Observations of Multiple Transformation Kits

Bryan Smith (LaLumiere School)
Teresa Pairitz (Marian High School)
Shelly Gregory (South Bend Adams High School)
ND RET Molecular Biology Workshop 2009

Transformation

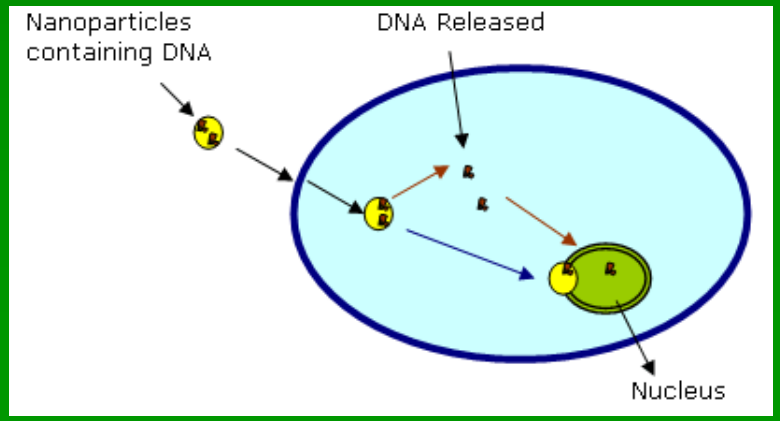
- Occurs when a cell takes up and expresses a new piece of DNA (plasmid) which it did not previously have
- Stumbled upon initially by F. Griffith (1928) while studying pneumonia in mice

Uses of Transformation

- GMOs—to deter from frost, pests; increase drought resistance; boost nutrient content;
  potential for crops to deliver
  vaccines for infectious diseases

- Bioremediation—oil-digesting bacteria

- Medicine—human insulin, clotting factor, growth hormone production by bacteria; target and destroy hard-to-reach cancer cells (3/2009)



Basic Transformation Procedure Common to the 3 Kits

- Bacteria and plasmid chilled on ice (4 C) in $CaCl_2$ to allow cell membrane permeability to plasmid
- Heat shock (42 C), times variable, to improve plasmid permeability
- Ice, times variable
- Recovery, times variable, with LB
- Plating, incubation (37 C overnight)

Bio-Rad: pGLO Bacterial Transformation

- Transform bacteria with jellyfish gene (GFP)
- Study gene selection and regulation (amp/ara)
- Restriction enzyme and ligation concepts
- Advanced lab techniques
- Possible extentions (GFP chromatography)
- Complete in two 45 minute lab sessions

Pros and Cons

+Highlights the central molecular framework of biology: (DNA→RNA→Protein→Trait)

+Problem solving opportunities

+/-Procedure is just complicated enough to have both and positive and negative outcomes (working v. not)

+Extention to GFP chromatography

Carolina Biological: E-Z Gene Splicer

- Splice genes for ampicillin and kanamycin resistance into a recombinant plasmid
- Transform E.Coli with the new plasmid
- Isolate transformed bacteria by growing them on plates with ampicillin and kanamycin
- Additional concept of ligation (gene slicing)

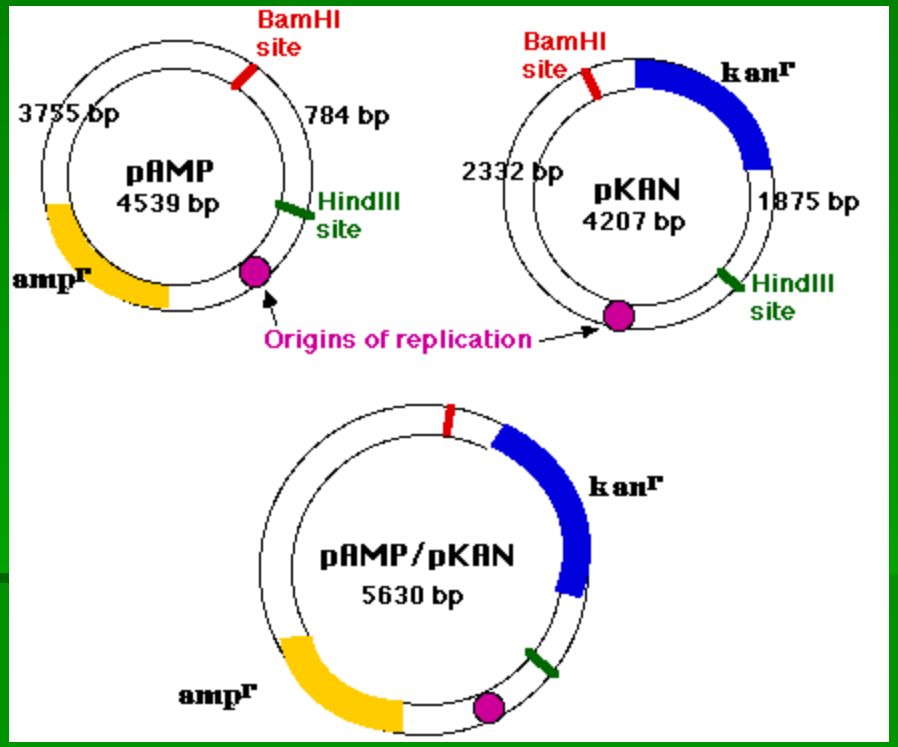
Ligation

Digested pAmp w/ampicillin resistance gene

+

Digested pKAN w/kanamycin Resistance gene

+



Ligase w/ATP ---1 hour → plasmid w/ampicillin & kanamycin resistance!

Carolina Pros and Cons

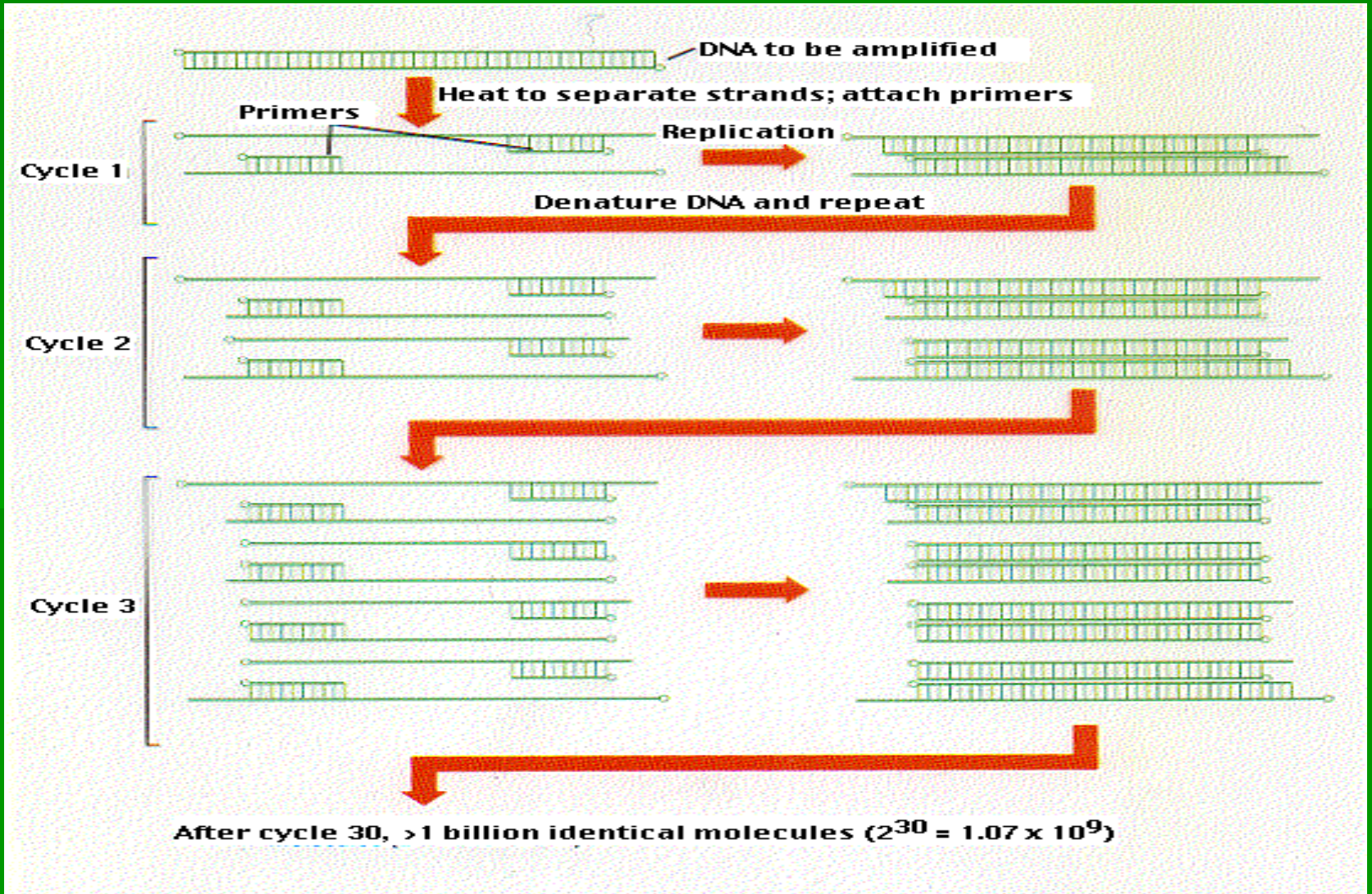
+ All necessary supplies included

- Must streak plate bacteria ahead of time to obtain fresh colonies for transformation

Peyer Lab Systems: Cloning a Fluorescent Gene (Granger!)

- Amplification of GFP using PCR
- Ligation of GFP to a vector to make recombinant plasmid for transformation
- Transform E.Coli bacteria with the recombinant plasmid
- Isolate transformed bacteria by growing on LB agar plate with ampicillin
- Pix here

PCR


DNA to be amplified
Heat to separate strands; attach primers
Primers
Replication
Cycle 1
Denature DNA and repeat
Cycle 2
Cycle 3
After cycle 30, >1 billion identical molecules (2^{30} = 1.07 x 10^9)

Peyer Pros and Cons

+ Excellent lab manual
+ Can lease high cost equipment (thermocycler, fluorescing light source, micropipettes)
+ Pre-prepared agar plates
- PCR process and ligation must be successful for lab to work effectively

Wards: Glowing Bacteria: Transformation with a Firefly Gene

- Introduction to biotechnology/plasmids and their applications
- Emphasizes precision in lab techniques
- Bioluminescence using the *luc* gene

Pros & Cons

- + interesting result if it works
- + luciferin/luciferase relationship – discuss the action of enzymes
- - high cost
- - procedure has a lot of wait time; need additional activity to fill this time