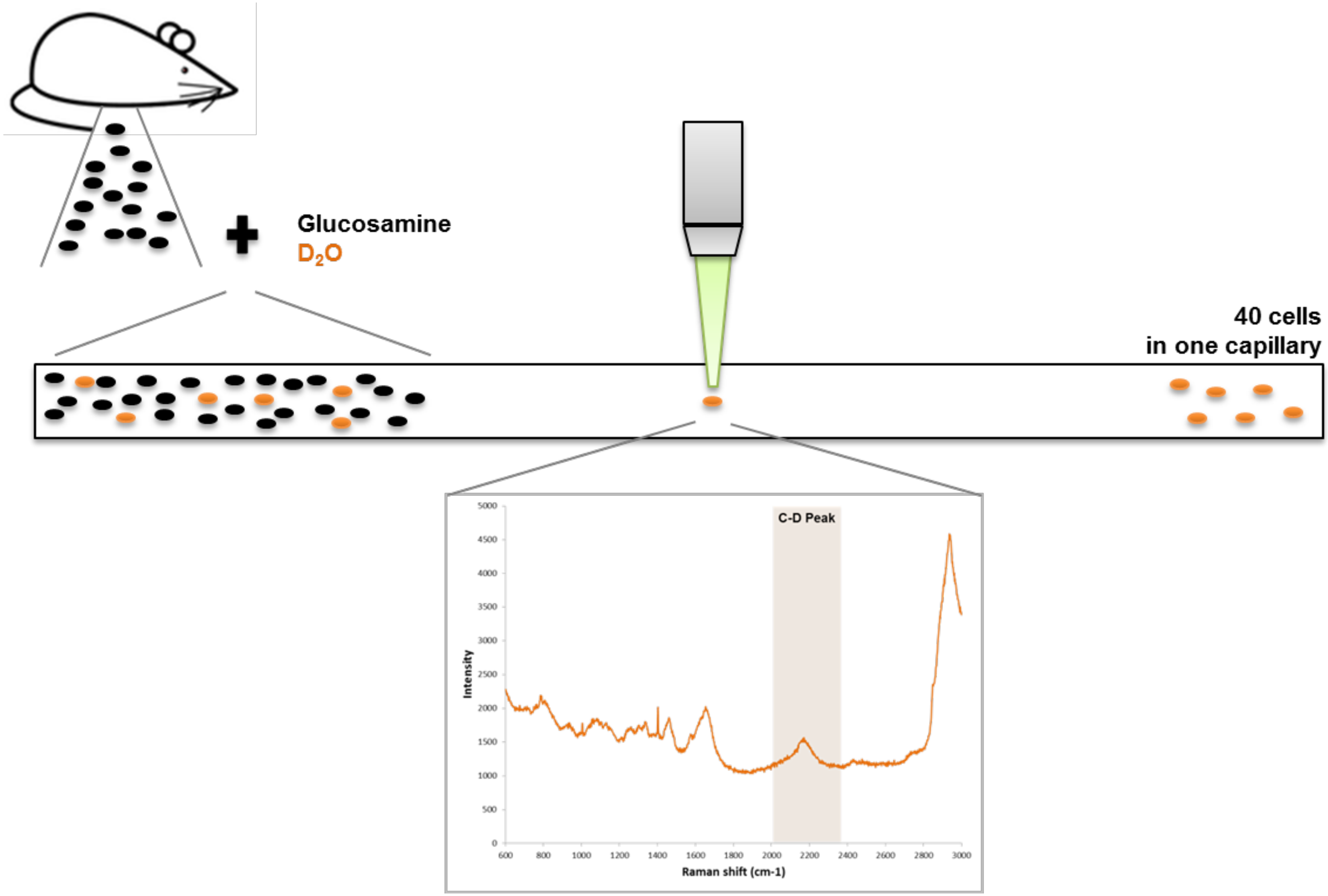
Raman active single cell sorting from mouse gut



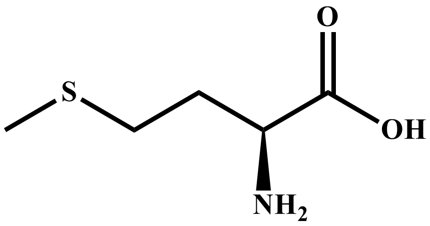
Glucosamine

D_2O

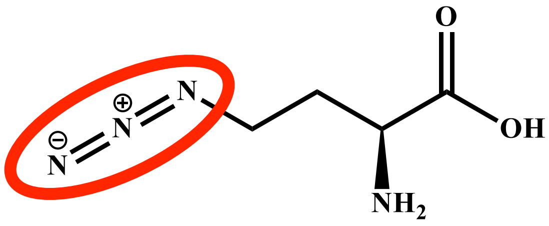
40 cells
in one capillary

Barry et al 2015

Bioorthogonal non-canonical amino acid tagging (BONCAT)

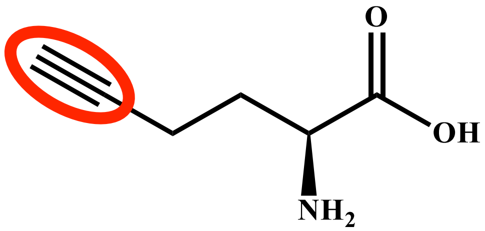


***L*-Methionine**
Met



***L*-Azidohomoalanine**
AHA

or

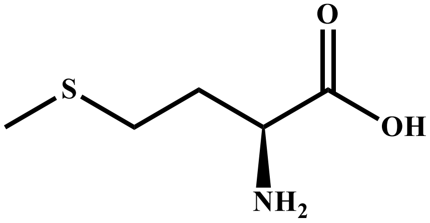


***L*-Homopropargylglycine**
HPG

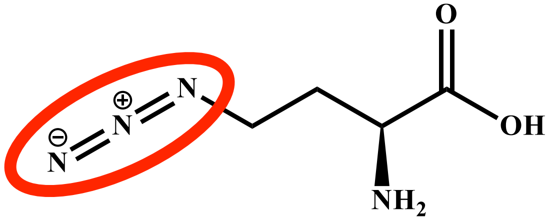
incorporated into new proteins instead of Met

Hatzenpichler *et al.*, 2014 **Hatzenpichler & Orphan, 2015**

Bioorthogonal non-canonical amino acid tagging (BONCAT)



***L*-Methionine**
Met



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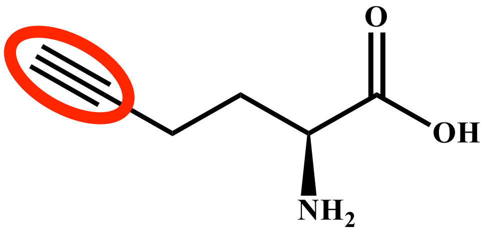

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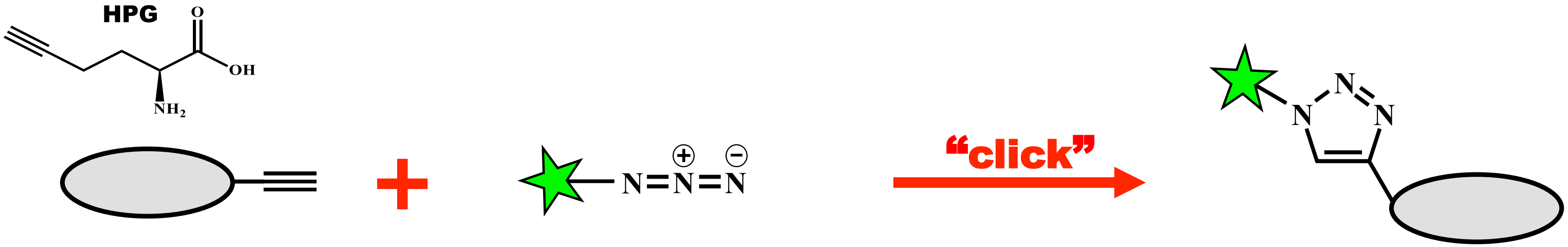


Alkyne-labeled protein

Azide-modified dye

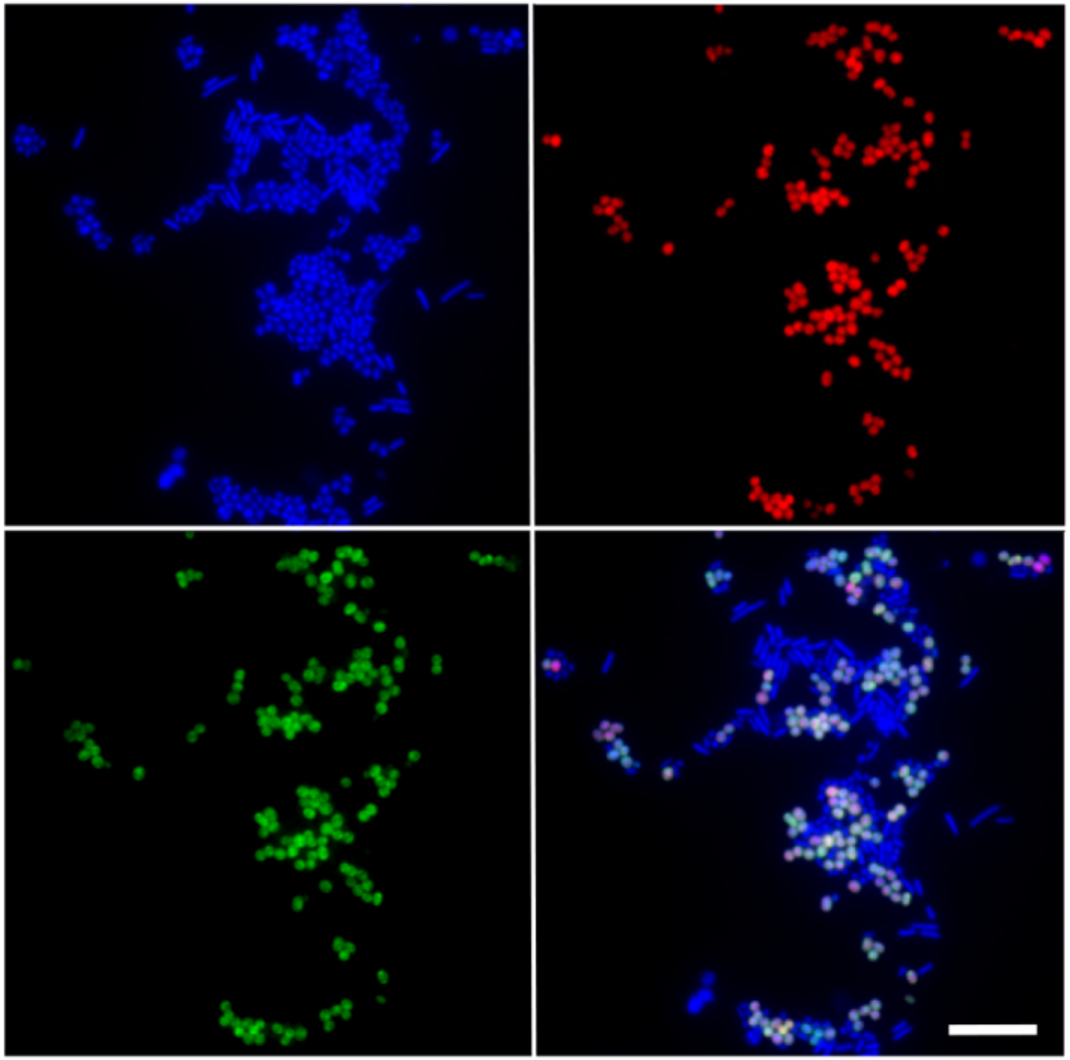
Triazole conjugate

Hatzenpichler *et al.*, 2014 Hatzenpichler & Orphan, 2015

Identification of translationally active cells

DAPI
(DNA)

γ-Proteo WF-1
(rRNA)



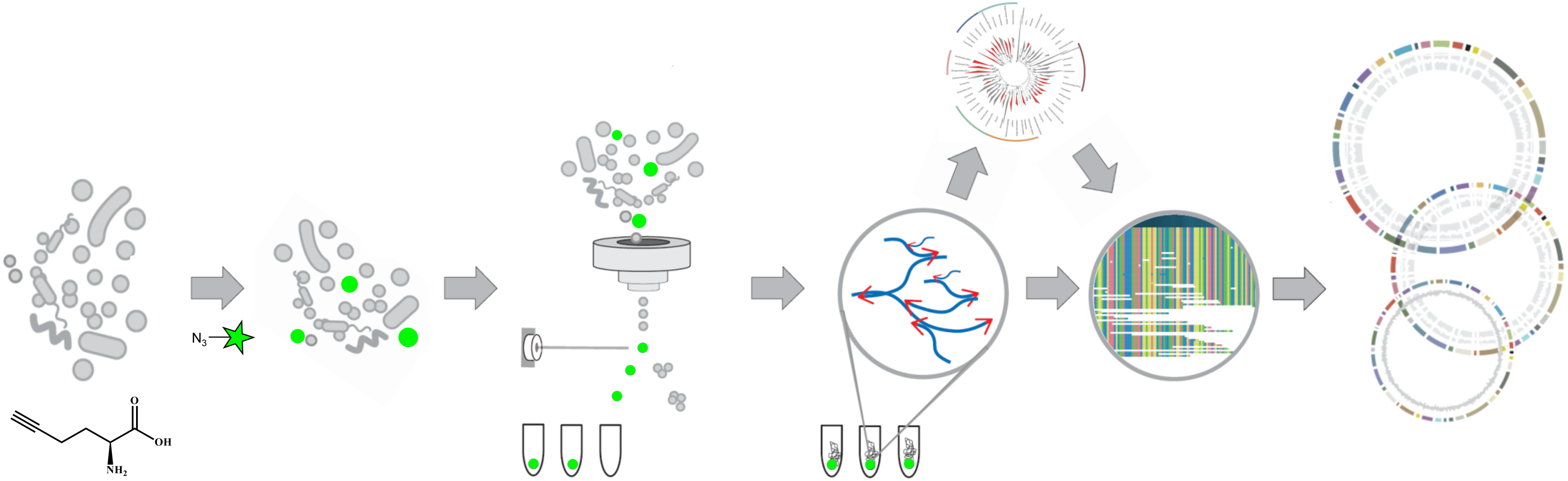
BONCAT
(new proteins)

overlay

Bar = 10 µm

Hatzenpichler *et al.*, 2014

Sorting and genomic sequencing of translationally active microbial consortia



16S rRNA gene tag screening

Incubate with HPG

BONCAT

Isolate via FACS

Cell lysis and WGA

Genome sequencing

Bio-informatics

figure modified from Rinke *et al.*, 2013

Questions?