20.106 Midterm 2 Review Session

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Topics

- Nitrogen Cycle
- Communities, symbiosis, genome reduction
- Horizontal Gene Transfer
- Biotechnology

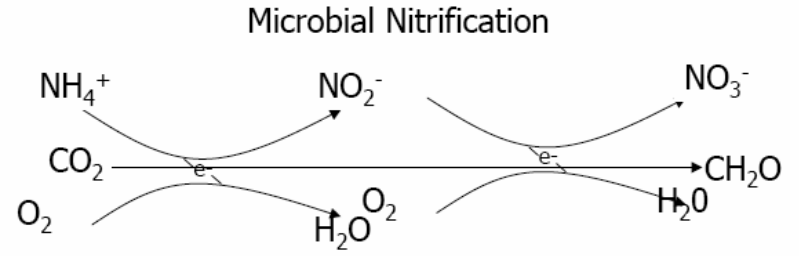
Nitrogen Cycle

Important Reactions

- ✓Nitrogen Assimilation Incorporation of to NH_3 into organic molecules ($R\text{-}NH_2$)
- ✓Deamination Deamination of organic molecules releasing NH_3
- ✓Nitrification
- ✓Denitrification
- ✓N_2 Fixation

Nitrogen Cycle: Nitrification

Oxidation of NH_3 to NO_2^- then NO_3^-

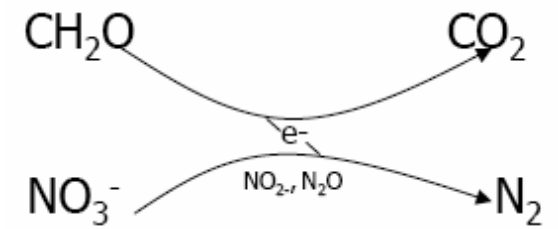


Only carried out by microbes

- Soil and aquatic bacteria
- Aerobic chemolithoautotrophic two step process
  - Ammonia oxidizers (*Nitrosomonas*, *Nitrosococococcus*, *Nitrosospira*, archaea)
  - Nitrite oxidizers (*Nitrobacter*, *Nitrospira*)
- Enzyme is ammonia monooxygenase

Nitrogen Cycle: Denitrification

Nitrate reduction, conversion of NO_3^- to N_2 gas



Only carried out by microbes

- Anaerobic chemoheterotrophic process
- Facultative anaerobes in sediment and oxygen-limited water
- Anaerobic respiration (using Nitrate and Nitrite as TEAP)
- Oxidation of organic matter for energy
- Several enzyme systems--dissimilatory nitrate reductase
- Loss of Nitrogen from system
- Nitrate to ammonia NOT denitrification

Plants perfom assimilatory nitrate reduction

✓ Convert NO_3^- to organic nitrogen (R-NH_2)

Nitrogen Cycle: N_2 Fixation

Molecular conversion of N_2 gas to NH_3

(exist in solution as NH_4^+)

- **ANAEROBIC**
- Proteobacteria, Cyanobacteria, Archaea
- Requires nitrogenase enzyme
  - 21 genes
  - 8 subunits/accessory proteins
  - Molybdenum and iron cofactors
- High energy cost (16 ATP per N_2)

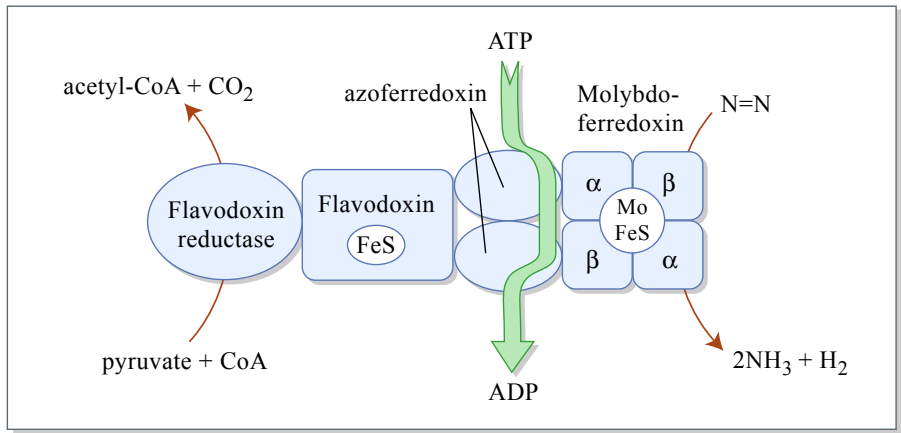


Figure by MIT OCW.

$$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16\ MgADP + 16\ P_i$$

Rhizobium

- Free-living are aerobic, not N_2 fixers
- When symbiotic
  - *Rhizobium* turn on plasmid-based *Nod* genes
  - Become anaerobic N_2-fixing, bacteroid form
  - Legumes form nodules to control symbiotic relationship

Images of free-living Rhizobium and bacterioids in nodule removed due to copyright restrictions.

Symbiosis and Genome Reduction

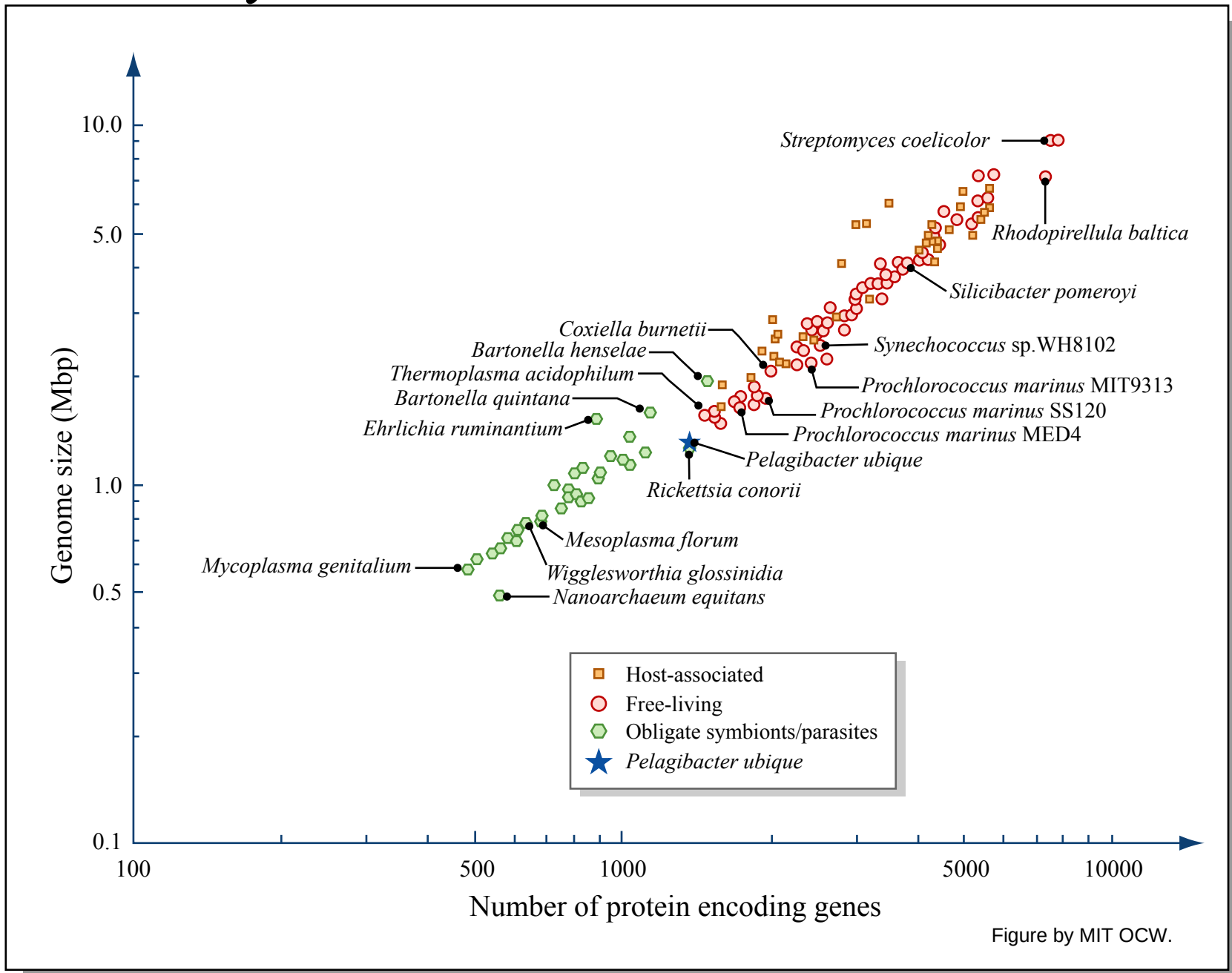
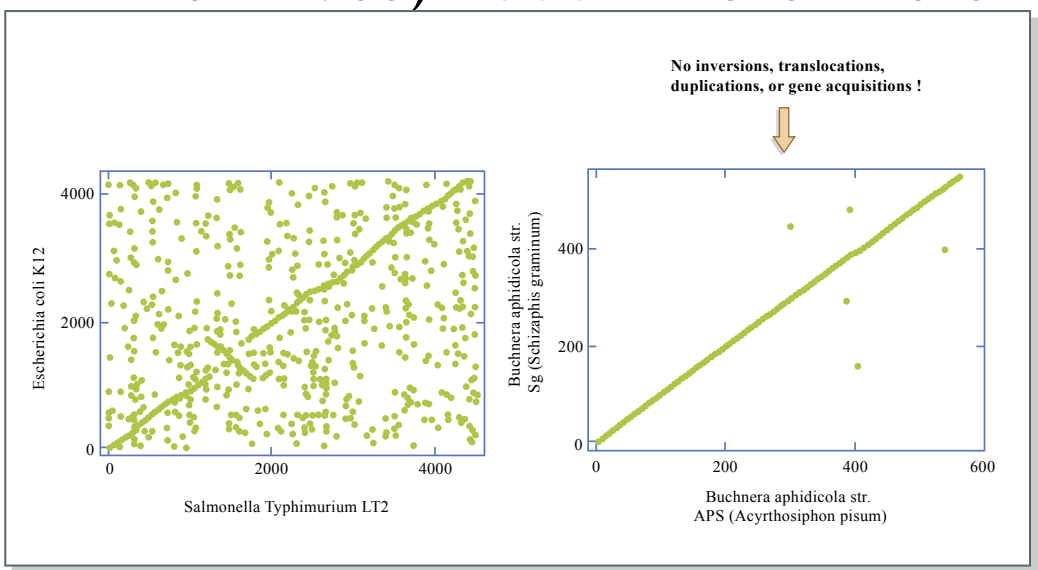


Figure by MIT OCW.

Symbiosis and Genome Reduction

- *Buchnera aphidicola* from two aphids *Schizaphus graminum* (Sg) and *Acyrthiosiphon pisum* (Ap)
- 70 million years
  - No chromosomal rearrangements
  - Sequence divergence (9^{-9} synonymous substitutions/yr
  - 1.65^{-9} non-synonymous substitutions/yr
- *E. coli* and *Salmonella* spp. (closest free-living relatives) 2000x more liable



50 Million Years of Genomic Stasis in Endosymbiotic Bacteria

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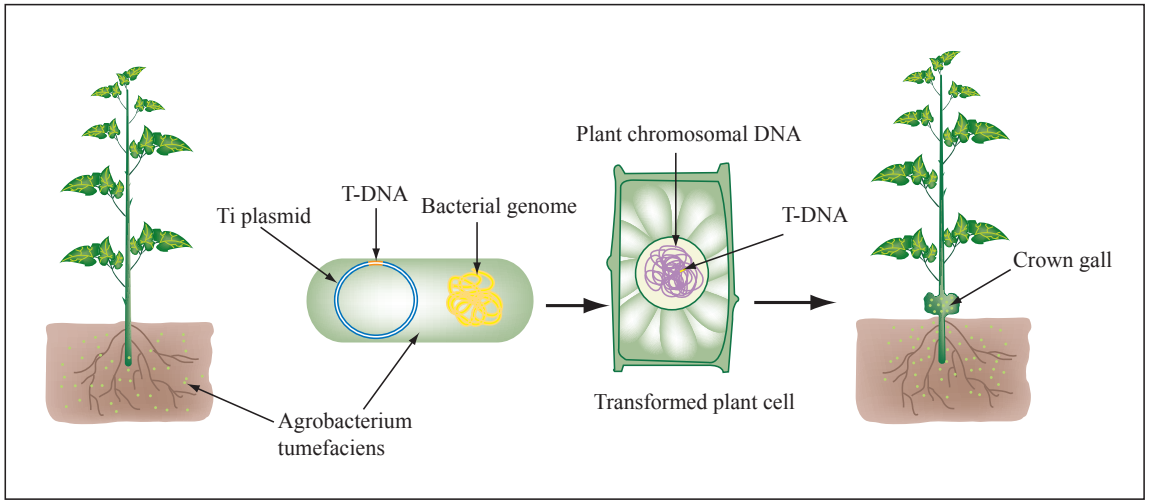
Figure by MIT OCW.

Genome Dynamics in *Buchnera*

Obligate endosymbiont

- Substantial sequence divergence
- Prominence of pseudogenes
- Loss of DNA repair mechanisms
- Stable genome architecture HOW??
  - Gene transfer elements reduced/eliminated
    - Reduced phage
    - Reduced exhange w/other genomes
    - Fewer repeat sequences
    - Fewer transposons
  - Lack of recombination mechanisms (no recA, recF)
  - Lower frequency of recombination

Argobacterium



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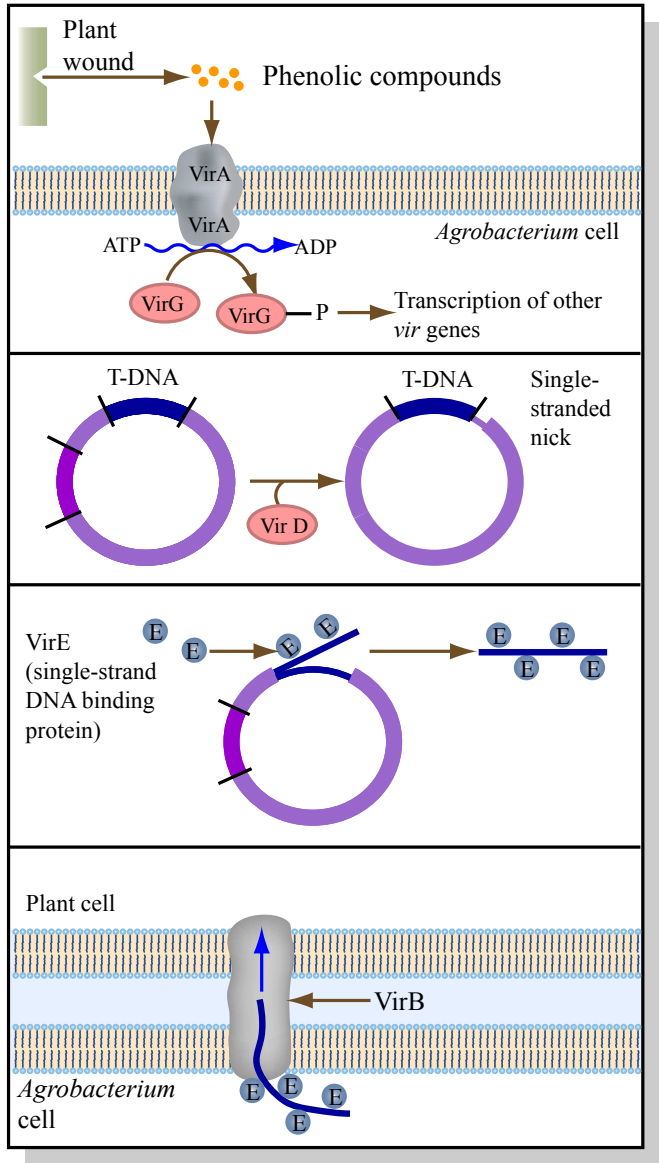

- Ti plasmid & crown gall disease
  - A portion of the Ti plasmid is inserted into the plant chomosome causing the formation of the tumor or gall.

Figure by MIT OCW.

Horizontal Gene Transfer: Transformation

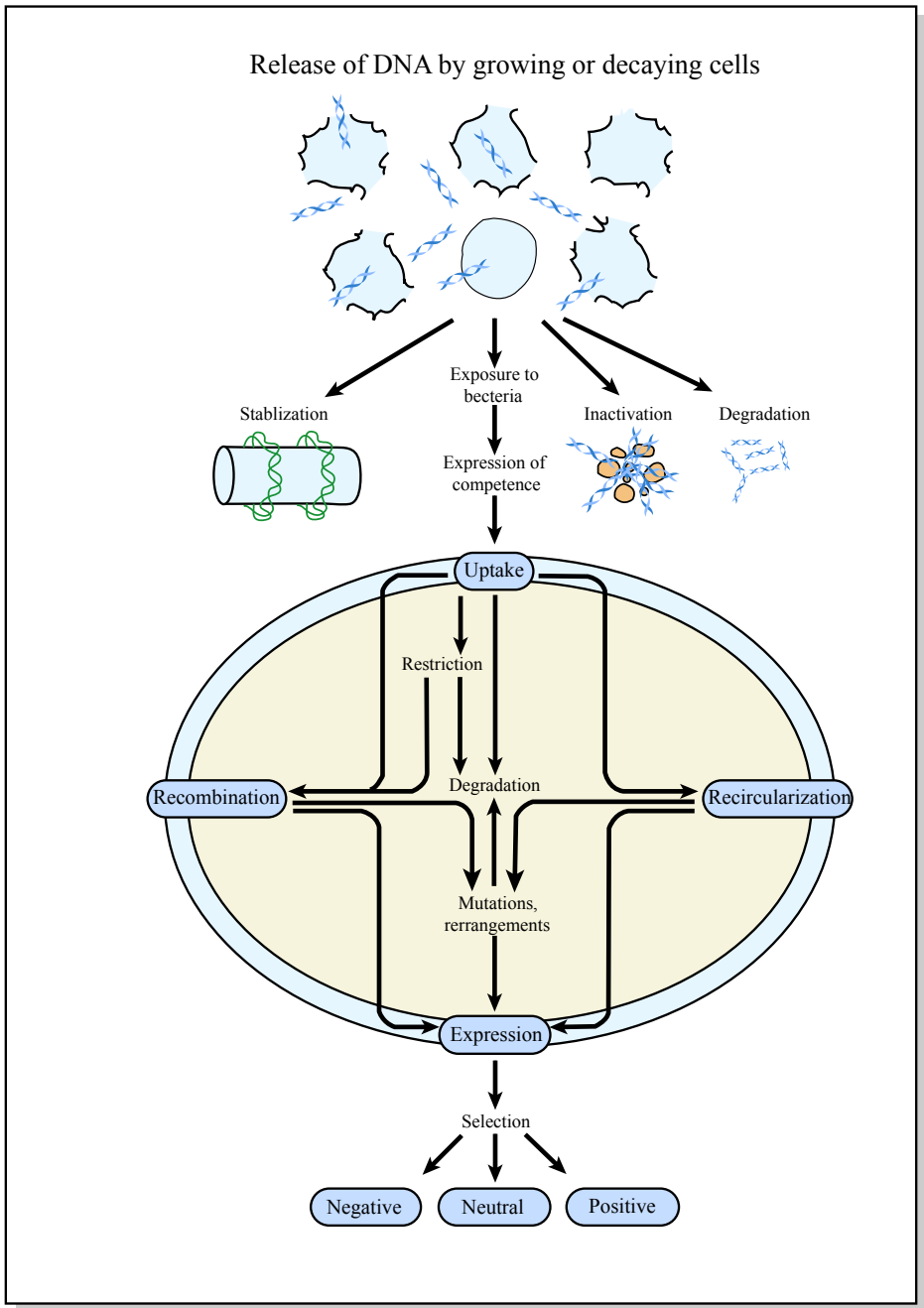

Figure by MIT OCW.

Horizontal Gene Transfer: Conjugation and Transduction

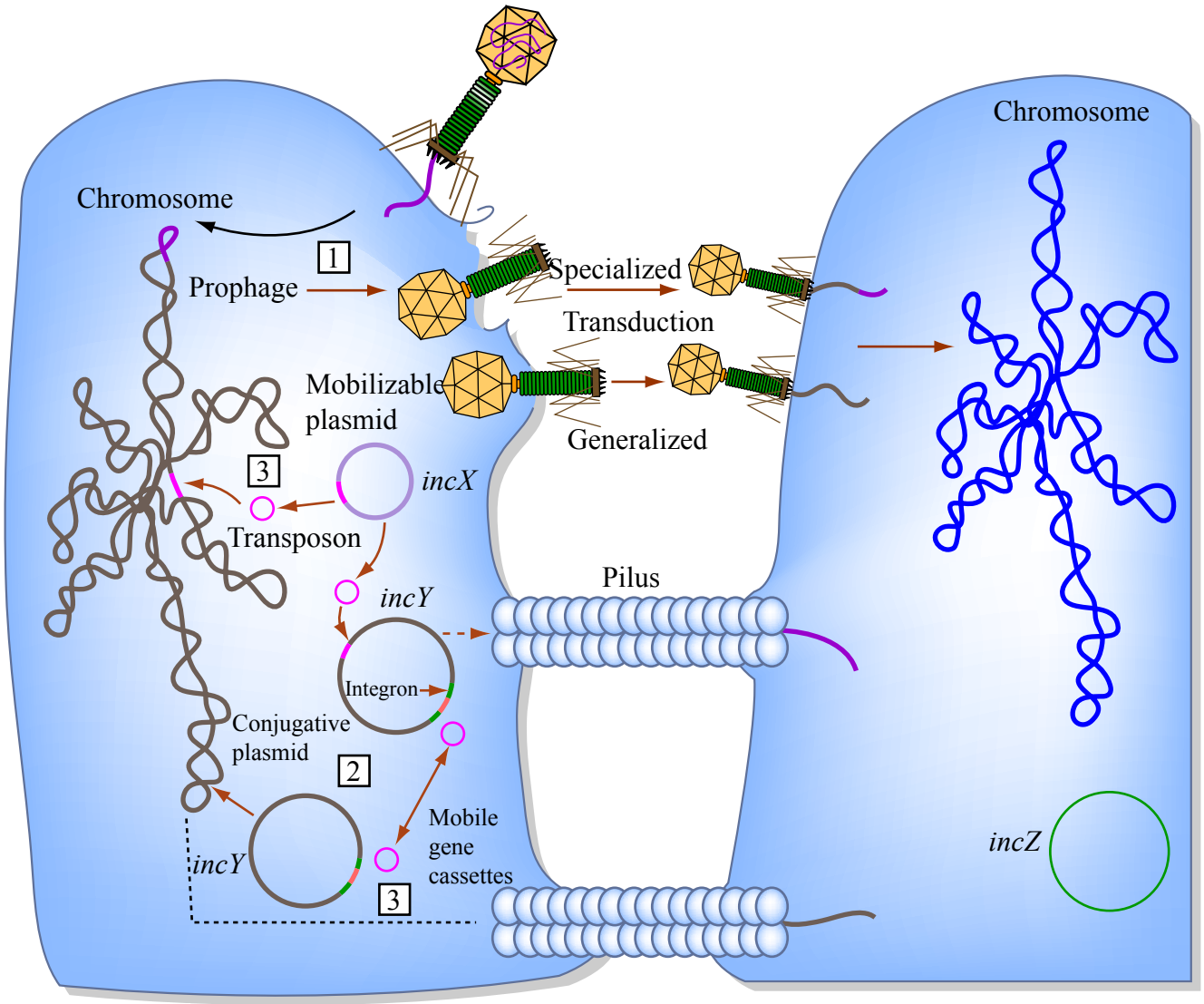

Figure by MIT OCW.

DNA Technologies

- Basics of Sequencing
  - Sanger method
  - Shotgun sequencing
- Cloning (cloning vectors)
  - Plasmids
  - Phage
  - Bacterial Artificial Chromosomes (BAC)
  - Yeast Artificial Chromosomes (YAC)