Cloning

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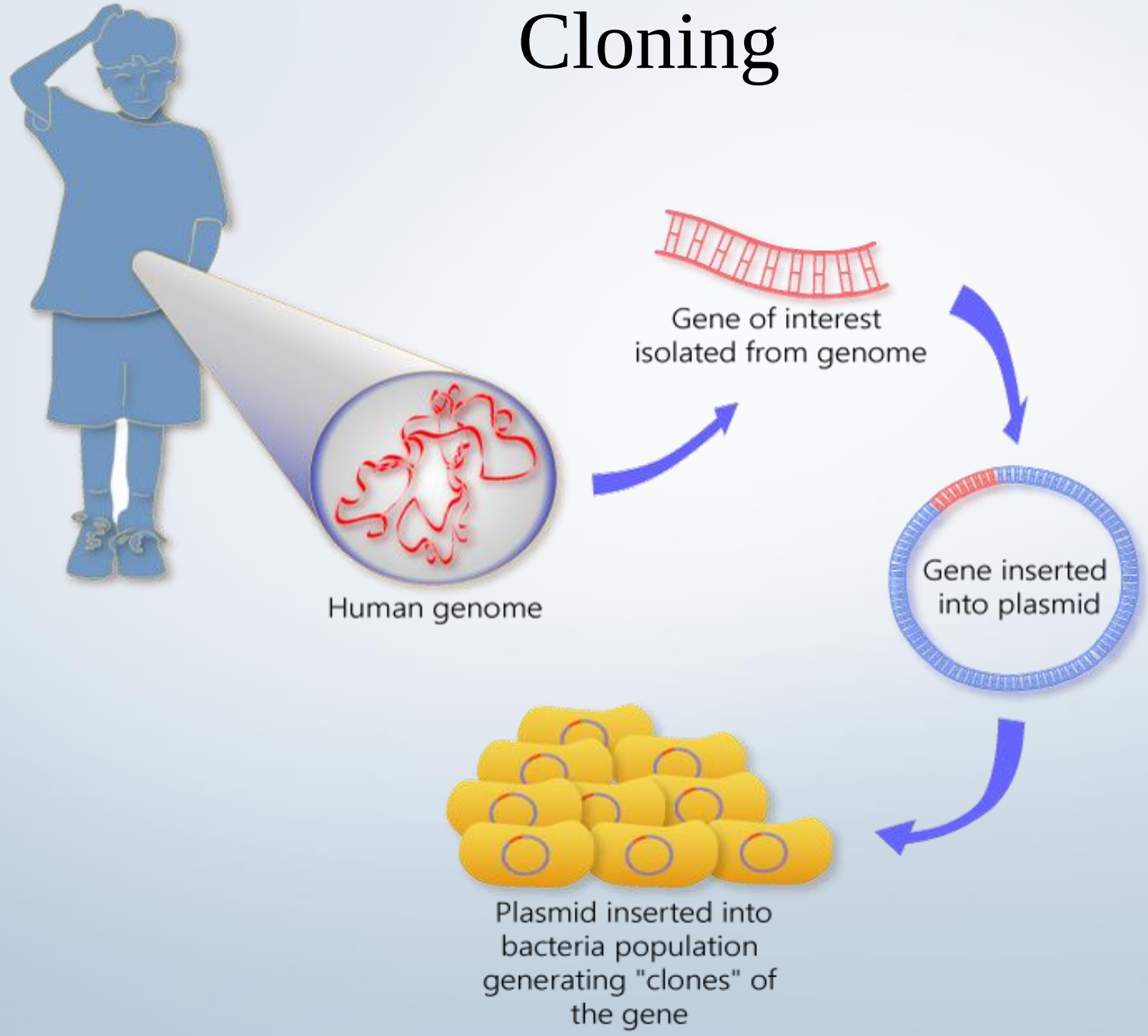
Cloning

- **Insert** (Part/Whole DNA)
- **Host Cell**
- **Vector**
- **Goal:** Bind **Insert** into **Vector** and move to the **Host Cell**

Cloning



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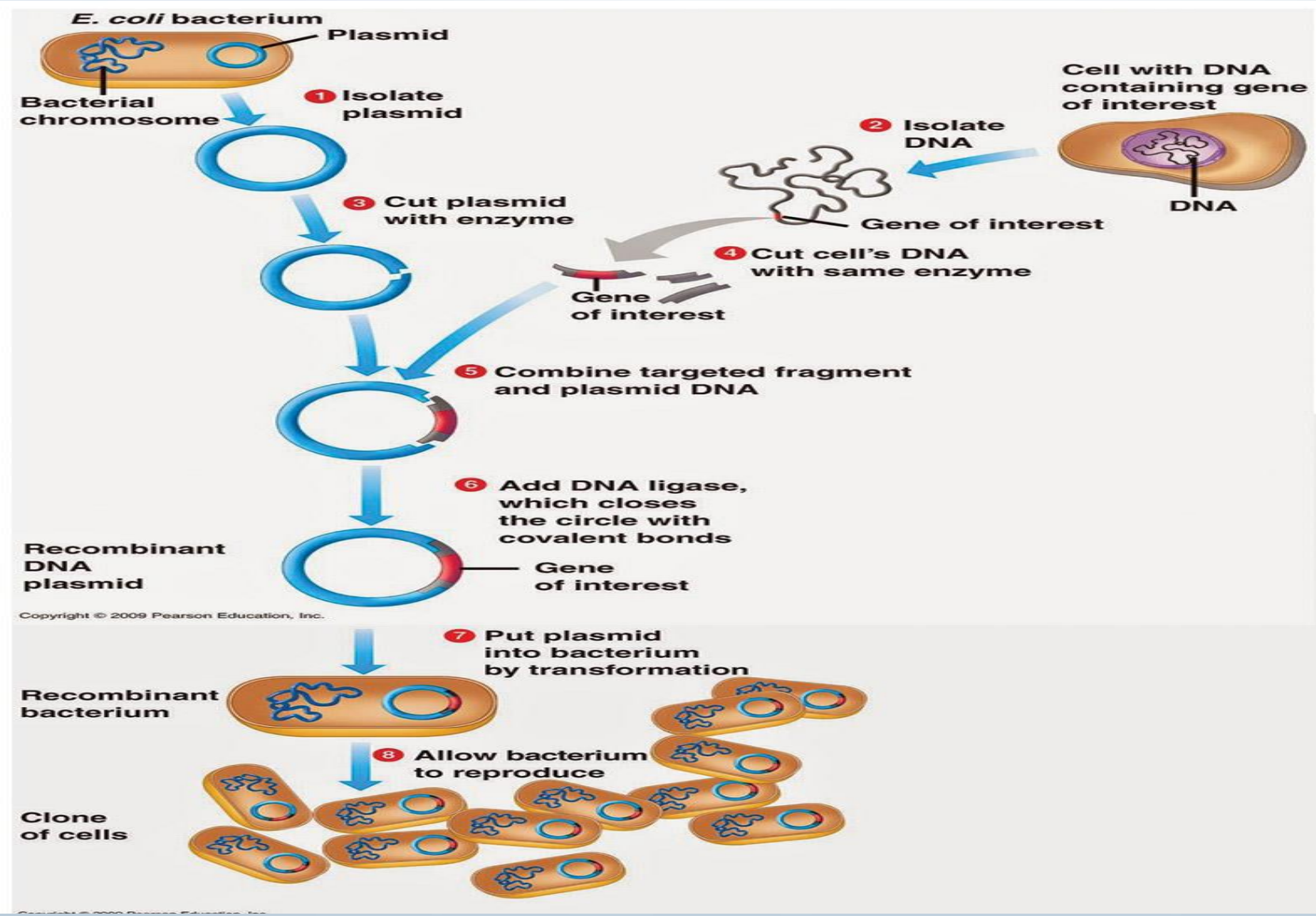
Cloning



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Vector vs Plasmid

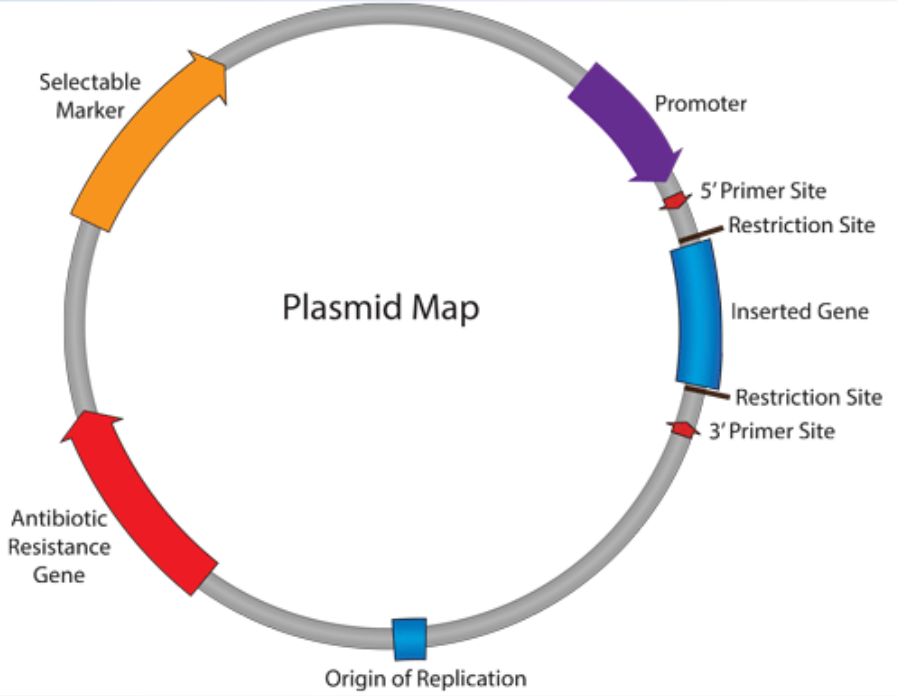
- **Vector** is a plasmid or manipulated artificially after ligation and digestion reaction series, whereas a **plasmid** naturally occurs in bacterial cells.
- There are several vectors, which can be used in recombinant DNA, whereas all plasmids may NOT be used directly in recombinant DNA technology.
- All vectors are plasmids, but not all plasmids are vectors

Types of Plasmids

- **Simple Plasmids**
  - Cloning Plasmids
  - Expression Plasmids

- **Complex Plasmids**
  - Epitope-tagged or Fusion Plasmids
  - Reporter Plasmids
  - Gene Knockdown Plasmids
  - Viral Plasmids

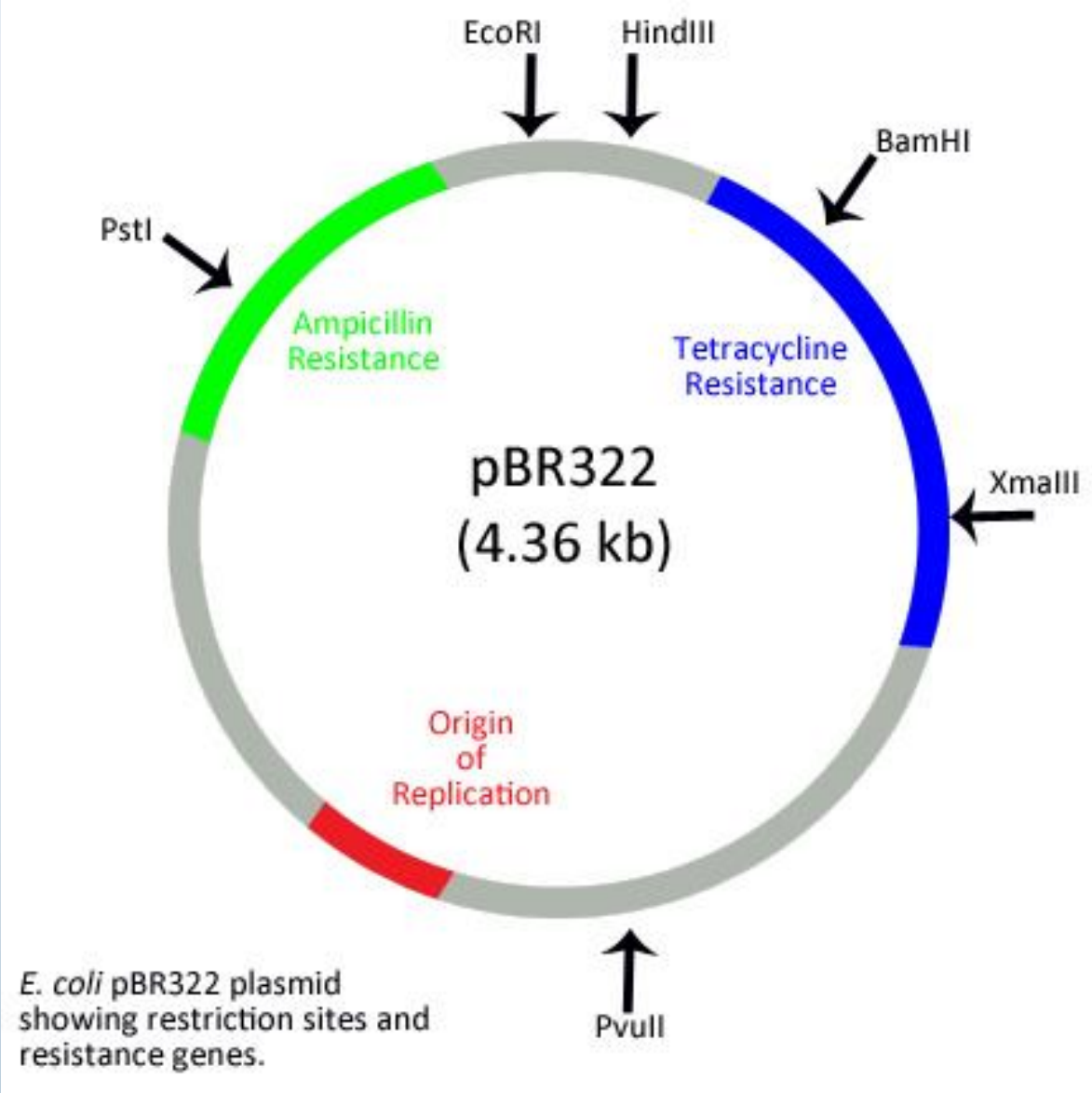
Simple Plasmids



Vector Element	Description
Origin of Replication (ORI)	DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins
Antibiotic Resistance Gene	Allows for selection of plasmid-containing bacteria.
Multiple Cloning Site (MCS)	Short segment of DNA which contains several restriction sites allowing for the easy insertion of DNA. In expression plasmids, the MCS is often downstream from a promoter.
Insert	Gene, promoter or other DNA fragment cloned into the MCS for further study.
Promoter Region	Drives transcription of the target gene. Vital component for expression vectors: determines which cell types the gene is expressed in and amount of recombinant protein obtained.
Selectable Marker	The antibiotic resistance gene allows for selection in bacteria. However, many plasmids also have selectable markers for use in other cell types.
Primer Binding Site	A short single-stranded DNA sequence used as an initiation point for PCR amplification or sequencing. Primers can be exploited for sequence verification of plasmids.

https://www.addgene.org

Simple Plasmids – (example: pBR322)



E. coli pBR322 plasmid showing restriction sites and resistance genes.

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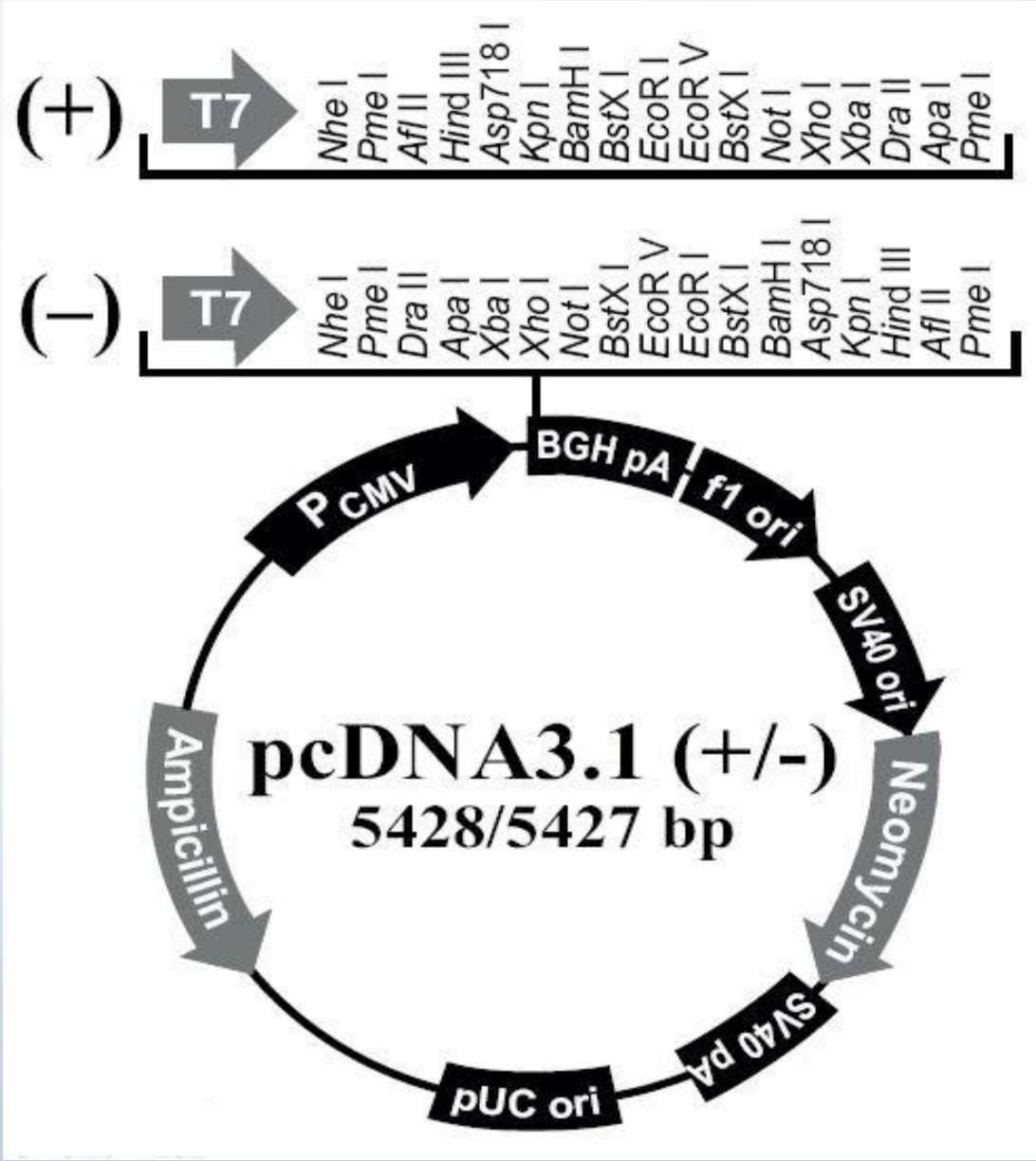
Simple Plasmids

- **Expression Plasmids**
  - Ori (pro)
  - MCS
  - Resistance Gene (pro)
  - Promoter (pro/eu)
  - Transcription Terminator
  - Might contain
    - Ori(eu)
    - Resistance Gene (eu)
  - Example: pcDNA3.1



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pcDNA3.1

addgene
The nonprofit plasmid repository

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Plasmid: pcDNA3.1(+)

Source/Vendor:	Invitrogen
Alt Name:	pcDNA3.1+, pcDNA3.1, pcDNA™3.1(+), pcDNA 3.1 neo
Analyze:	Sequence
Plasmid Type:	Mammalian Expression
Promotor:	CMV
Expression Level:	High

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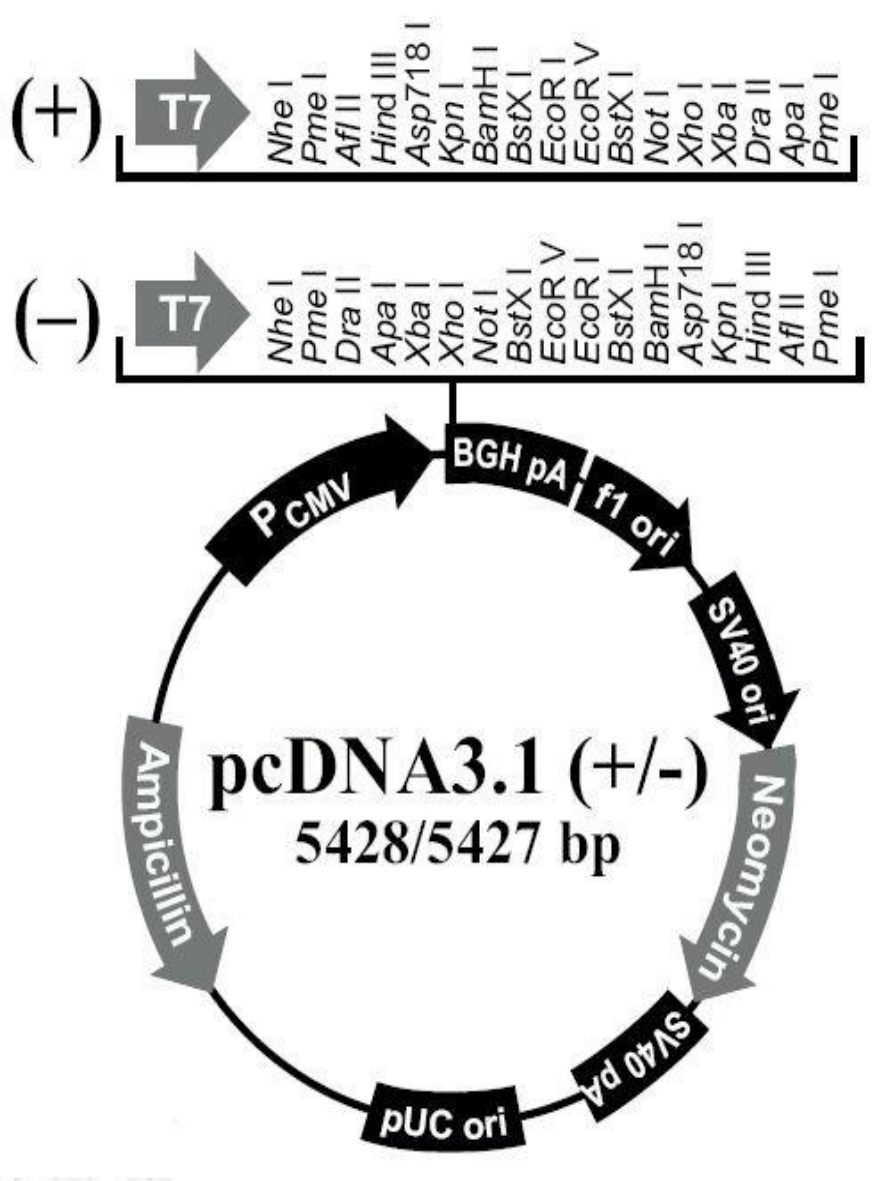
pCMV.SPORT6 vs. pcDNA3.1



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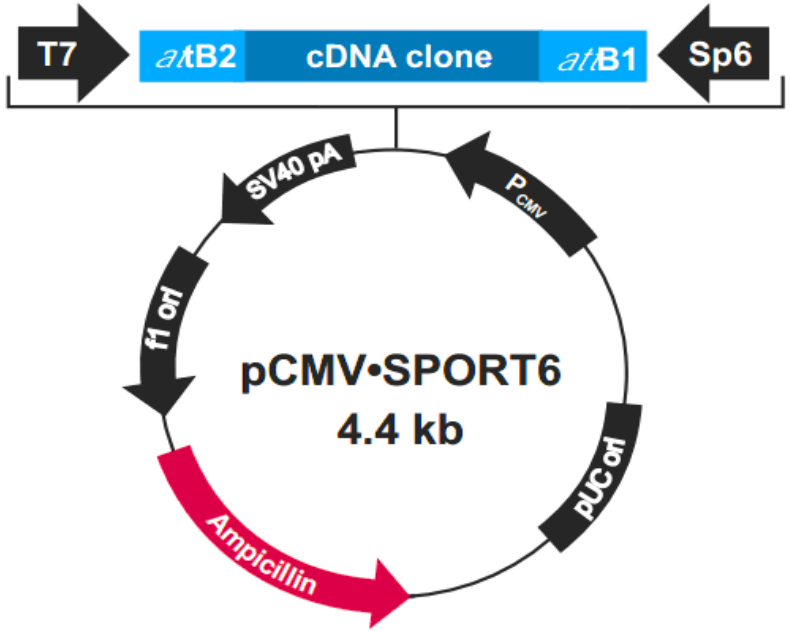

Comments for pCMV•SPORT6 (no insert)
4396 nucleotides

T7 promoter/priming site: bases 16-35
*att*B2: bases 36-60
*att*B1: bases 153-177
Sp6 promoter/priming site (c): bases 177-196
CMV promoter (c): bases 266-859
pUC origin: bases 1236-1908
Ampicillin (*bla*) resistance gene (c): bases 2112-2977
bla promoter (c): bases 2972-3029
f1 origin (c): bases 3260-3720
SV40 early polyadenylation signal (c): bases 3847-4113
(c) = complementary strand

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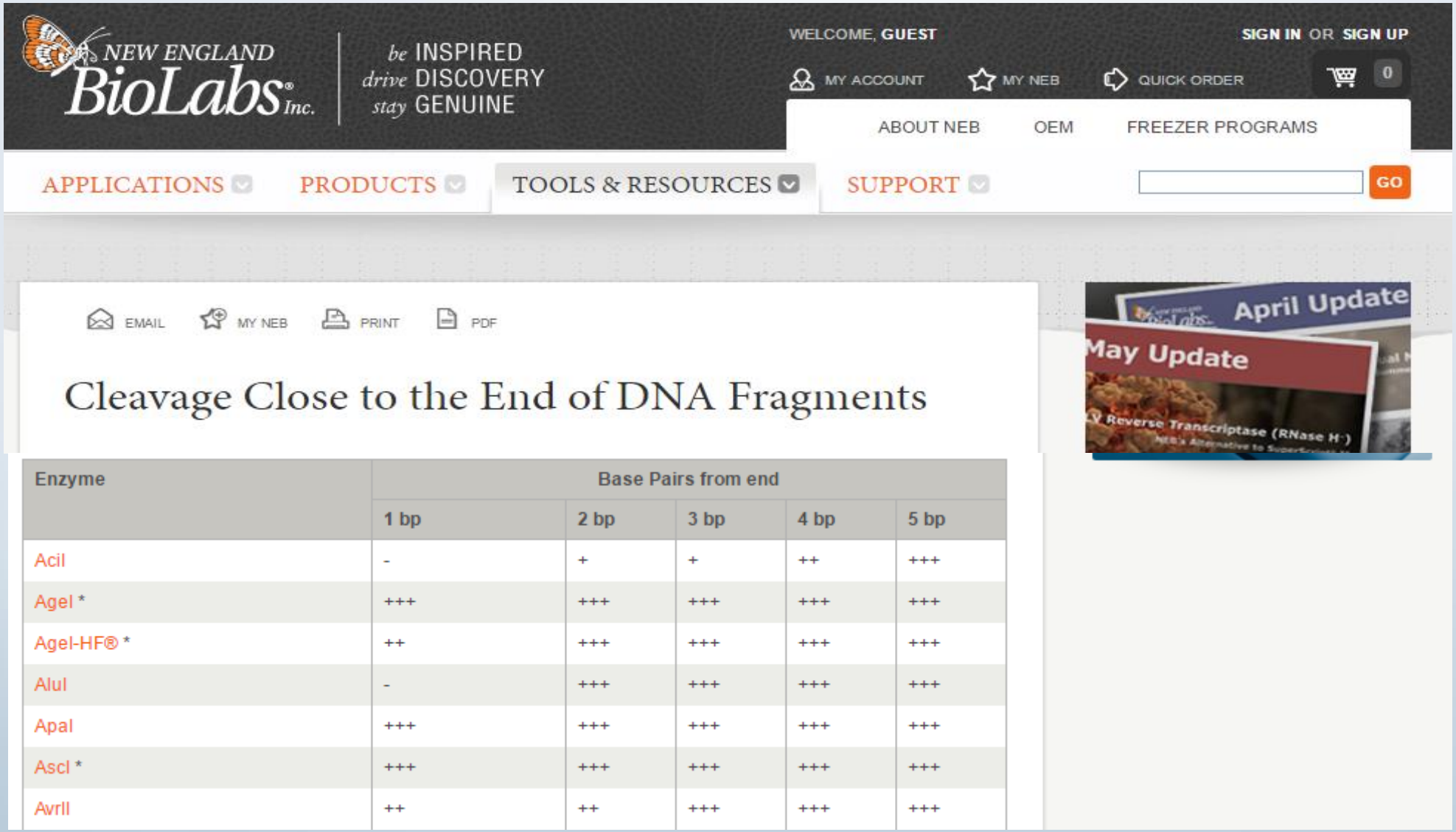
Junk DNA Properties



Enzyme	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
AciI	-	+	+	++	+++
AgeI *	+++	+++	+++	+++	+++
AgeI-HF® *	++	+++	+++	+++	+++
AluI	-	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI *	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++

https://www.neb.com/tools-and-resources/usage-guidelines/cleavage-close-to-the-end-of-dna-fragments