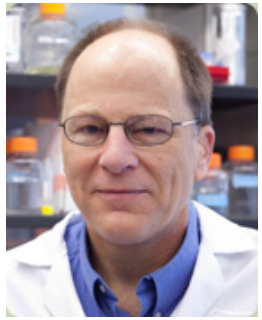
Microfluidic genome transplantation

James Pelletier, Elizabeth Strychalski, Nacyra Assad-Garcia, Vanya Paralanov, Andreas Mershin, Neil Gershenfeld, John Glass



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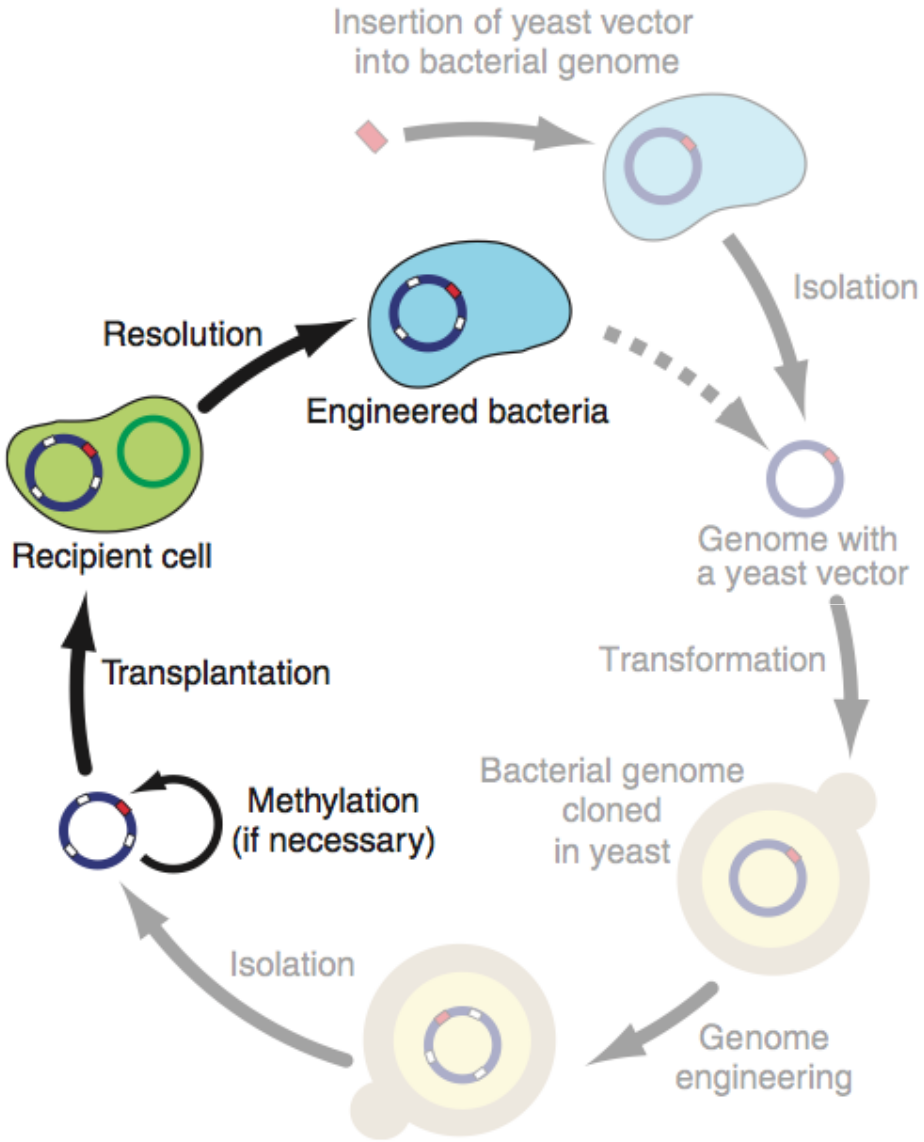
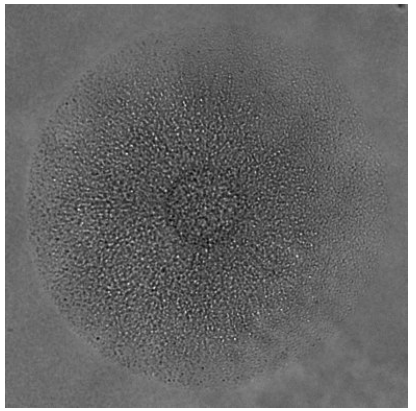
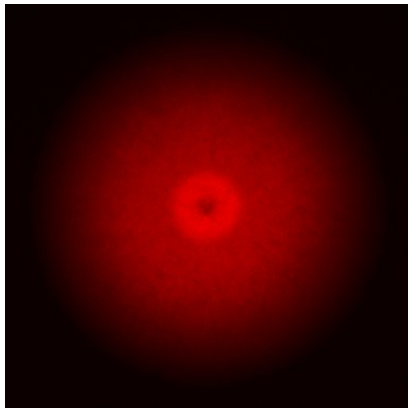
How can we transfer megabases of DNA to bacteria?



Mycoplasma mycoides genomes
to *Mycoplasma capricolum* cells

a colony after
transplantation



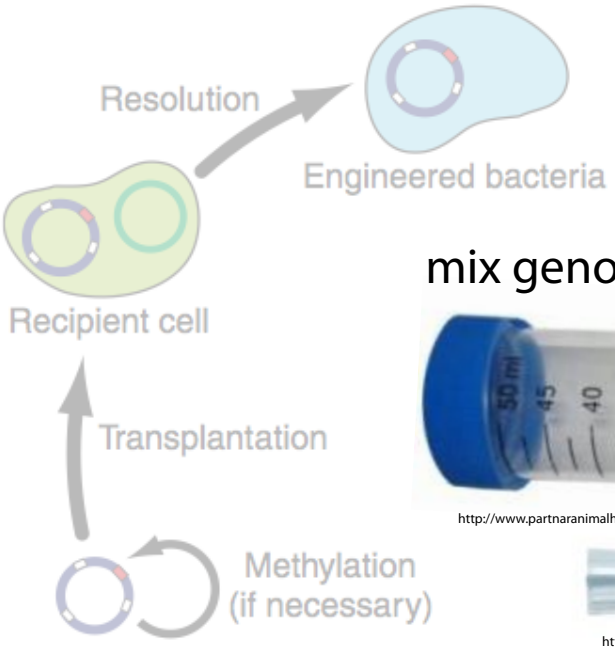


Lartigue et al. *Science* 2007, 2009

How can we transfer megabases of DNA to bacteria?

http://www.avena-medica.com/ProductVault/product_1351077166__mg_3651_S4.jpg

plate and recover transplanted cells



- sensitive
- low yield
- mechanism unclear

mix genomes and recipient cells

http://www.partnaranimalhealth.com/osCommerce/images/DCE-0016S%20Centrifuge%20Tube.jpg

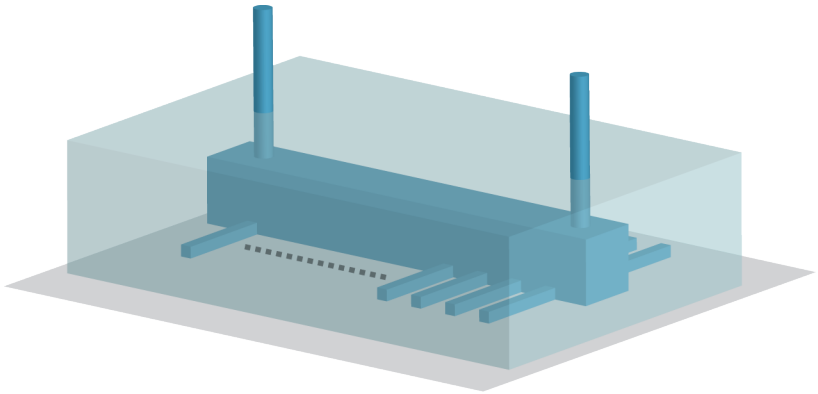
http://ecx.images-amazon.com/images/I/215d1cMFryL._SX342_.jpg

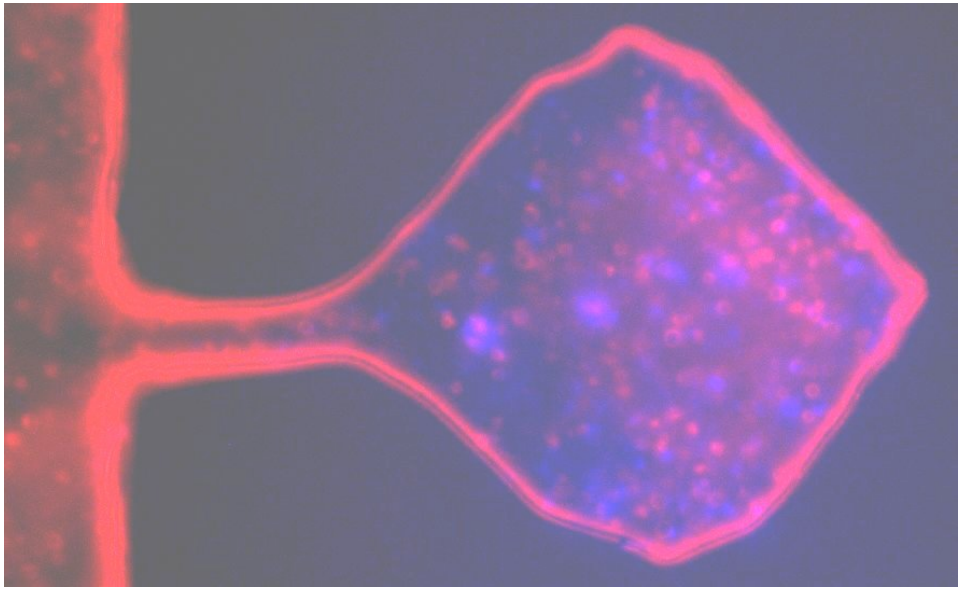
http://incubator.rockefeller.edu/wp-content/uploads/2014/03/Mouth.png

genome isolation in agarose plugs

http://www.bio-rad.com/webroot/web/images/lsr/products/electrophoresis/sku_view/global/170-3713_view.jpg

Microfluidics to transfer whole genomes to bacteria





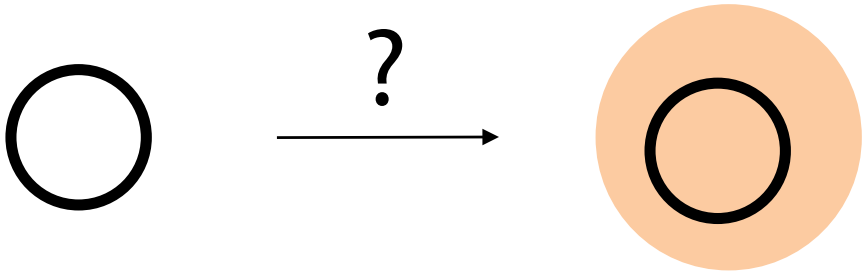
advantages

- fast buffer exchange
- real-time visualization
- high local concentrations
- shelter donor genomes from shear forces

big disadvantage

- many fewer cells than in a test tube

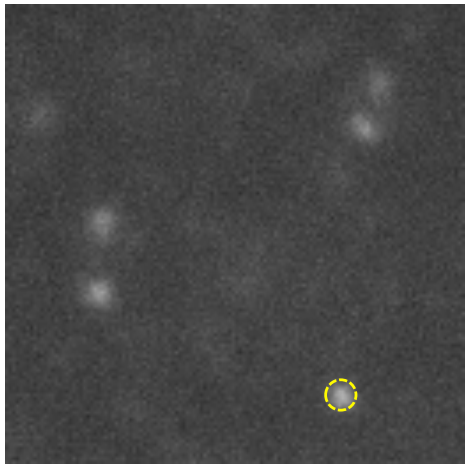
How does the donor genome enter the recipient cell?



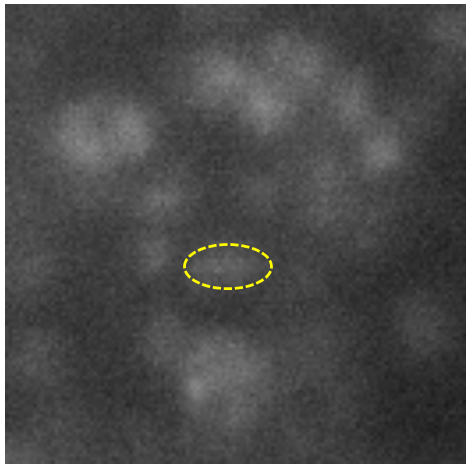
Plasmids are smaller than cells, whereas whole genomes are about the same size as cells.

For intuition, if we assume ideal chains $\frac{R_{\text{chromosome}}}{R_{\text{plasmid}}} \sim \left(\frac{N_{\text{chromosome}}}{N_{\text{plasmid}}}\right)^{1/2} \approx 10$.

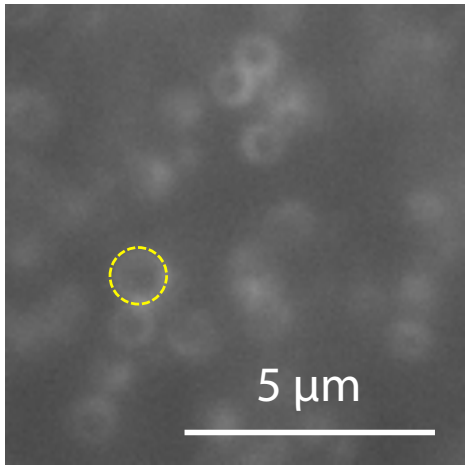
plasmids



chromosomes

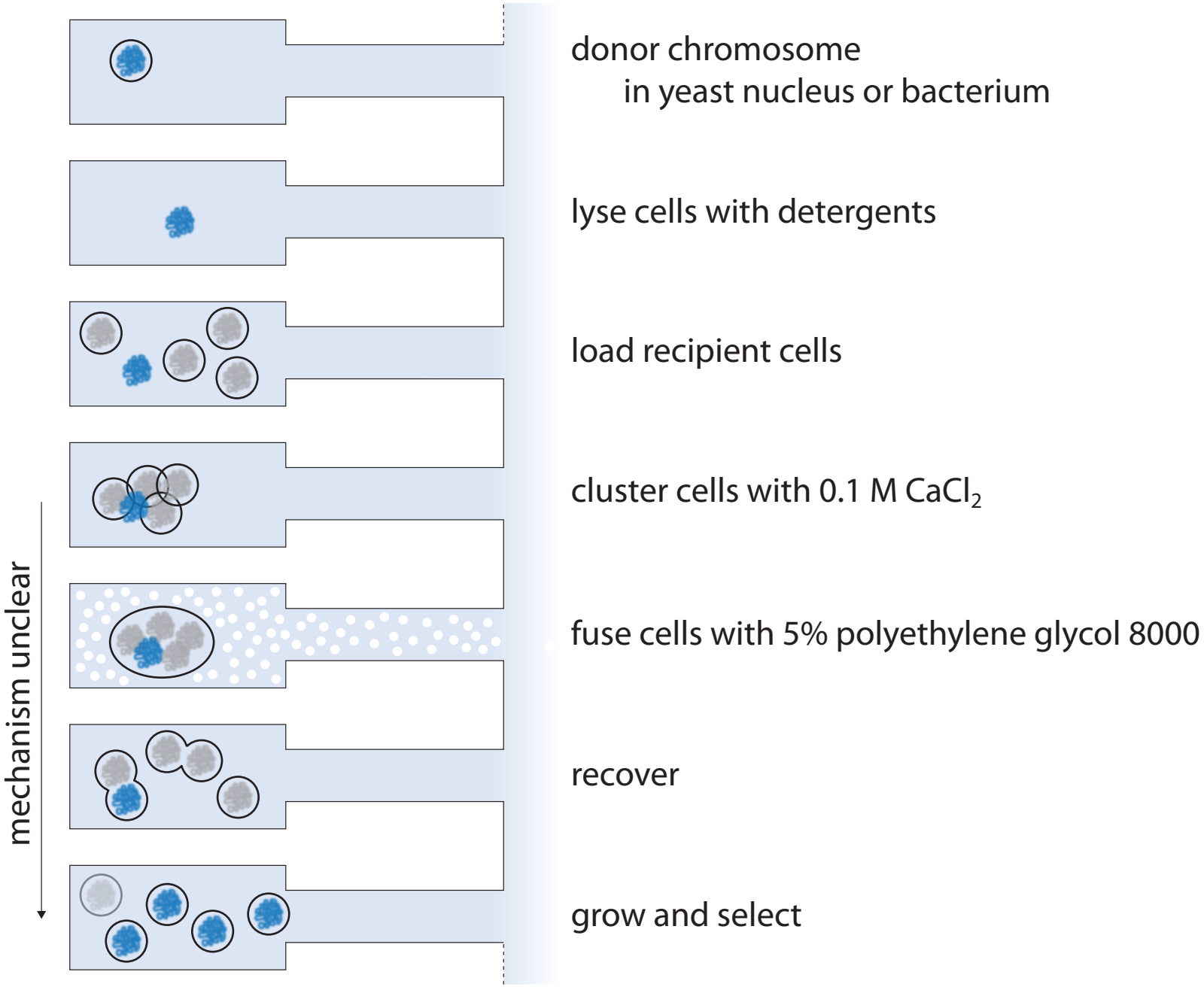


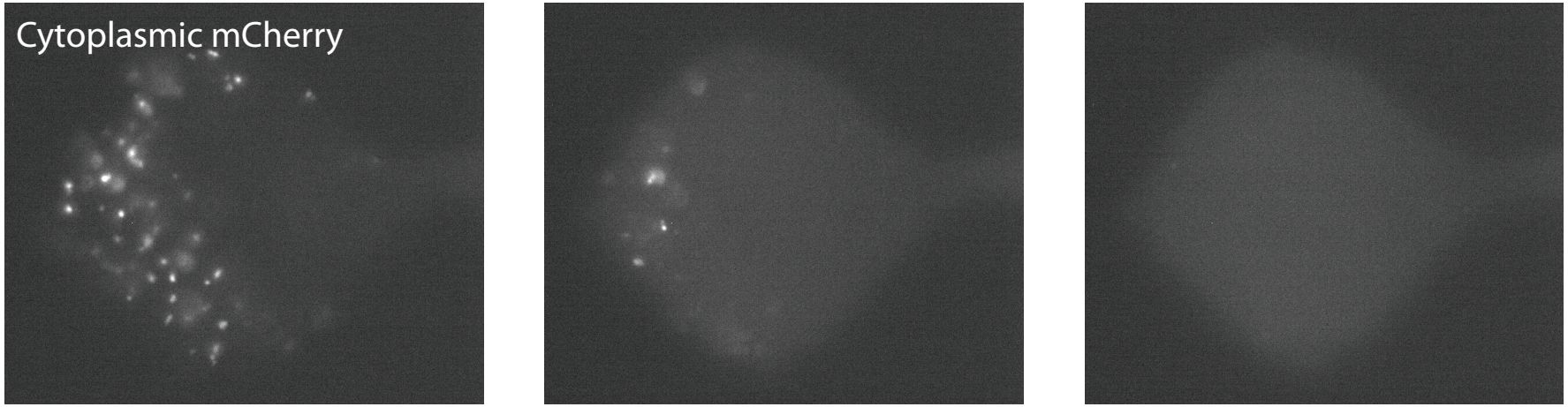
cells



Positive control: *Acholeplasma laidlawii* (BL1) grows in chambers

Transplantation of whole bacterial genomes in microfluidics

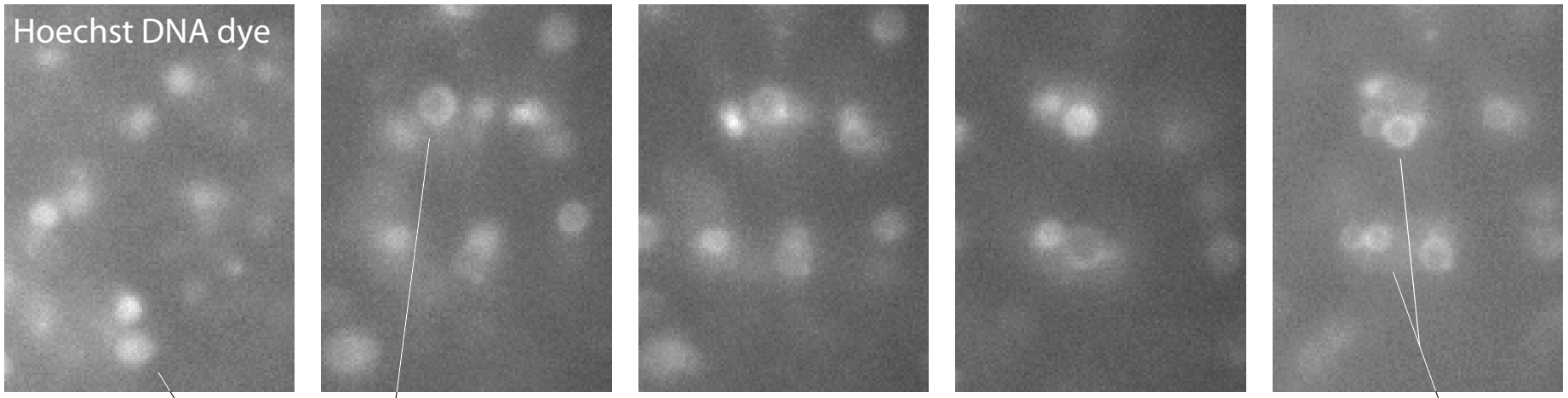




when cells lyse, fluorescent proteins in cytoplasm disperse

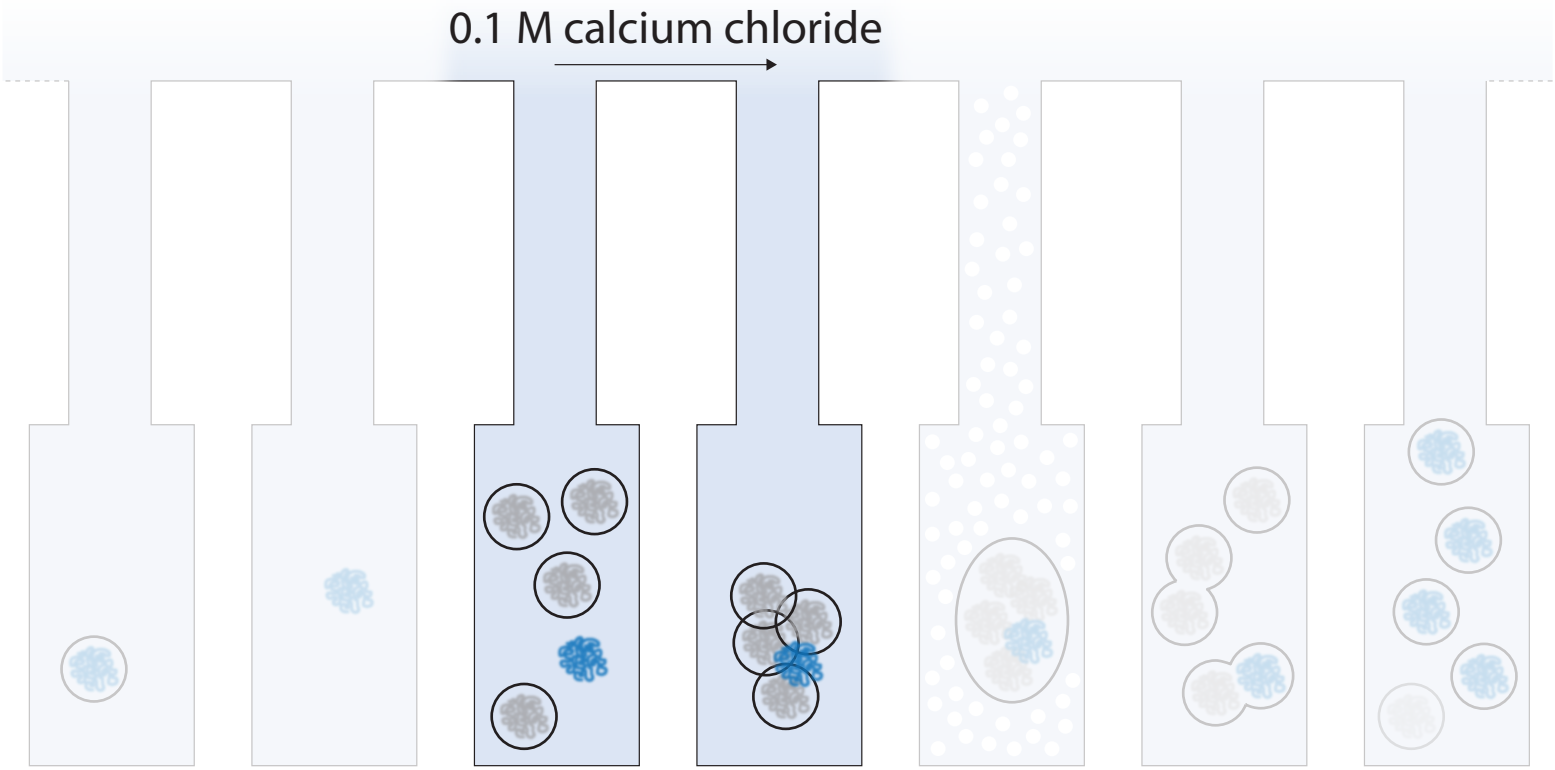
Lysis of *Mycoplasma mycoides* donor cells

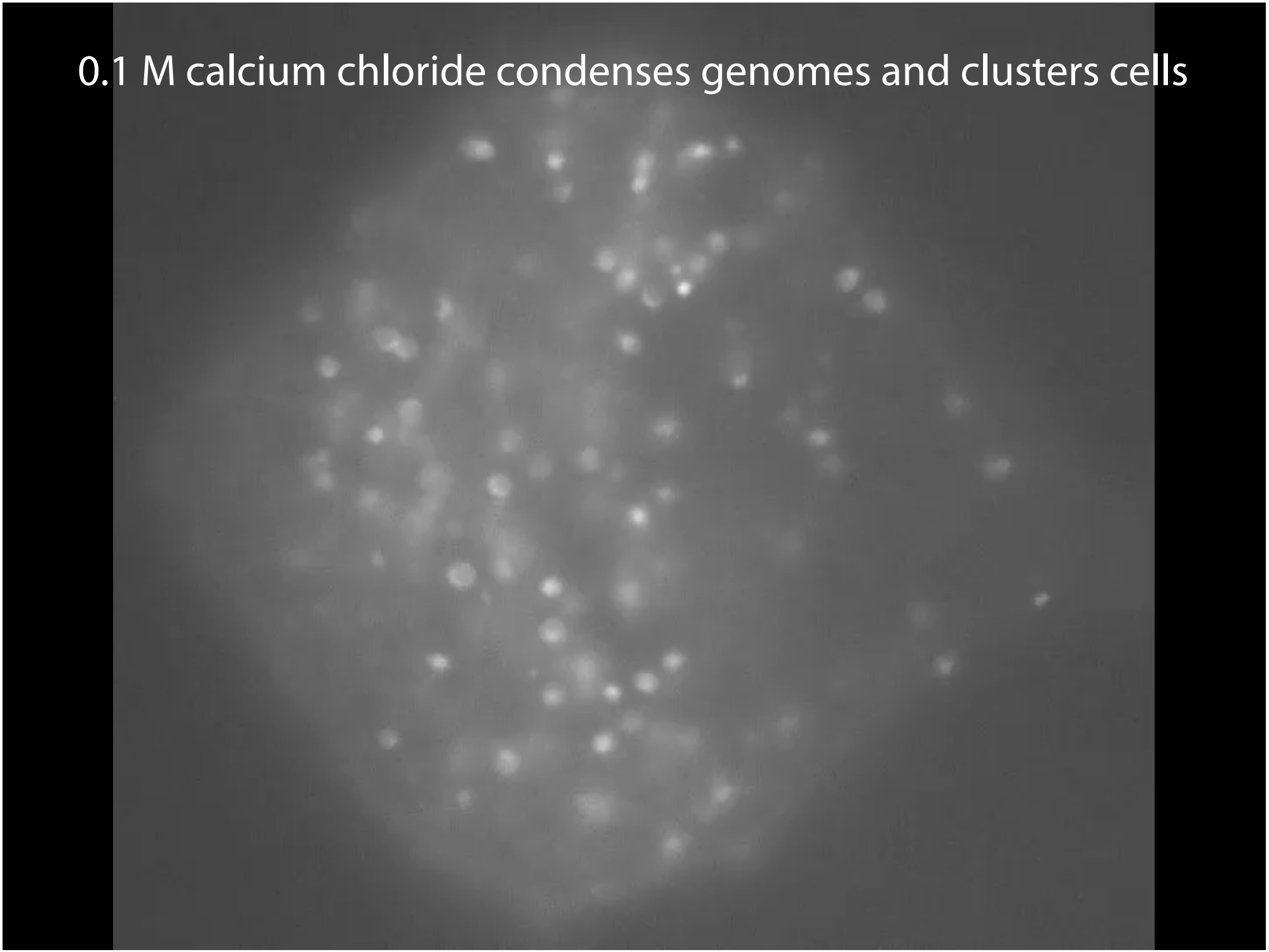
After lysis, whole genomes remain in chambers

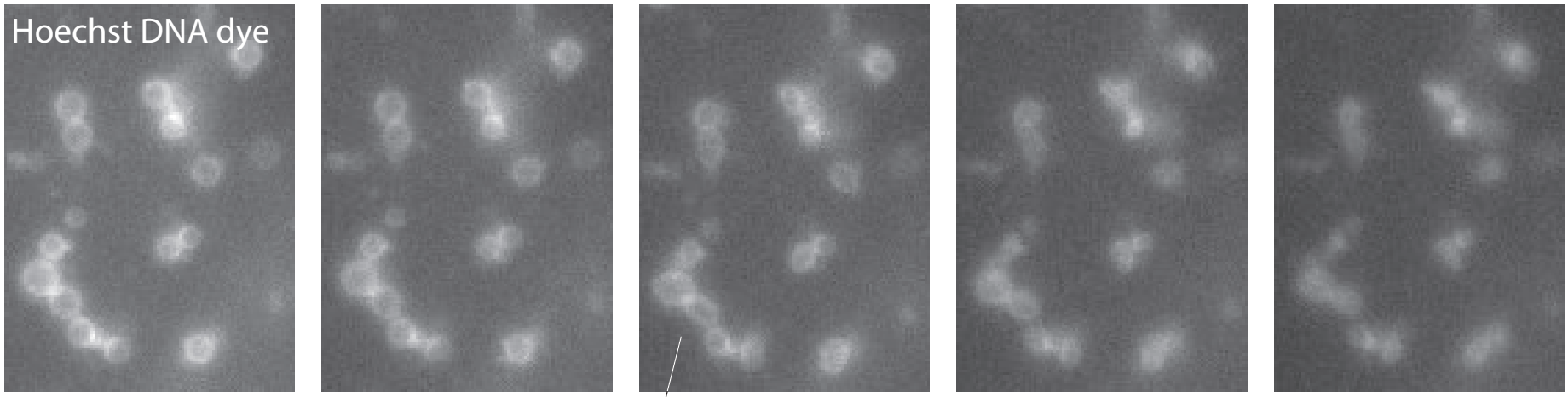


clouds to rings: chromosomes condense on cells

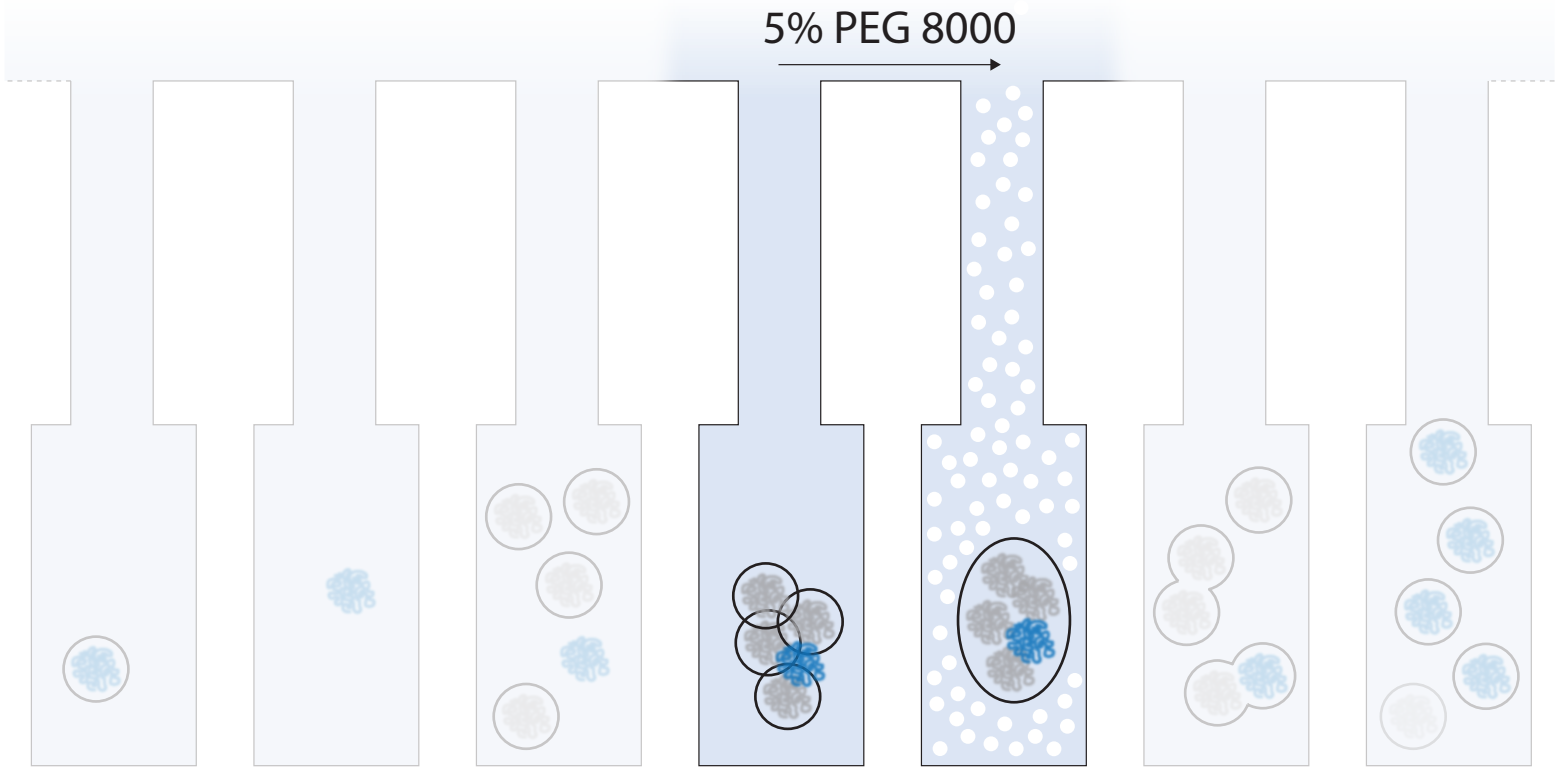
cells cluster

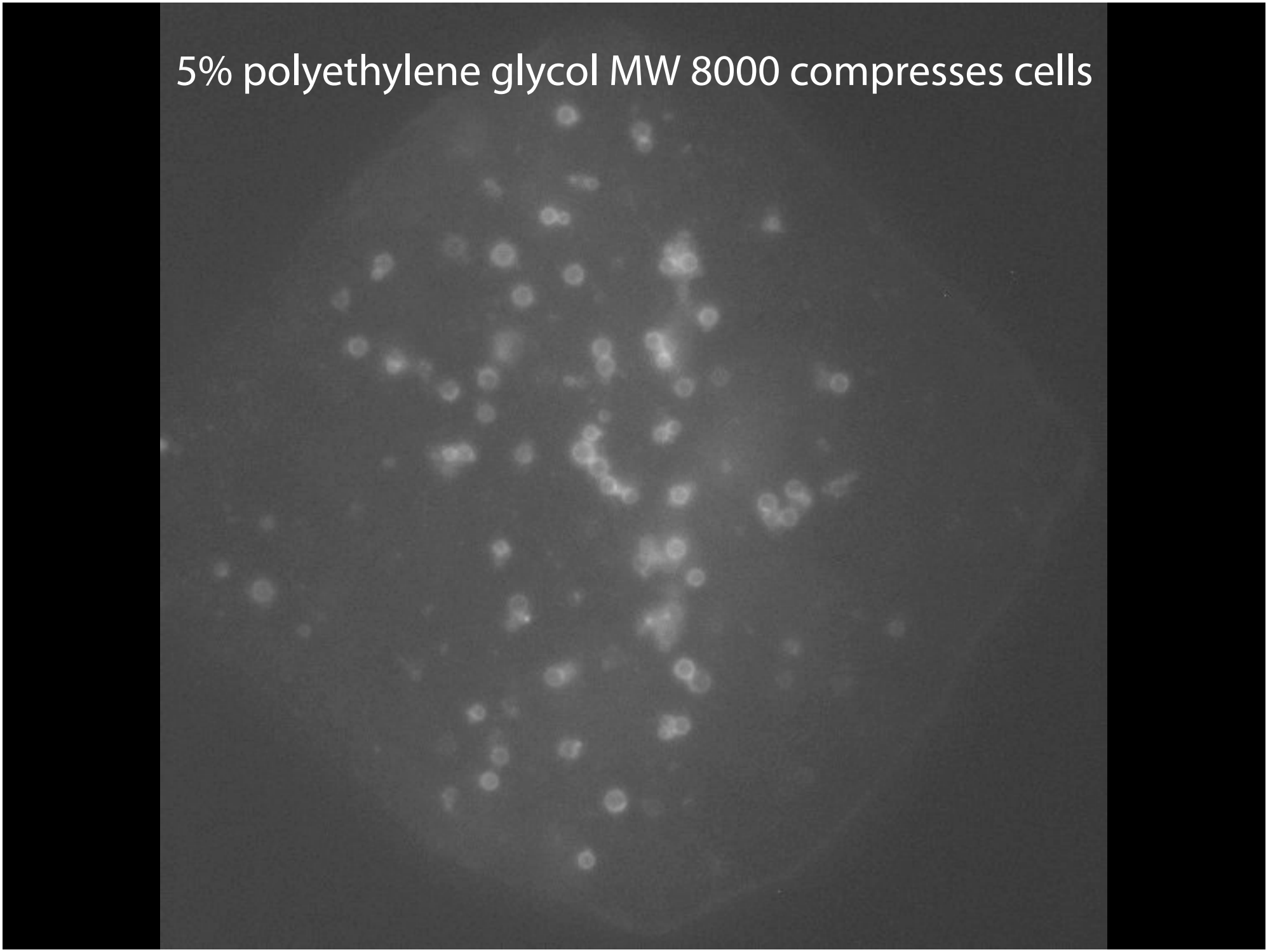



0.1 M calcium chloride condenses genomes and clusters cells

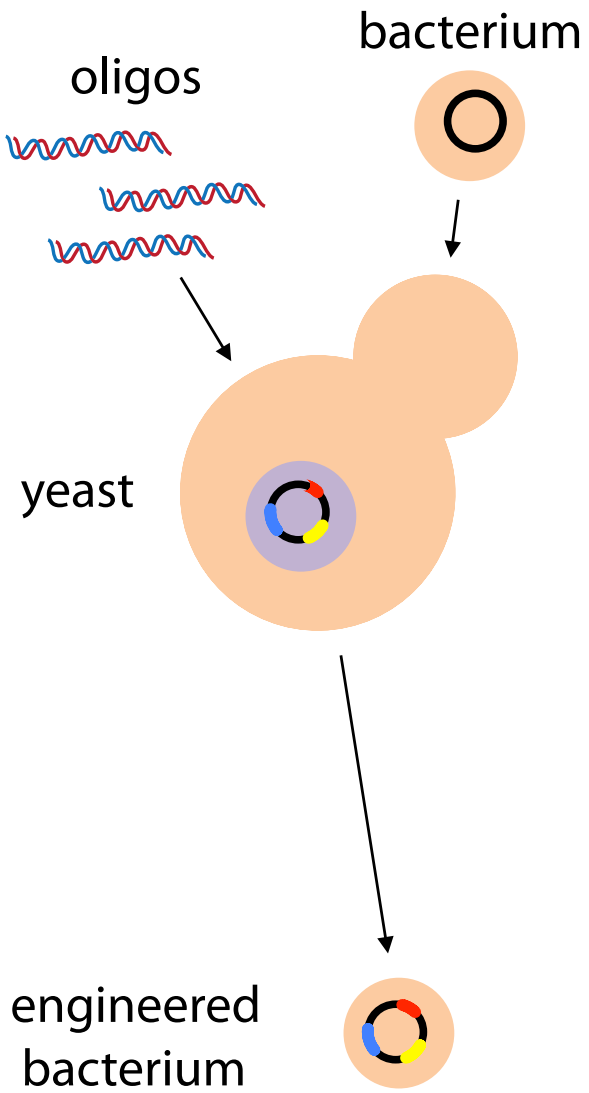


crowding agent distorts membranes and may fuse cells

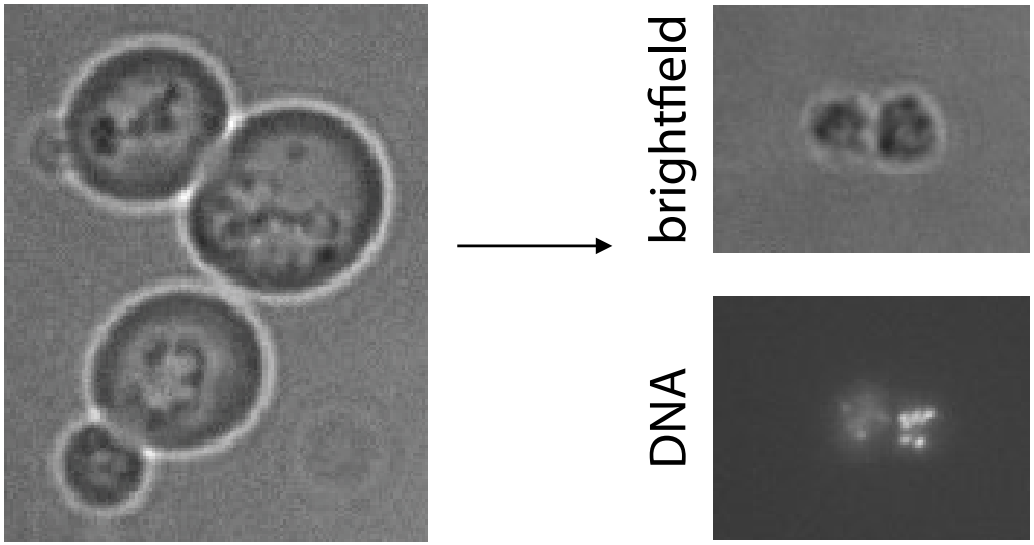



5% polyethylene glycol MW 8000 compresses cells

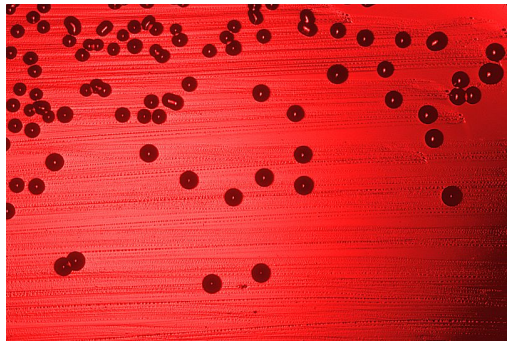
Outlook: genome transplantation in other species?



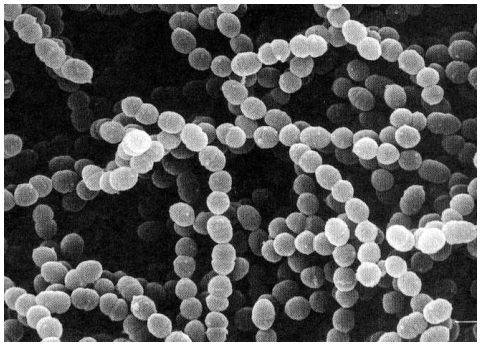
isolation of yeast nuclei



Gram-negative
H. influenzae



Gram-positive
S. thermophilus



Thank you very much!

John Glass
Nacyra-Assad Garcia
Vanya Paralanov
Evgeniya Denisova
David Brown
Adriana Jiga

Elizabeth Strychalski
Jason Kralj
Javier Atencia

Andreas Mershin
Neil Gershenfeld
Will Langford
Prashant Patil
Charles Fracchia
Fei Chen
Paul Tillberg
David Feldman

John Waynelovich (Epsilon Micro Devices)

You!