

BIOL 311 Human Genetics

Recombinant DNA Methods: Restriction Enzymes, Cloning Vectors

Reading: Chap. 5 pp. 129-133; 139-146

Lecture Outline:

1. Restriction enzymes
2. Conventional cloning vectors and applications
3. Cloning vectors for genomics

Lecture:

1. Restriction enzymes

- a. Discovered in bacteria and blue-green algae.
- b. Enzymes that recognize and cleave specific sites on DNA. Are endonucleases--cleave within a DNA duplex.
- c. Function to protect organism from virus infections; the host's DNA is typically modified by methylation to prevent restriction enzymes from damaging host DNA.
- d. Useful type for recombinant DNA known as type II restriction enzymes.
- e. Most of the commonly used restriction enzymes recognize 4-6 bp palindromic restriction sites. "Rare cutters" such as Not I and Sfi I recognize larger (8 bp) sites and are useful for genome mapping.
- f. May produce 5' overhangs, 3' overhangs or blunt ends.
- g. Uses of restriction enzymes: Create fragments for cloning (recombinant DNA), create fragments for end-labeling--used in restriction mapping, genomic maps, RFLP analysis.

Examples:

<i>EcoRI</i>	<i>Escherichia coli</i> R factor	5'G AATT C3' 3'C TTAA G5'	5' overhang
<i>PstI</i>	<i>Providencia stuarti</i>	5'C TGCA G3' 3'G ACGT C5'	3' overhang
<i>AluI</i>	<i>Arthrobacter luteus</i>	5'AG CT3' 3'TC GA5'	Blunt end

Naming: 1st letter is genus name, 2nd and 3rd letter is species name, numbered in order of discovery.

Small scale restriction digests: 20 ul reaction

- 2 ul of 10x restriction buffer (buffer, Mg²⁺, salts)
- 1-10 ul of DNA sample (0.5-1 ug)
- 1 ul (1-2 units) restriction enzyme

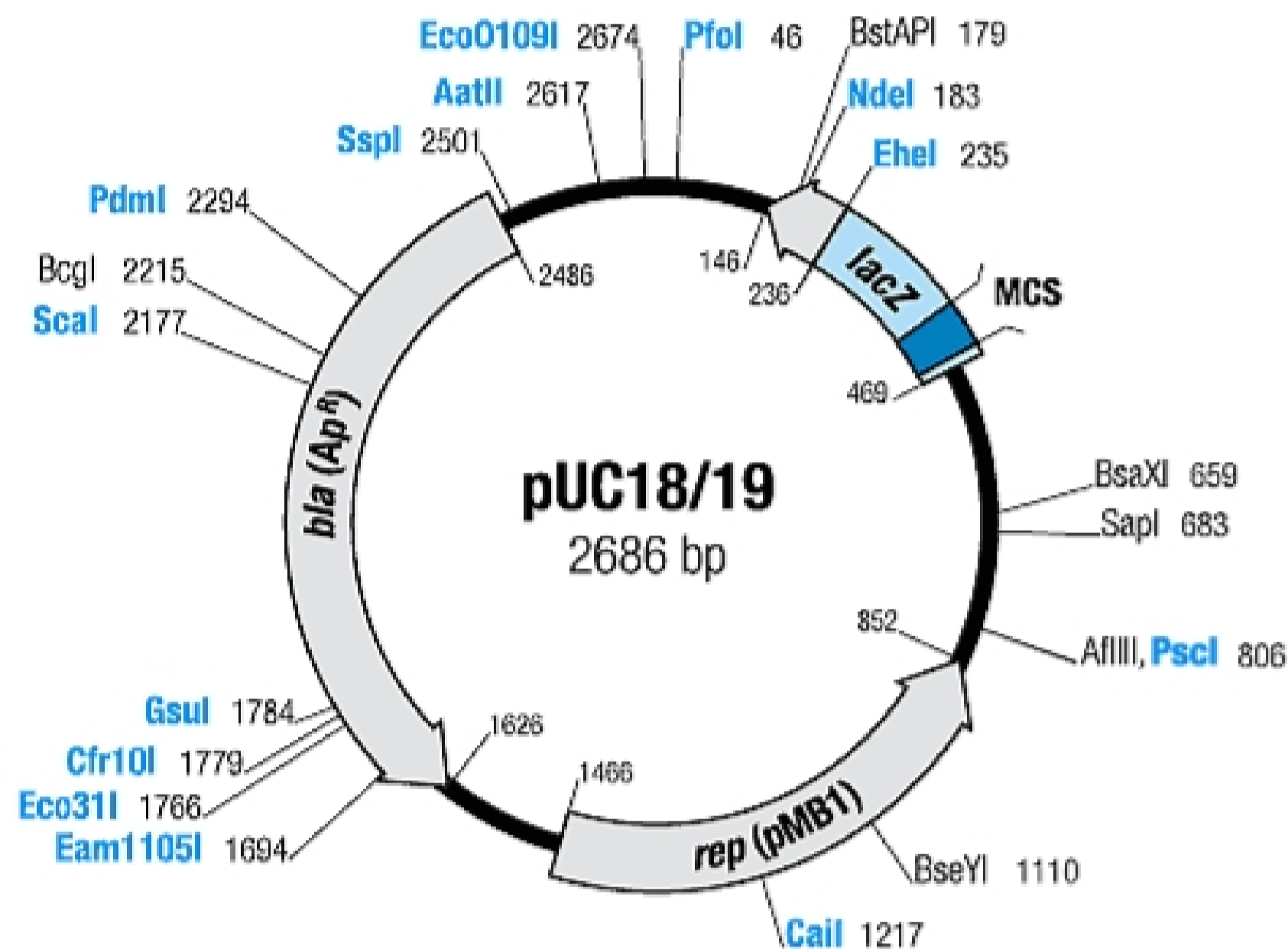
Each restriction enzyme unique; use a special buffer or for convenience, use a buffer supplied by manufacturer that has been tested for its efficiency.

2. Conventional cloning vectors and applications

a. Different cloning vectors used for different applications:
 plasmids--analyzing small DNA regions, expressing genes in cell
 viruses--cloning larger regions (lambda virus), gene therapy (adenovirus)
 artificial chromosome vectors (BACs, PACs, YACs)--cloning chromosomal regions

b. Conventional *E. coli* plasmid cloning vectors typically have:
 origin of replication that functions in bacteria
 antibiotic resistance gene(s)
 selectable marker gene (often lacZ, encoding beta-galactosidase)
 polylinker (also known as a multiple cloning site)

example: pUC18 or pUC19



One would typically clone gene into polylinker within marker gene using restriction enzymes and DNA ligase, transform *E. coli* competent cells, then use antibiotic resistance and the marker phenotype to identify recombinants.

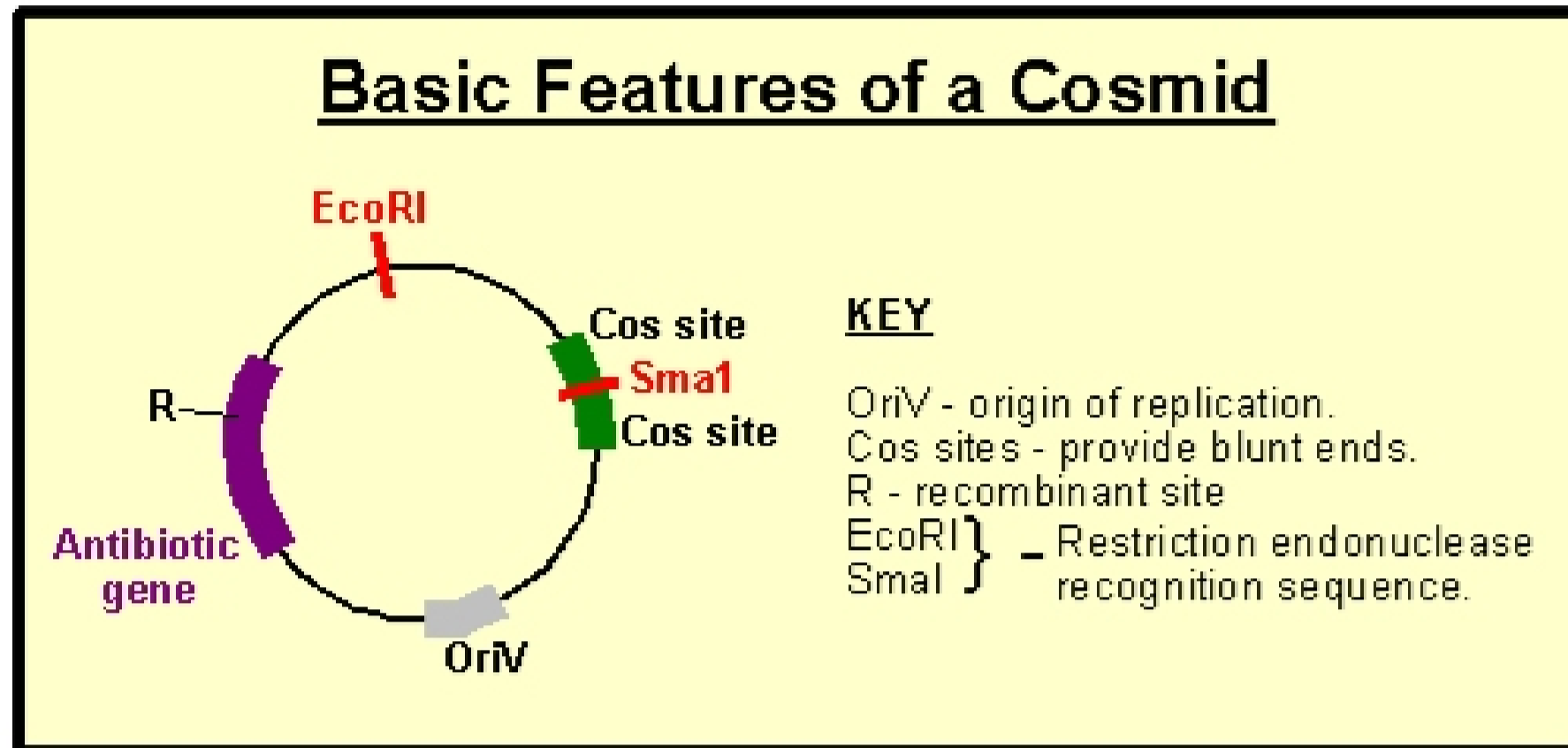
c. Bacteriophage lambda is used to clone larger DNAs or complementary DNAs
 capacity about 10-20 kb
 replace internal section required for lysogeny, but not for lytic life cycle, with foreign DNA

d. Cosmids

capacity 30-44 kb

plasmid vector with lambda packaging site (cos)

not as popular for use because they are unstable and prone to rearrangements



e. M13 virus

small single stranded virus of *E. coli*

replication intermediate (RF) is double-stranded and can be used as cloning vector (much like a plasmid)

after transforming *E. coli*, single stranded virus is produced

single stranded DNAs readily sequenced using dideoxysequencing; also provide a template for site directed mutagenesis protocols.

3. Cloning vectors for genomics

a. Capacity of conventional cloning vectors limiting for studying genomes, particularly the human genome.

b. Large capacity vectors for cloning in bacterial make use of bacterial artificial chromosomes (BACs) and bacteriophage P1 artificial chromosomes (PACs).

Figure of BAC

