Microfluidic genome transplantation

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How can we transfer megabases of DNA to bacteria?

Mycoplasma mycoides genomes to Mycoplasma capricolum cells

a colony after transplantation

How can we transfer megabases of DNA to bacteria?

- sensitive
- low yield
- mechanism unclear

plate and recover transplanted cells

mix genomes and recipient cells

genome isolation in agarose plugs
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

$$\frac{R_{\text{chromosome}}}{R_{\text{plasmid}}} \sim \left(\frac{N_{\text{chromosome}}}{N_{\text{plasmid}}} \right)^{1/2} \approx 10$$
Positive control: *Acholeplasma laidlawii* (BL1) grows in chambers
Transplantation of whole bacterial genomes in microfluidics

- Lyse cells with detergents
- Cluster cells with 0.1 M CaCl$_2$
- Fuse cells with 5% polyethylene glycol 8000
- Load recipient cells
- Cluster cells with 0.1 M CaCl$_2$
- Fuse cells with 5% polyethylene glycol 8000
- Recover
- Grow and select

Donor chromosome in yeast nucleus or bacterium

Mechanism unclear
when cells lyse, fluorescent proteins in cytoplasm disperse
Lysis of *Mycoplasma mycoides* donor cells

Time: 15.35 sec.
After lysis, whole genomes remain in chambers
clouds to rings: chromosomes condense on cells

0.1 M calcium chloride

Hoechst DNA dye

cells cluster
0.1 M calcium chloride condenses genomes and clusters cells
crowding agent distorts membranes and may fuse cells

Hoechst DNA dye

5% PEG 8000
5% polyethylene glycol MW 8000 compresses cells
Outlook: genome transplantation in other species?

- Intact yeast
- Yeast nuclei
- Isolation of yeast nuclei
- DNA
- Gram-negative
  - H. influenzae
- Gram-positive
  - S. thermophilus
- Engineered bacterium
- Bacterium
- Oligos
- Yeast
Thank you very much!

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You!