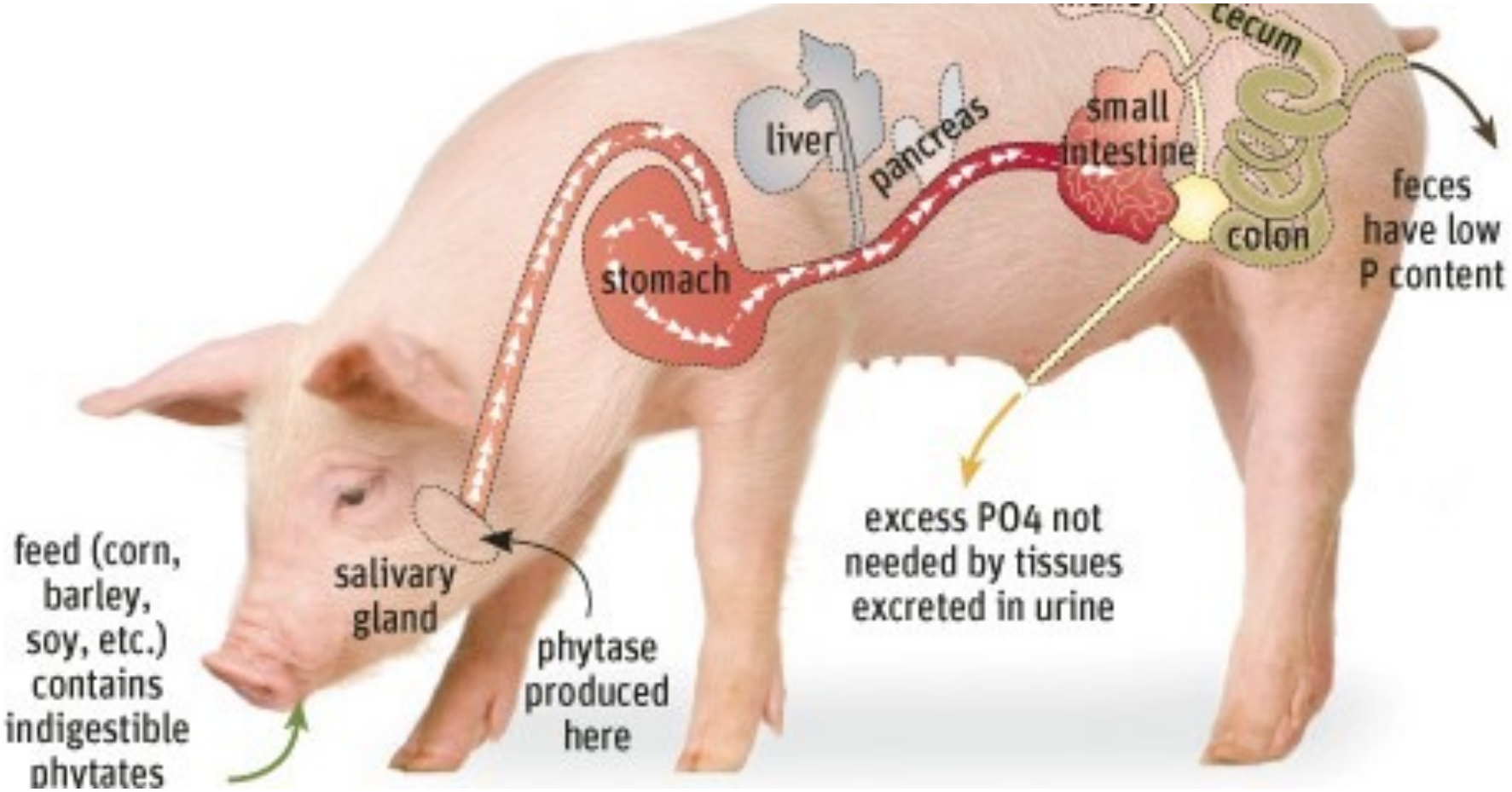
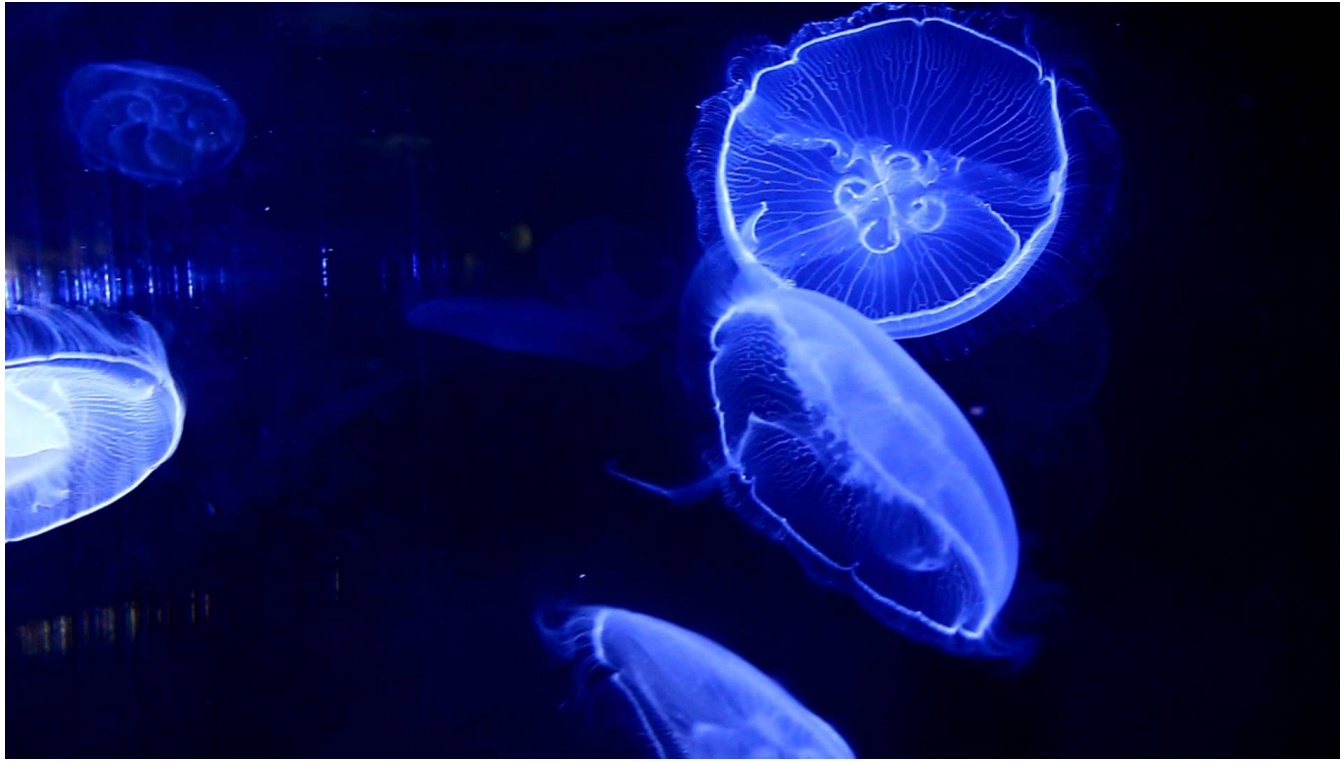
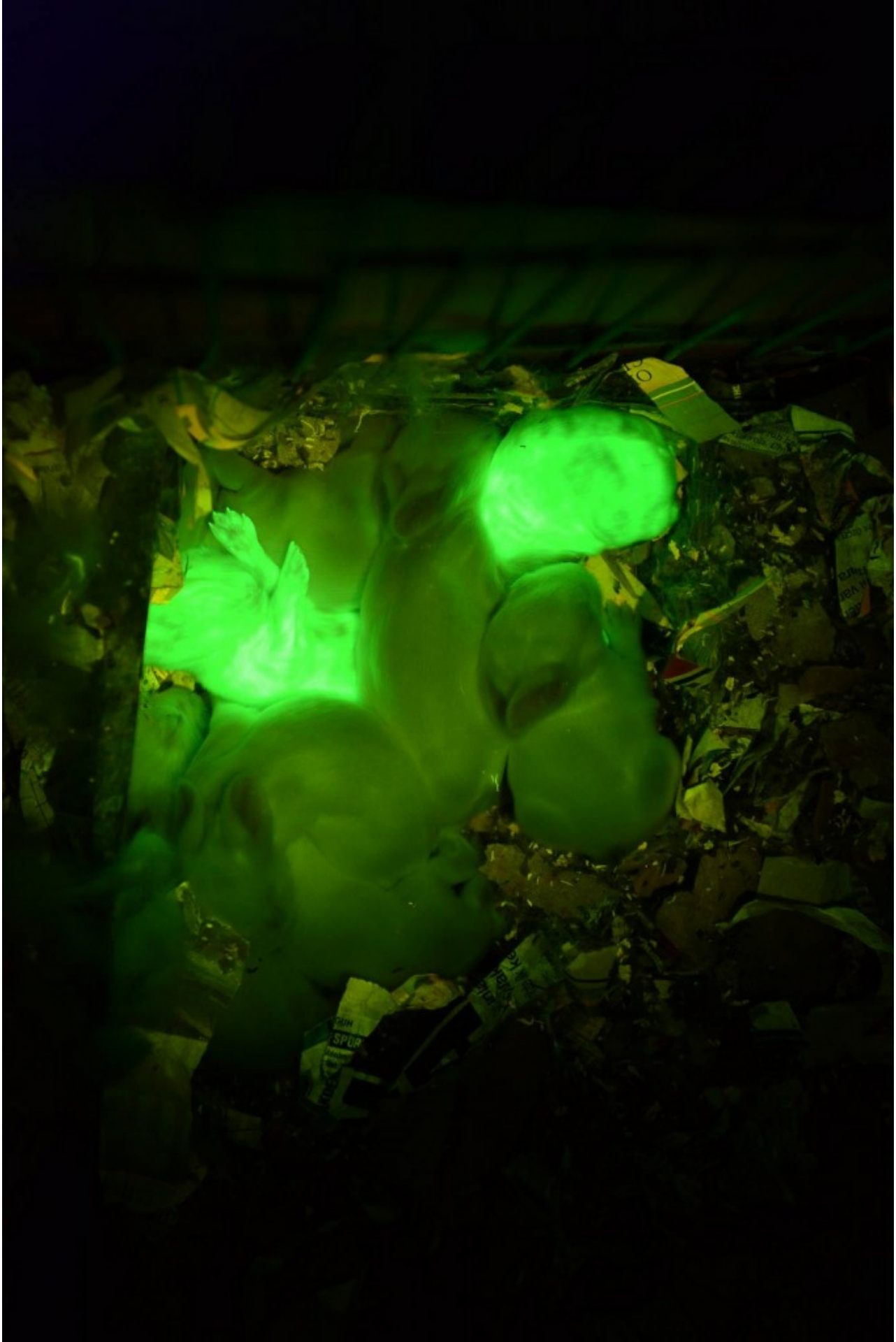
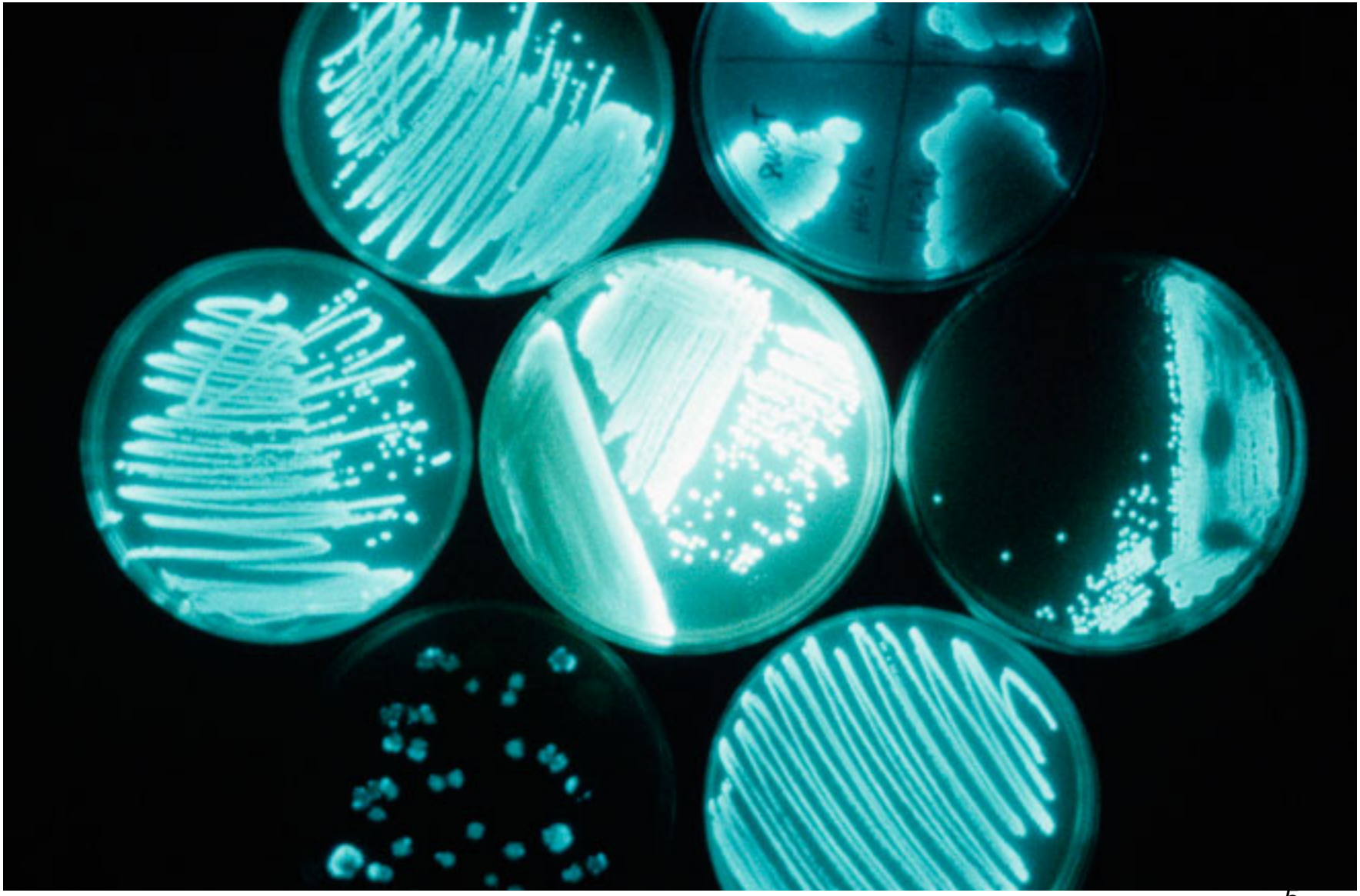
Manipulating & Cloning DNA in Bacteria

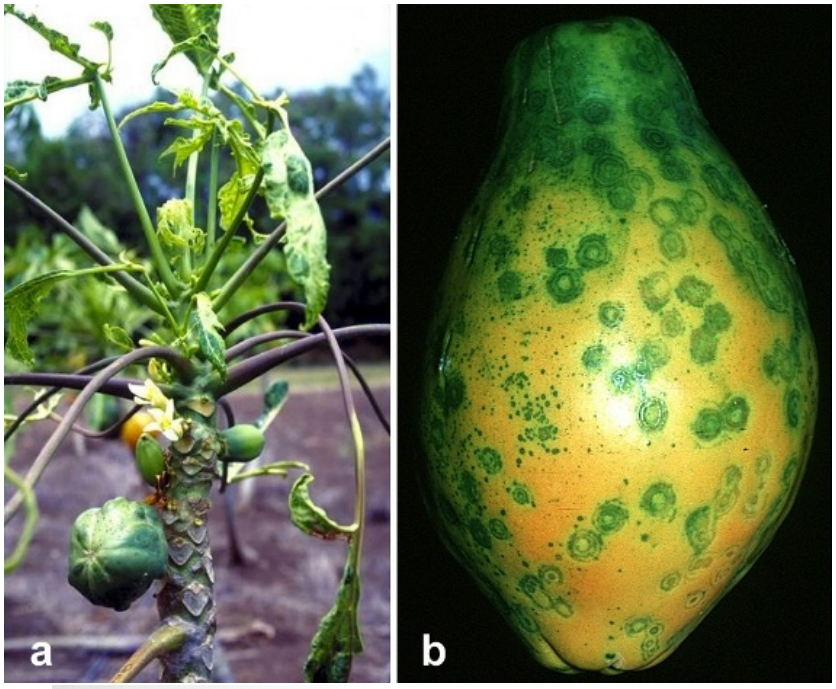
Genetic Technologies


cecum
liver
pancreas
small intestine
stomach
colon
feces have low P content
excess PO4 not needed by tissues excreted in urine
feed (corn, barley, soy, etc.) contains indigestible phytates
salivary gland
phytase produced here








a
b

Insulin

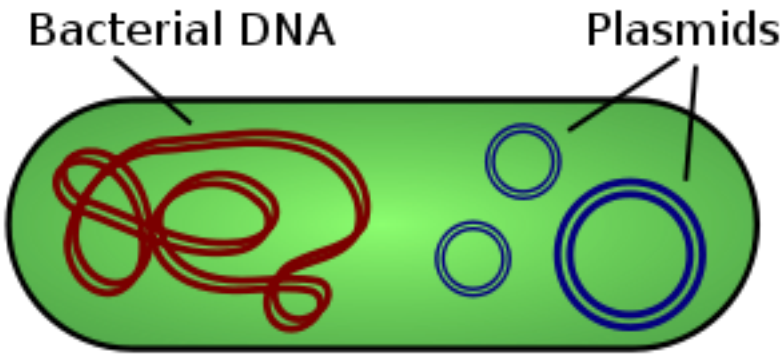
- *E. coli* and safflower plants have both been used to produce human insulin

- How?


kbtc

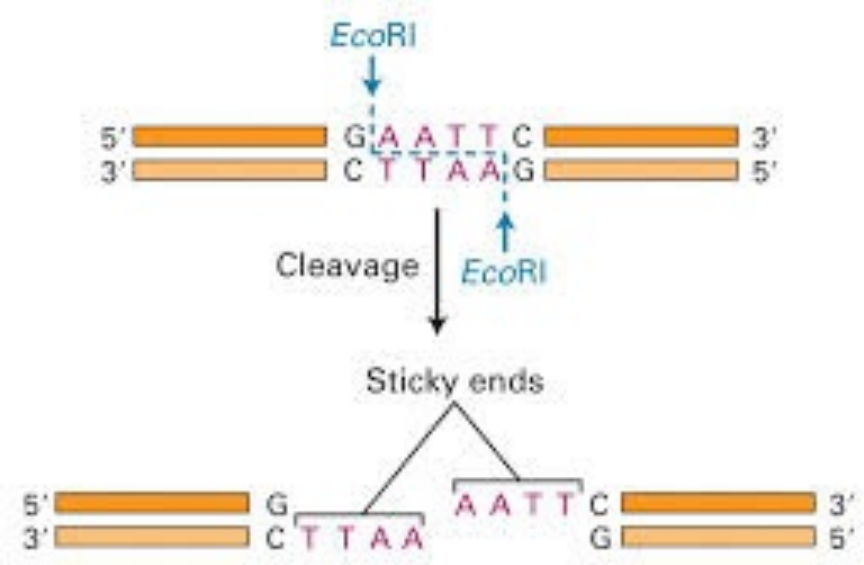
Genetic Engineering

- human insulin gene can be introduced to plasmids *(recombinant DNA)*
- plasmids can be introduced to bacteria cells
- By what process?



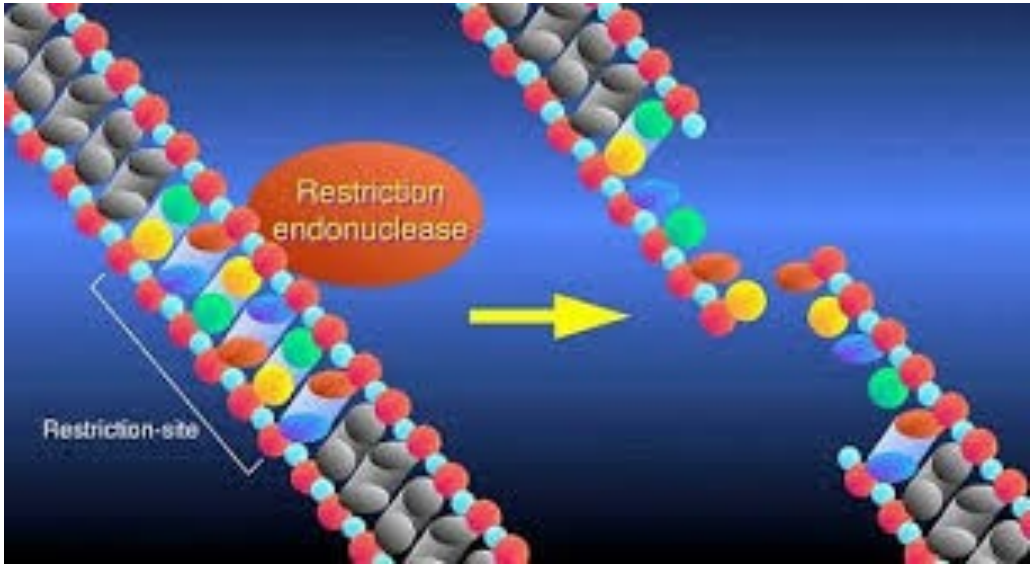
Restriction Enzymes

- restriction enzymes cut DNA at a specific **recognition site**
- recognition sites are always **palindromic**: (same sequence when read from the 5' to 3' direction on either strand)



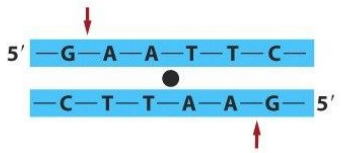
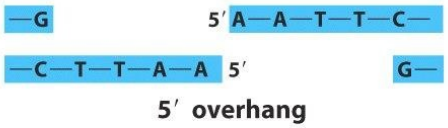
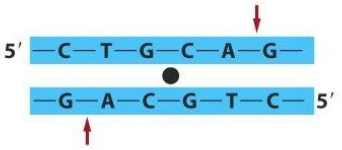
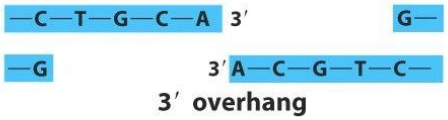
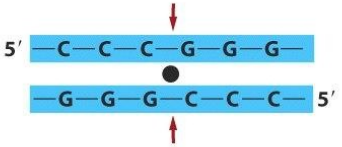
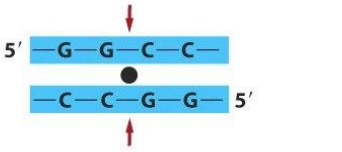
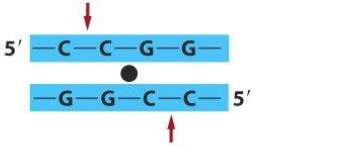
Restriction Enzymes

- Restriction enzymes are harvested by researchers & used in genetic engineering
- they are produced by bacteria to function as an "immune system" against invading viruses by cutting up the viral DNA or RNA

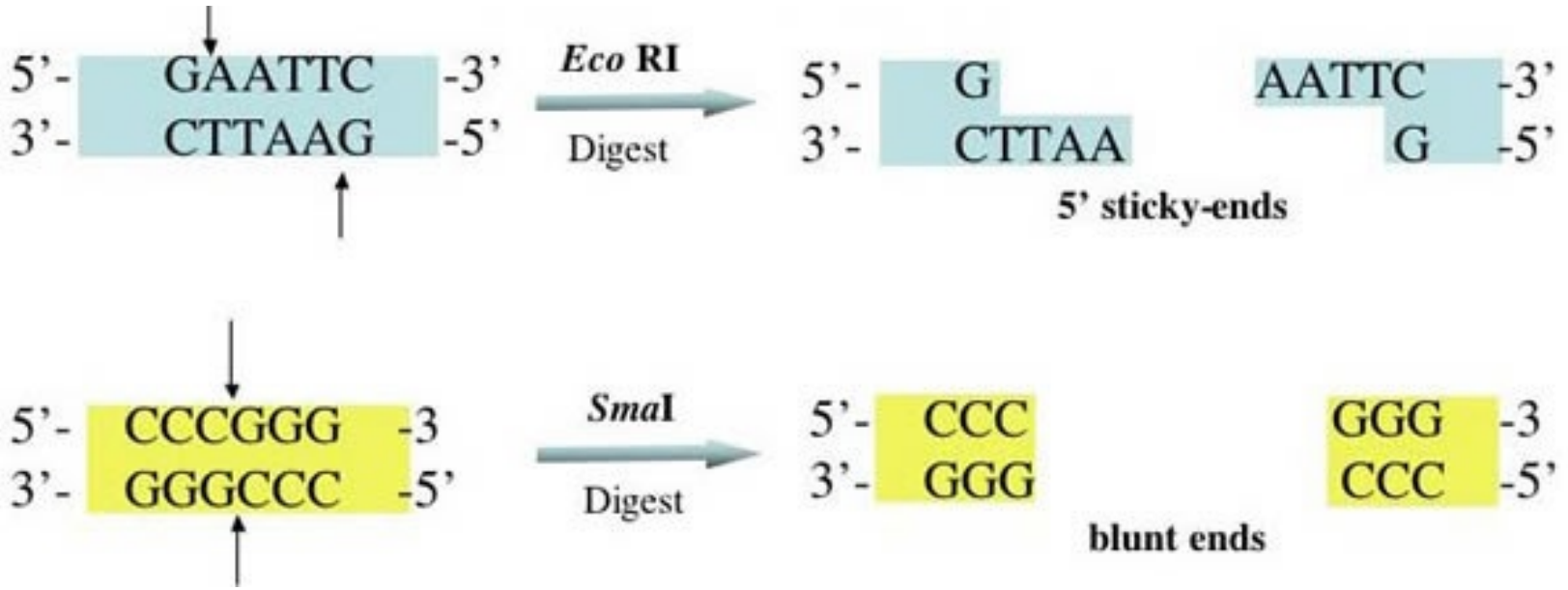


Restriction Enzymes *(pg 367)*

Some restriction enzymes

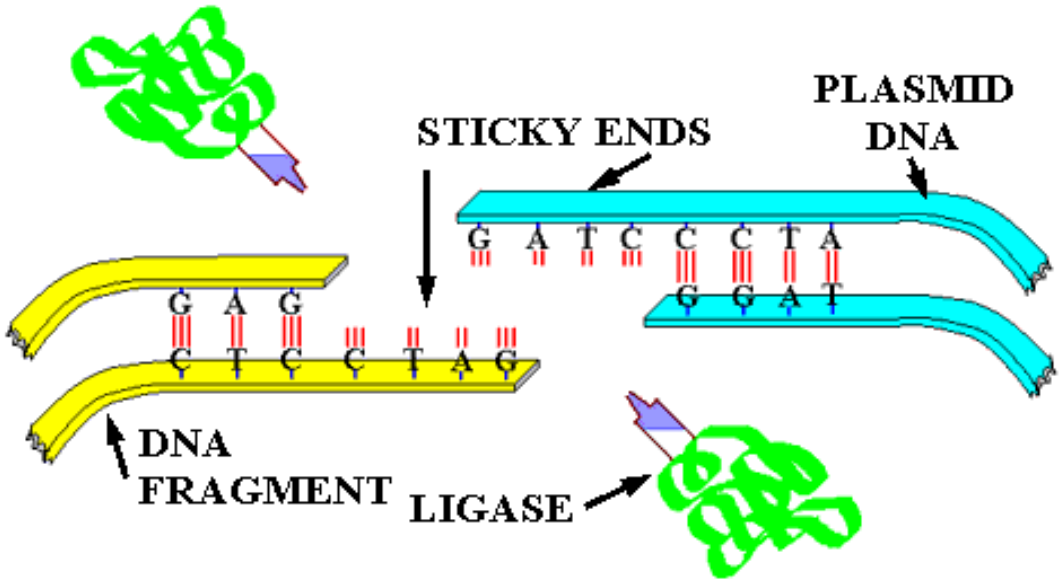
Enzyme	Source organism	Restriction recognition site in double-stranded DNA	Structure of the cleaved products
(a)			
*Eco*RI	*Escherichia coli*		
*Pst*I	*Providencia stuartii*		
*Sma*I	*Serratia marcescens*		
(b)			
*Hae*III	*Haemophilus aegyptius*		
*Hpa*II	*Haemophilus parainfluenzae*		

Blunt Ends vs. Sticky Ends



DNA Ligase

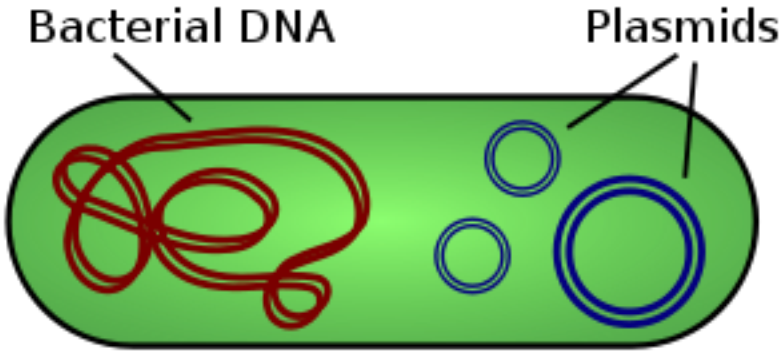
- after cut **DNA ligase** can be used to attach restriction fragments



http://www.dnalc.org/resources/animations/restriction.html

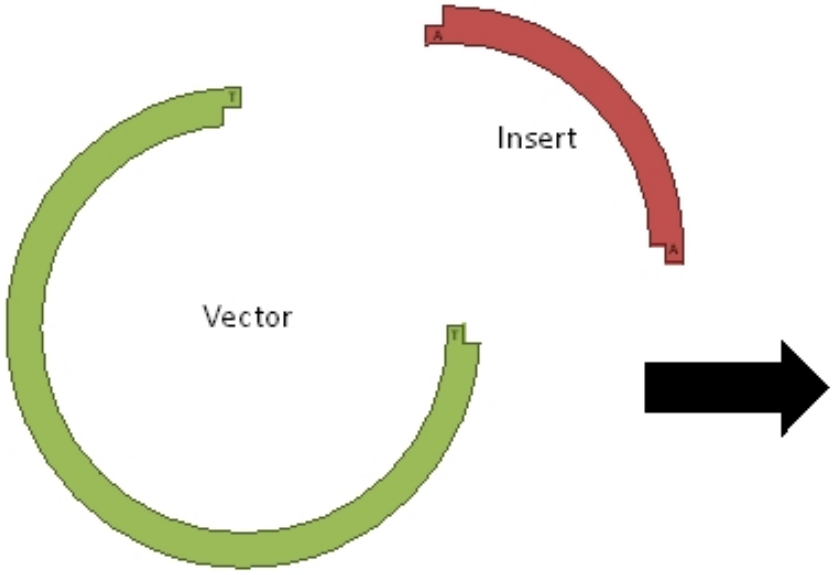
Plasmids

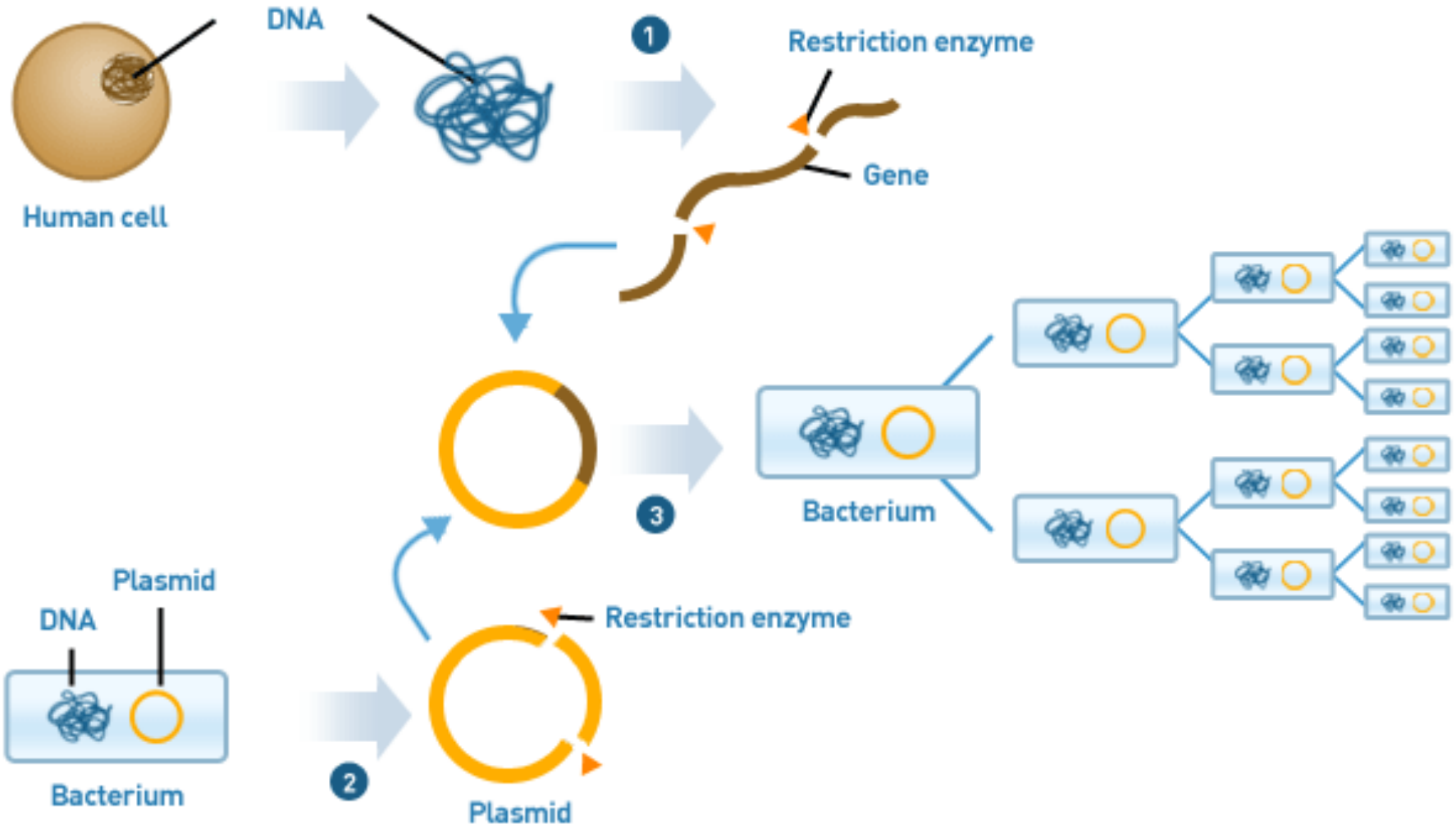
- circular pieces of non-chromosomal DNA found in bacteria cells



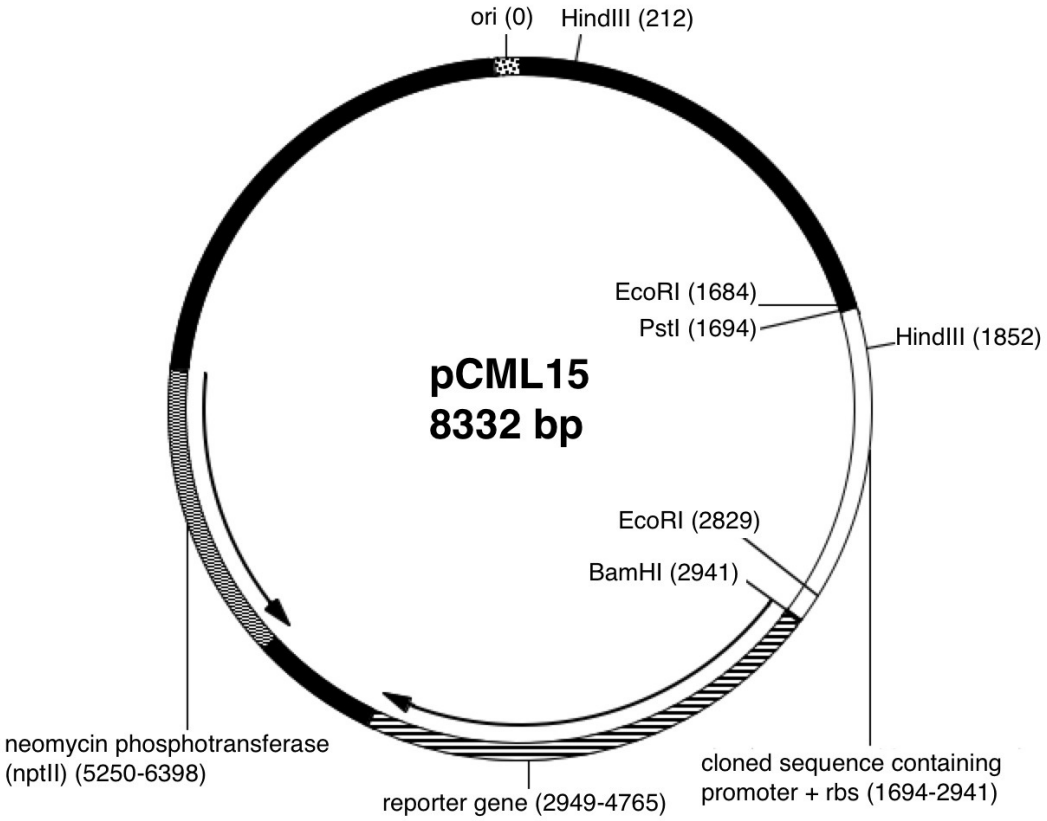
Plasmids as Vectors

- genes can be inserted into plasmids using restriction enzymes and DNA ligase
- can then introduce these genes into **host** bacterial cells




DNA
Human cell
1
Restriction enzyme
Gene
3
Bacterium
Plasmid
DNA
Bacterium
2
Restriction enzyme
Plasmid

Restriction Maps



Constructing Restriction Maps

- Sample Problem 1:

Plasmid X undigested	Plasmid X digested with EcoRI	Plasmid X digested with BamHI	Plasmid X digested with EcoRI and BamHI
14 kbp	14 kbp	6 kbp 8 kbp	1 kbp 6 kbp 7 kbp

Example 2:

EcoRI	BamHI	EcoRI + BamHI
6 kbp 12 kbp	6 kbp 12 kbp	2 kbp 2 kbp 4 kbp 10 kbp

Transformation

- successful introduction of DNA from another source
- bacterial cells can be made **competent** by placing in $CaCl_2$ solution (stabilizes phospholipid bilayer) and then rapid heating & re-cooling

http://www.dnalc.org/resources/animations/transformation1.html

Classwork/Homework