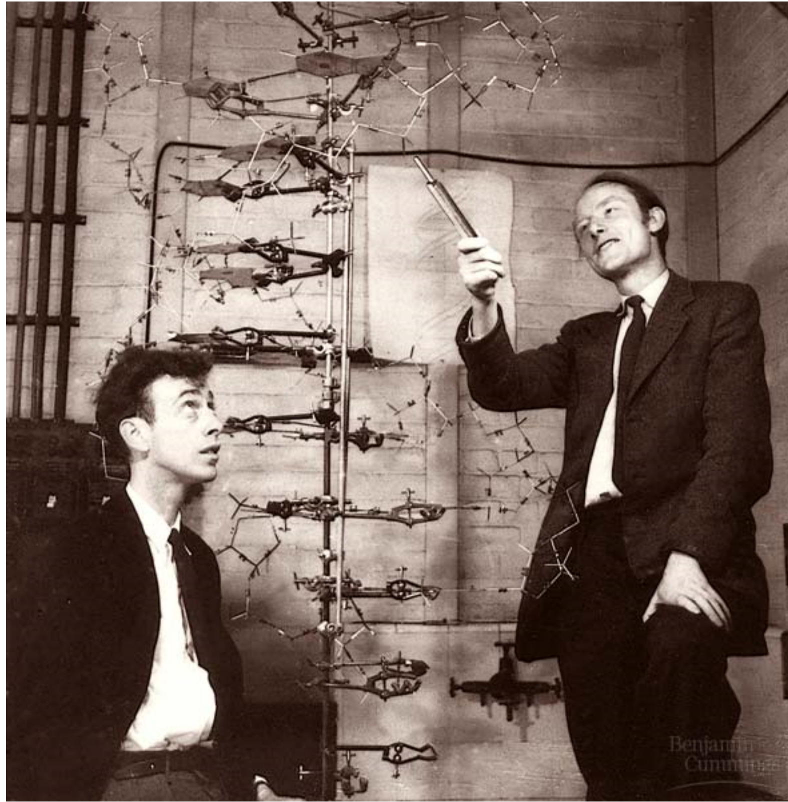
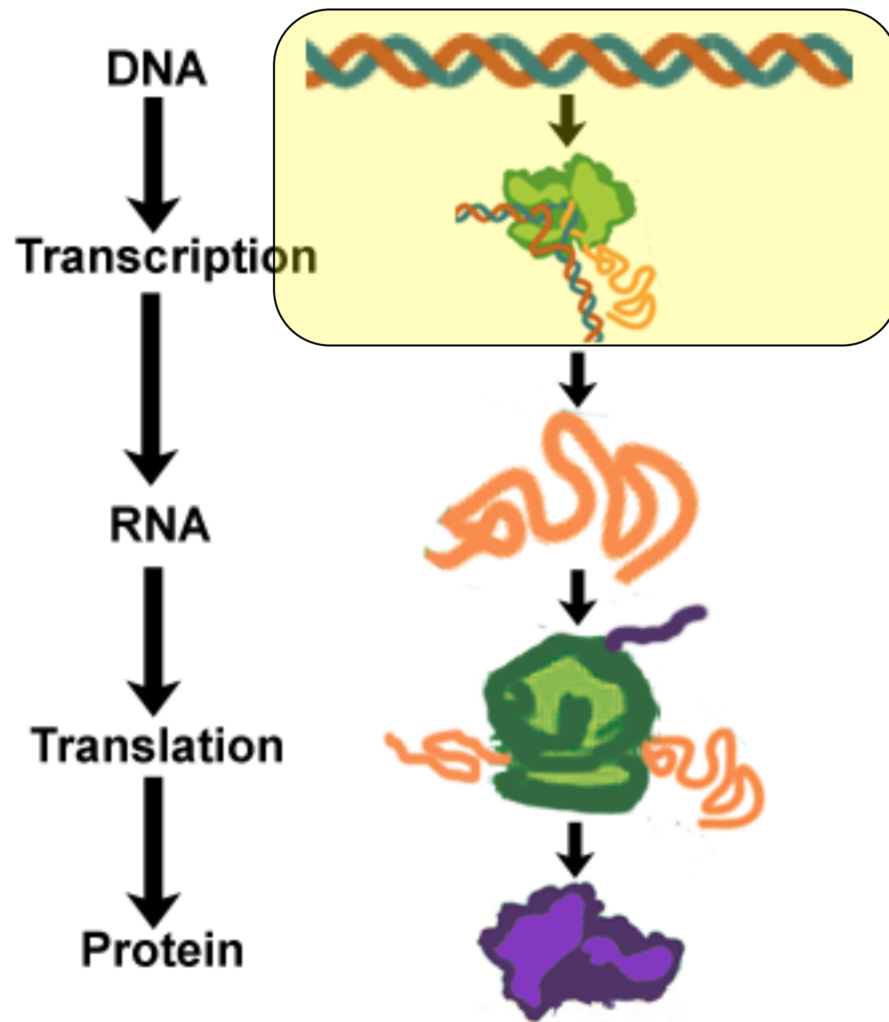


Molecular Biology

Aph 162 Winter 2009

The Central Dogma

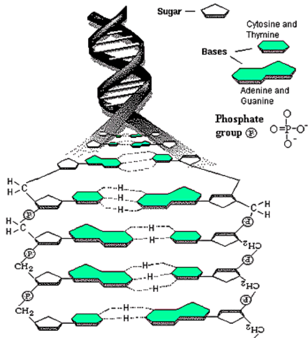


1) A simplified model.

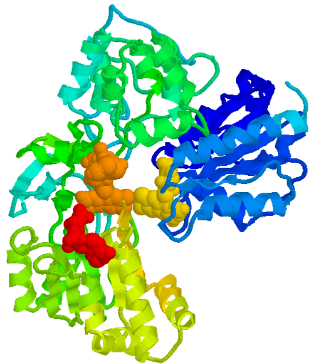
2) Played out across life.

3) Many distinct points for control.

Molecular Biology



DNA: four nucleotide bases (GC,AT) (2 bits)
genetic code in 3 base 'codons'
information storage and propagation,
genetic regulation



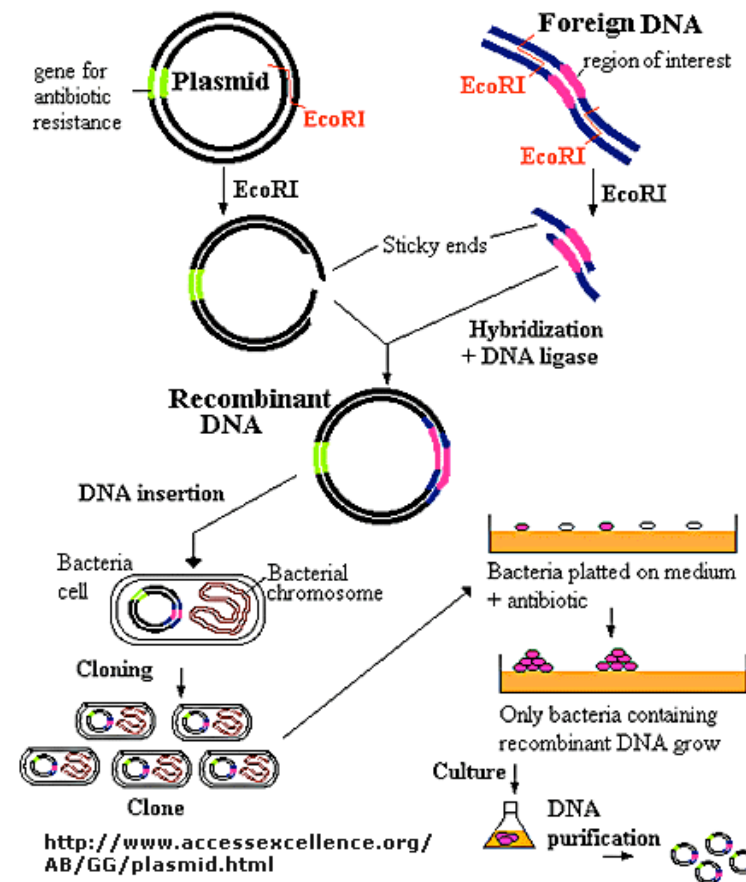
Protein: folded polypeptide of 20 amino acids
motility, metabolism, reproduction,

genetic regulation, transport, etc.

Manipulating DNA – Protein Relationships:
Revolutionized biological research (e.g. crystallography, fluorescent proteins as markers) and medicine (e.g. drug manufacture)

Overview of Cloning

- ***Steps to produce new DNAs that can be used as tools to ask deep biological questions.***
- ***We are going to see how to construct plasmids which include key features such as the GFP protein, antibiotic resistance, etc.***
- ***Big Message: Much of the brilliant trickery of modern molecular biology is tied to getting your DNA of interest into some organism.***



Cloning into a plasmid

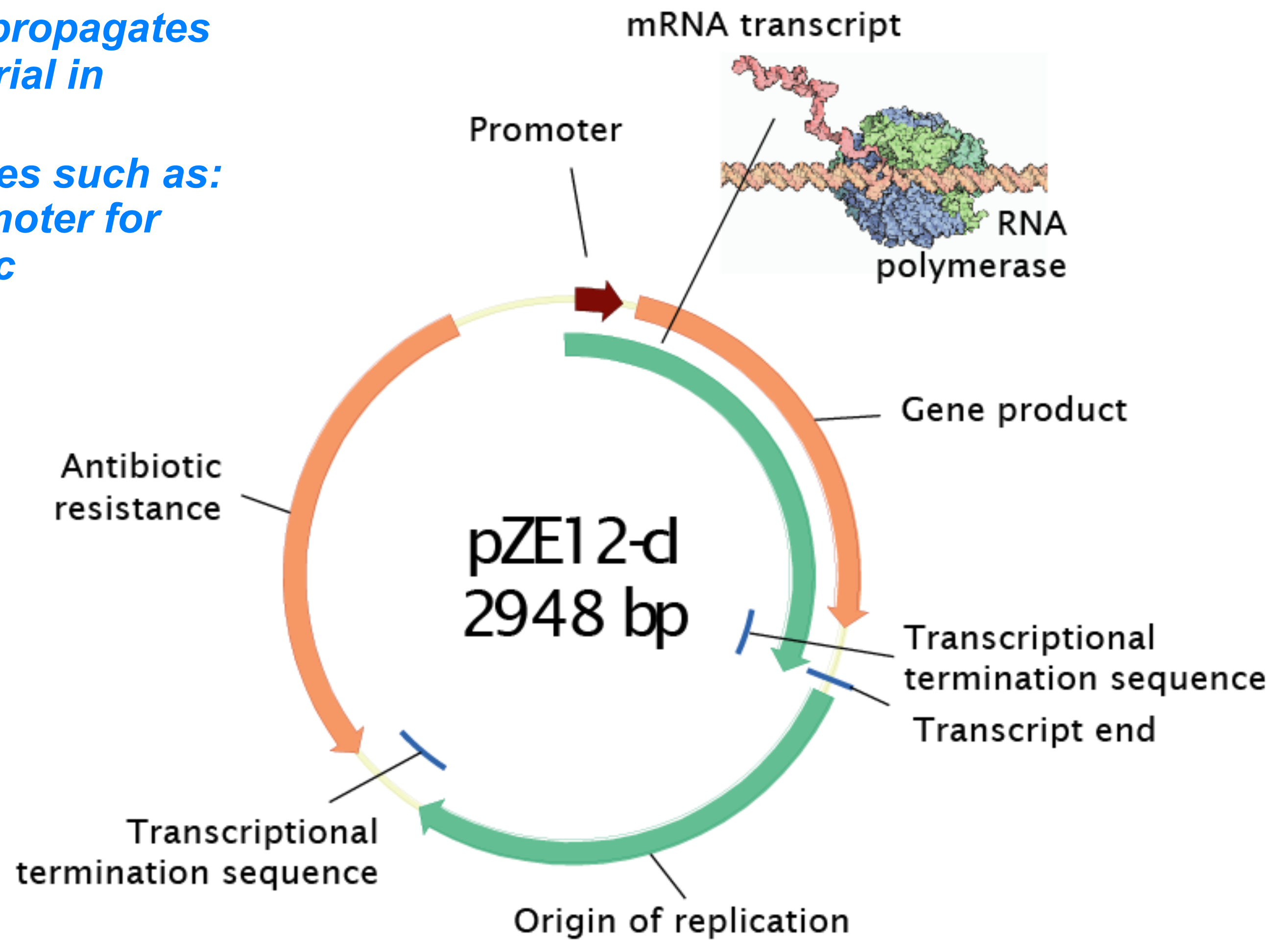
The tools of basic subcloning

1) Plasmids
2) Restriction Enzymes
3) Ligase
4) PCR
5) E. coli

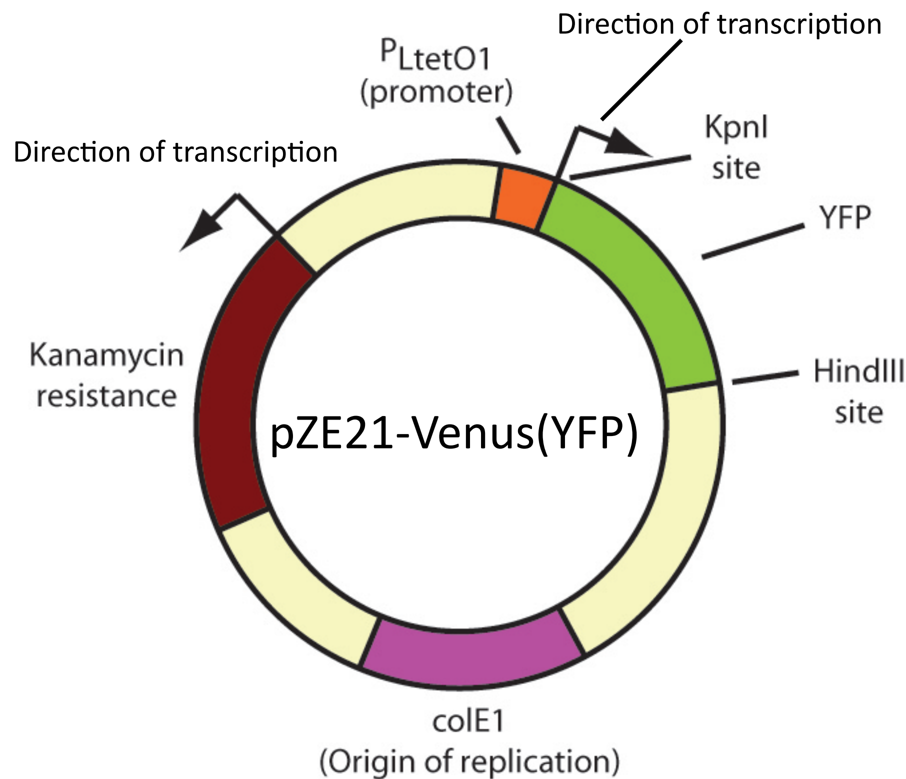
(and many more)

So, what's a plasmid?

- ***Plasmid: circular piece of DNA with origin of replication that propagates as separate genetic material in bacterial cells.***
- ***Plasmids have key features such as: origin of replication, promoter for gene of interest, antibiotic resistance,…***



Plasmid Structure



Promoter – RNA polymerase binding site, transcriptional regulator

Origin of Replication – site where plasmid replication begins for division, controls *copy number* and hence regulates

Restriction Sites – sequence-specific enzymatic DNA cleavage sites, leaves *sticky ends* for proper insert ligation

Kanamycin – encodes gene for Kanamycin (fungal) antibiotic resistance, imparts severe selective advantage in proper media

Non-descript DNA – contain other restriction sites for gene insertion

Building A Stock of Plasmids

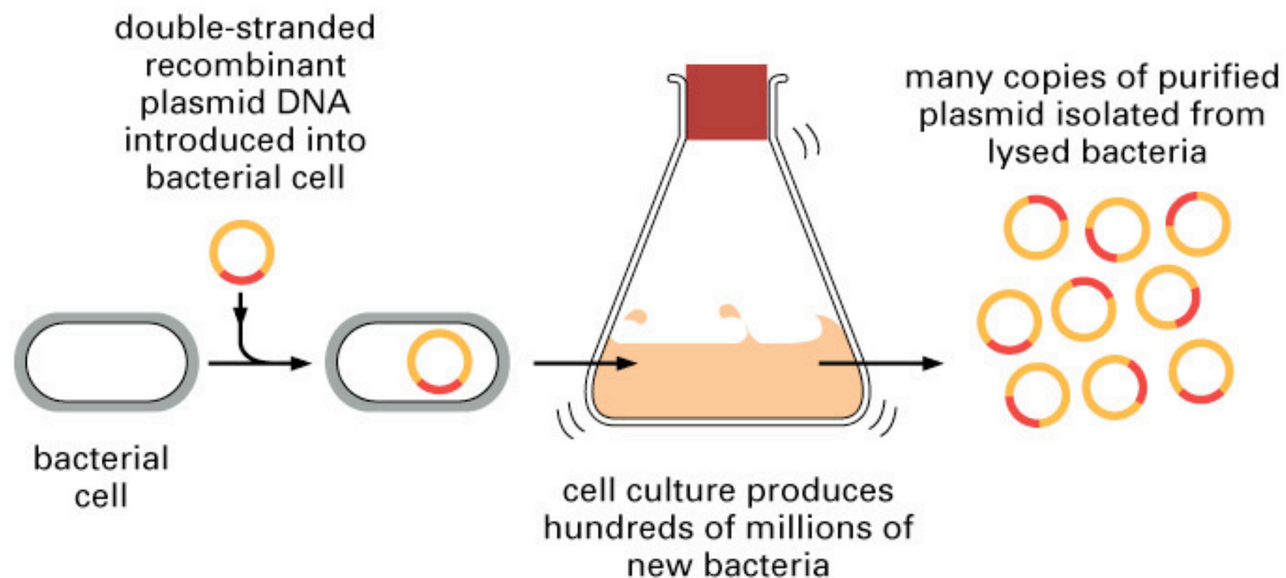


Figure 10-22 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Molecular Biology By Cut and Paste: Building a Plasmid

- ***In order to construct the relevant fusion, we need to do a variety of cutting, amplifying and pasting operations.***
- ***Once that is done, we need to do a series of controls and checks to make sure we produced what we think we did.***

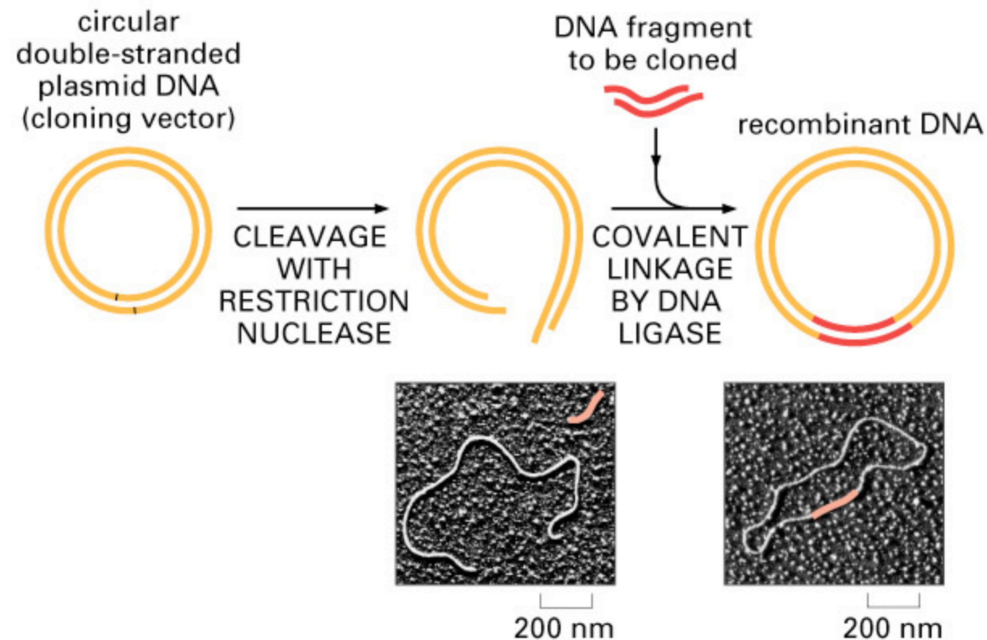
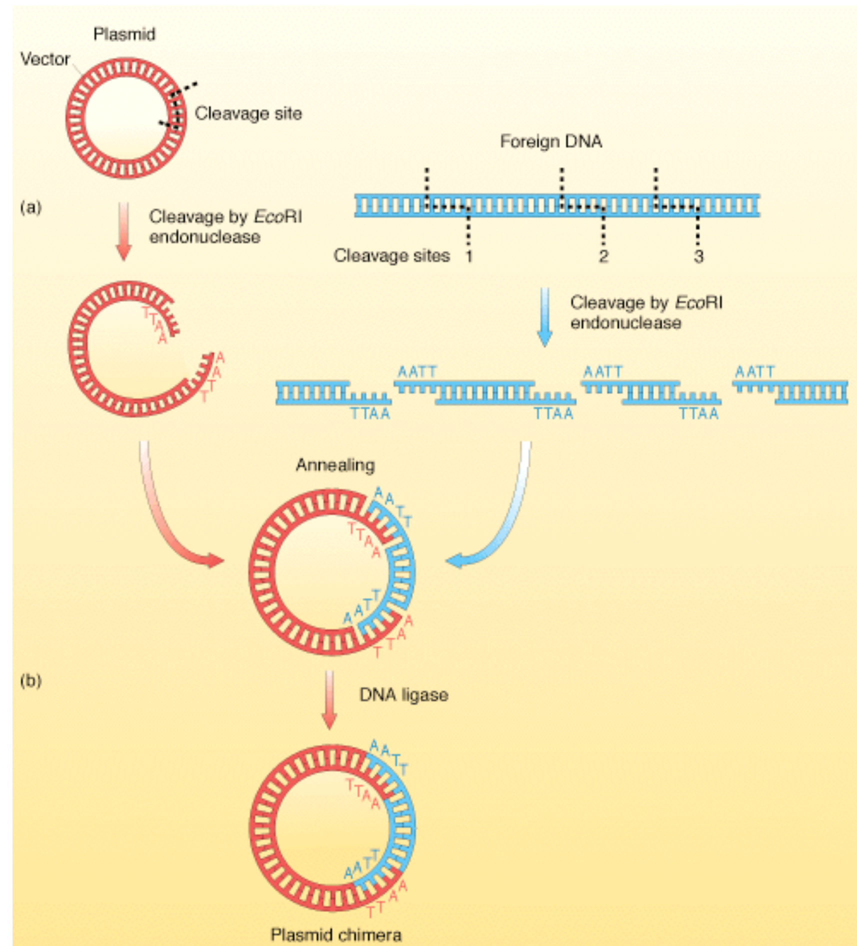


Figure 10-21 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Molecular Biology By Cut and Paste: Building a Plasmid

- ***Another view of the procedure***



http://fig.cox.miami.edu/Faculty/Dana/chimericDNA.gif

Restriction Enzymes and Cutting

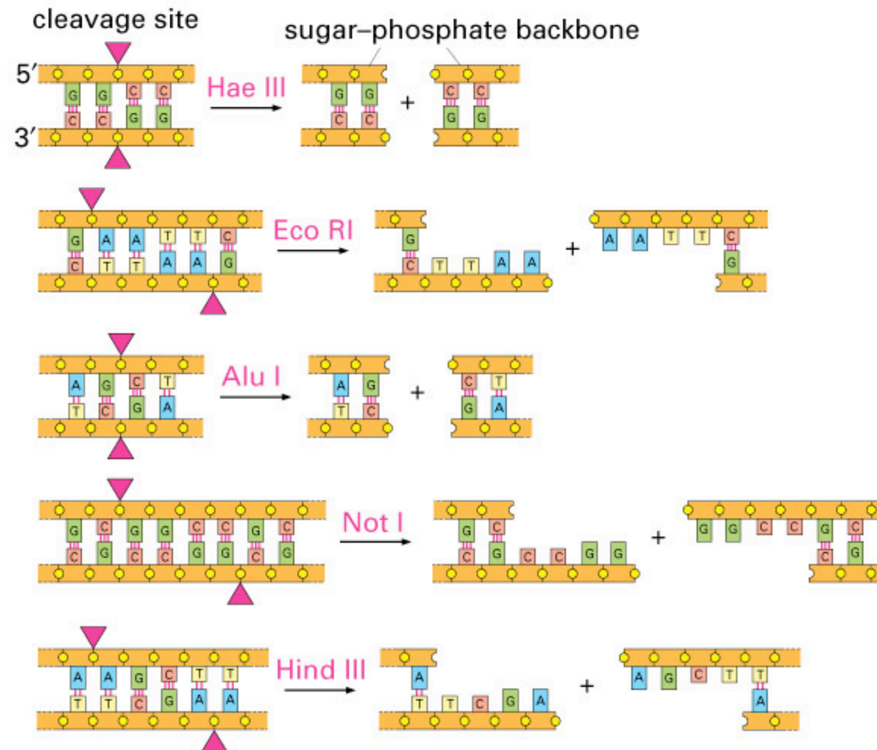


Figure 10-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

New England Biolabs Website

- www.neb.com
- *This list shows the sites on the phage lambda genome that will be cut by various restriction enzymes.*

Lambda DNA: Location of Sites

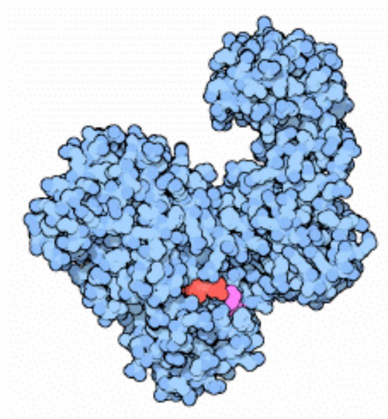
Enzyme	#	Locations
Apa I	1	10086
Bmt I	1	34679
BsiW I	1	19323
Kas I	1	45679
Nae I	1	20040
Nar I	1	45679
NgoM IV	1	20040
Nhe I	1	34679
PaeR7 I	1	33498
PspOM I	1	10086
SanD I (x)	1	28797
Sfo I	1	45679
SnaB I	1	12188
Tli I	1	33498
Xba I	1	24508
Xho I	1	33498
Acc65 I	2	17053 18556
Afe I	2	20995 37057
Asc I	2	3521 16648
Avr II	2	24322 24396
Bsa I	2	11424 42715
Bsu36 I	2	26717 34318
Eag I	2	19944 36654
FspA I (x)	2	21804 21825
Kpn I	2	17053 18556
Pci I	2	628 39395
PflF I	2	11202 36120
Pme I	2	8459 16293
Sac I	2	24772 25877
Sal I	2	32745 33244
Tth111 I	2	11202 36120
Afl II	3	6540 12618 42630
Bst Z17 I	3	15260 18834 19473
Drd I	3	5116 9104 11090
EcoO109 I	3	2815 28797 48473
Pml I	3	26529 41482 42362
PpuM I	3	2815 28797 48473
Pvu I	3	11933 26254 35787
Sma I	3	19397 31617 39888
Xma I	3	19397 31617 39888
ApaL I	4	5619 21798 27173 40216
Bmr I	4	7054 11608 25691 30332
Nco I	4	19329 23901 27868 44248
Sacl I	4	20320 20530 21606 40386
BamH I	5	5505 22346 27972 34499 41732
BsrG I	5	5220 6142 15855 29392 32496
EcoR I	5	21226 26104 31747 39168 44972
Nru I	5	4590 28050 31703 32407 41808
Rsr II	5	3800 6041 13983 19288 22242
Sbf I	5	2555 2819 11834 19832 37000
Sca I	5	16421 18684 25685 27263 32802
SexA I	5	22264 31009 32838 40497 44408
Bgl II	6	415 22425 35711 38103 38754 38814
Blp I	6	10297 10682 11661 16518 20744 39450
BssH II	6	3522 4126 5627 14815 16649 28006
SgrA I	6	7064 8680 12878 15653 16974 31824
Sph I	6	2212 12002 23942 24371 27374 39418
Stu I	6	12434 31478 32997 39992 40596 40614
Acl I	7	13529 16290 22580 22595 24642 43392 43488
Ban II	7	581 10086 19763 21570 24772 25877 39453
BsvC I	7	8012 18147 18465 30916 31222 31836 35813
BstB I	7	18048 25884 27980 29150 30396 34331 42637
Hind III	7	23130 25157 27479 36895 37459 37584* 44141 (*cI857ind1 mutants only)
Mlu I	7	458 5548 15372 17791 19996 20952 22220
Nde I	7	27630 29883 33679 36112 36668 38357 40131
PshA I	7	8920 9394 13512 15412 36925 37889 48152
Ava I	8	4720 19397 20999 27887 31617 33498 38214 39888
Bcl I	8	8844 9361 13820 32729 37352 43682 46366 47942
BsoB I	8	4720 19397 20999 27887 31617 33498 38214 39888
BspH I	8	889 4650 4989 10249 18275 28860 31608 40642
BssS I	8	20356 25572 27956 29425 34430 35219 42416 42737
Mfe I	8	22687 22715 23054 25863 35764 37186 38332 47880
Acc I	9	2190 15260 18834 19473 31301 32745 33244 40201 42921
Ahd I	9	6398 11238 12477 12915 16588 18544 23480 30467 44869
EcoN I	9	13509 21292 22377 25174 25223 35521 38268 41842 47213
Aat II	10	5105 9394 11243 14974 29036 40806 41113 42247 45563 45592
Bae I	10	694 7965 13267 15775 16271 18732 19109 21779 43976 48385
Bme1580 I	10	5619 5664 10086 11414 13039 14897 21798 27173 32330 40216
Dra III	10	2954 5613 6635 8999 14477 30365 31909 41479 47312 48434
Sap I	10	2397 6489 8702 10370 13286 24769 27234 34327 34800 47712
Sty I	10	19329 21211 23901 24322 24396 27868 28793 35016 36505 44248
Zra I	10	5105 9394 11243 14974 29036 40806 41113 42247 45563 45592
Aar I (x)	12	554 10835 13941 14383 16164 16194 16233 21242 29897 36293 37003 38481
Psi I	12	2285 9011 18941 19573 22014 24667 25472 27746 29498 29656 33236 37756
Xcm I	12	958 4770 5065 5874 9193 9460 9490 14891 19329 23685 32530 36165

There are no restriction sites for the following enzymes:
AsiS I, Fse I, I-Ceu I, I-Sce I, Not I, PI-Psp I, PI-Sce I, Pac I, Sfi I, Spe I, Srf I (x), Swa I

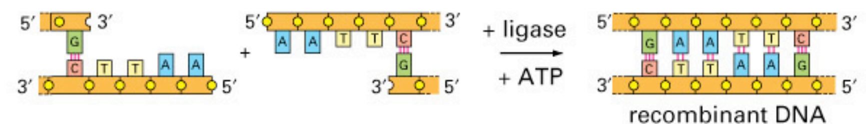
(x) = enzyme not available from NEB

The Idea of Ligation

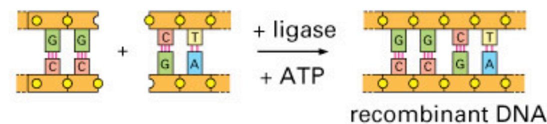
- ***When we have our fragments (YFP and protein) to insert into the plasmid, we have to glue them in using an enzyme called DNA ligase.***
- ***In living cells, this is relevant to supercoiling, recombination, DNA repair, replication, etc.***



(A) JOINING TWO COMPLEMENTARY STAGGERED ENDS



(B) JOINING TWO BLUNT ENDS



(C) JOINING A BLUNT END WITH A STAGGERED END

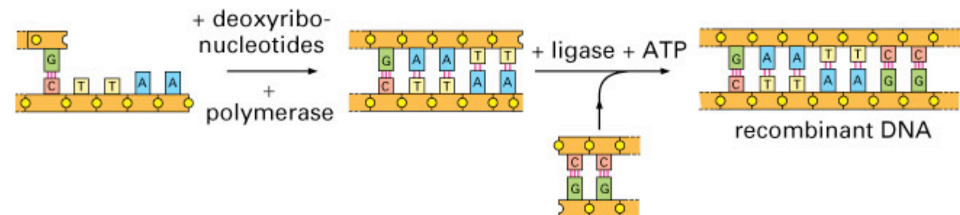
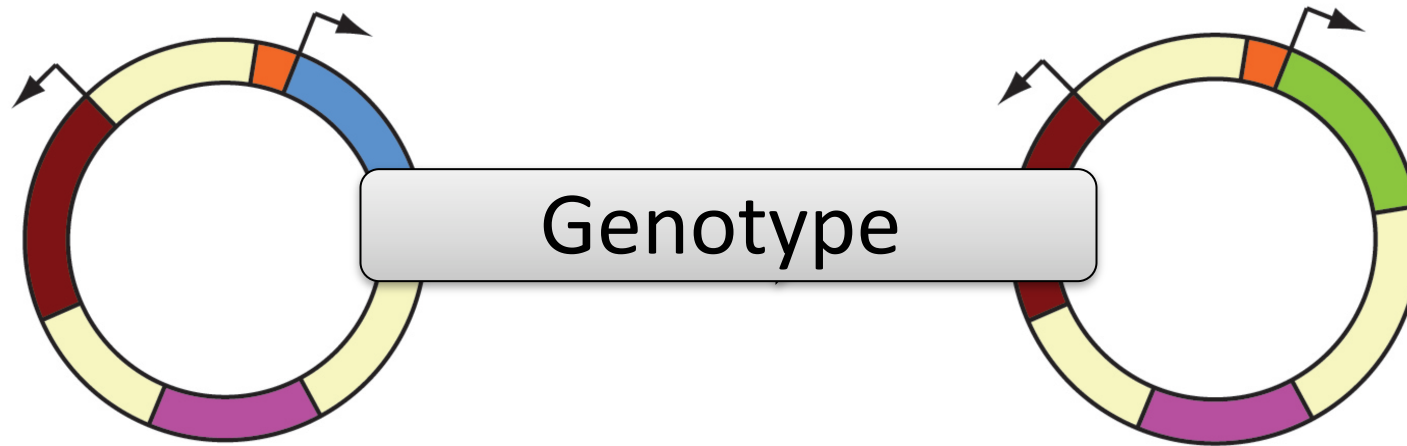
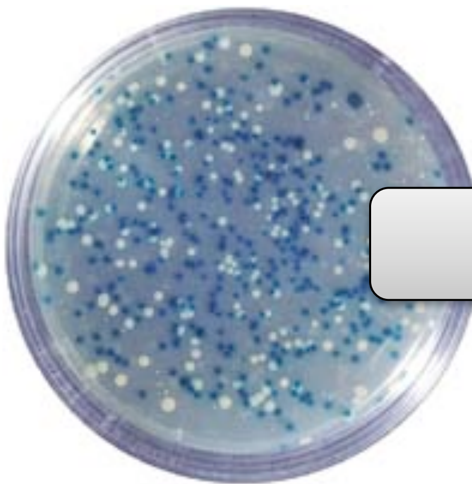


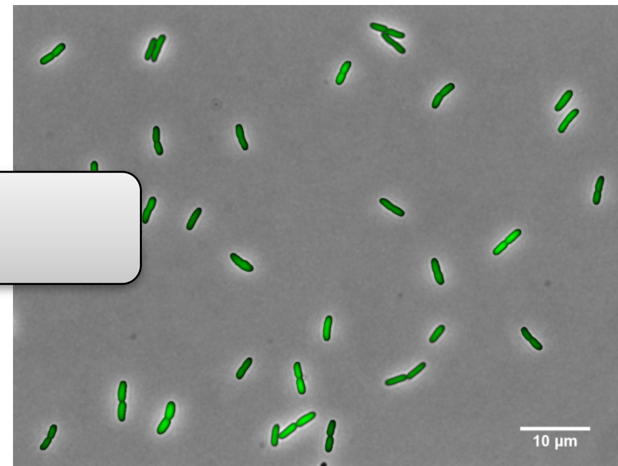
Figure 10-18 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Aph 162 Subcloning





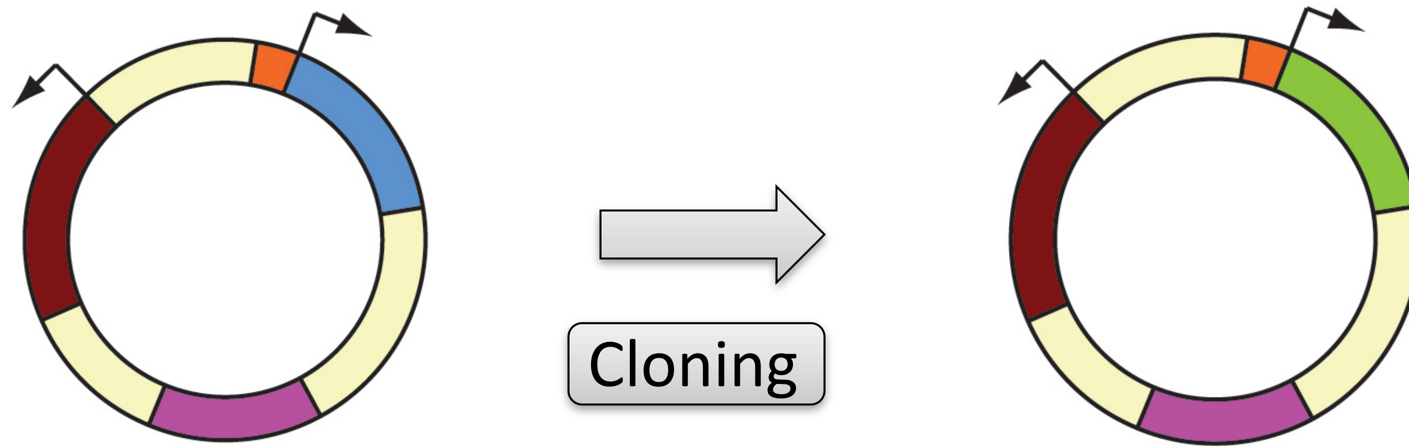
Phenotype

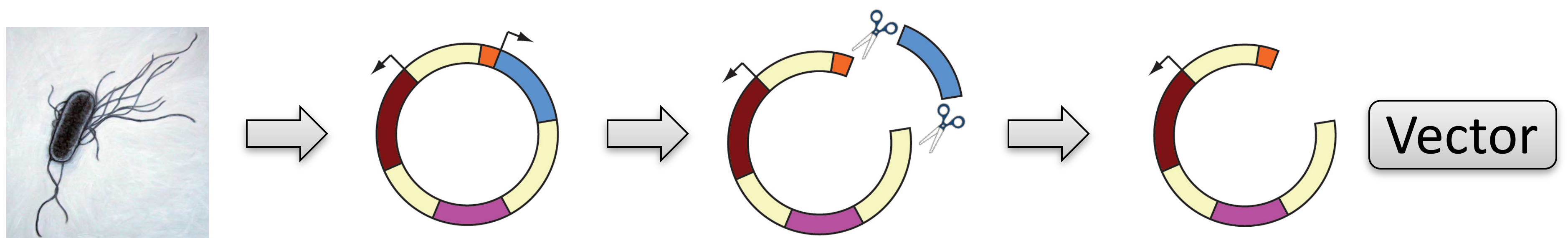


E. coli expressing protein β-galactosidase

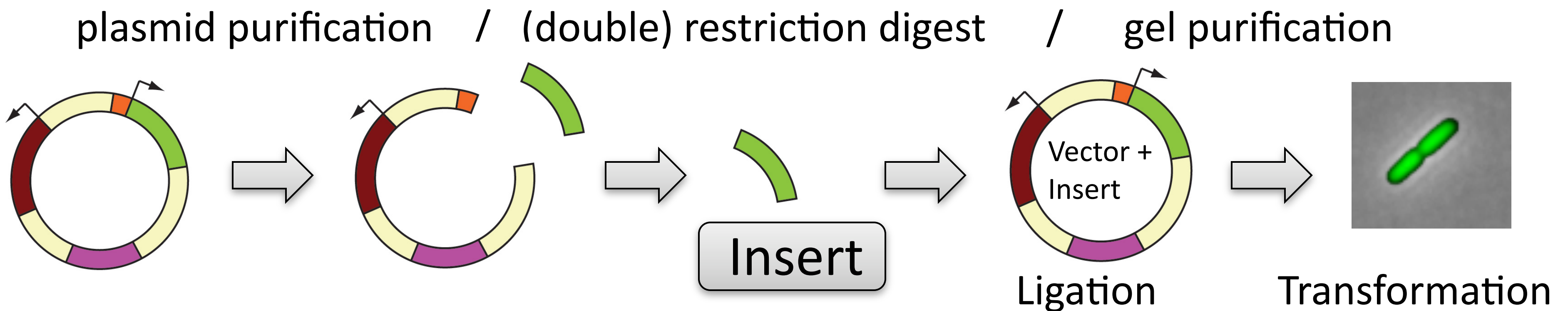
E. coli expressing fluorescent protein from jellyfish (YFP)

Subcloning

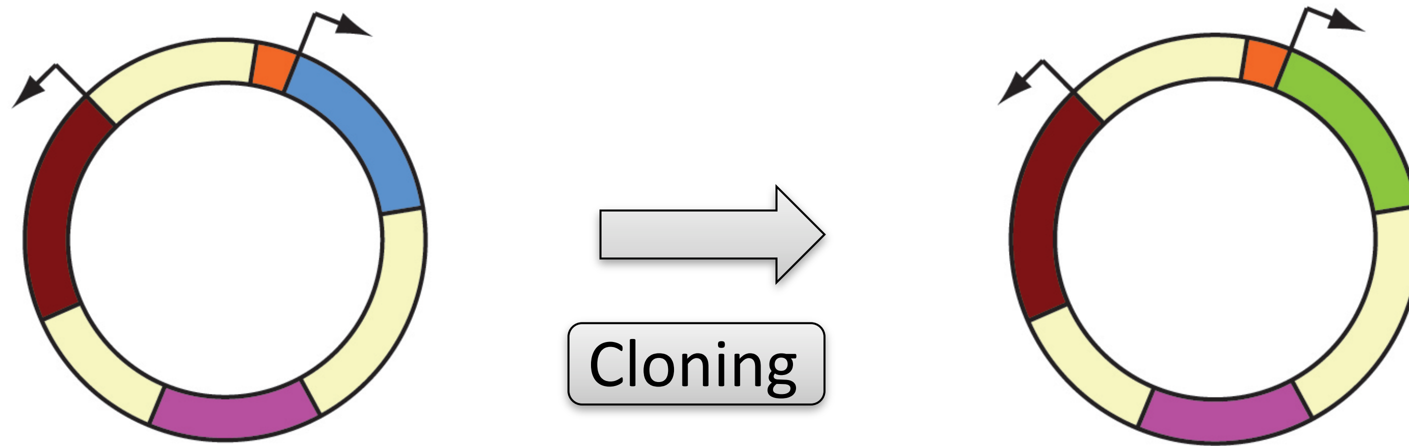


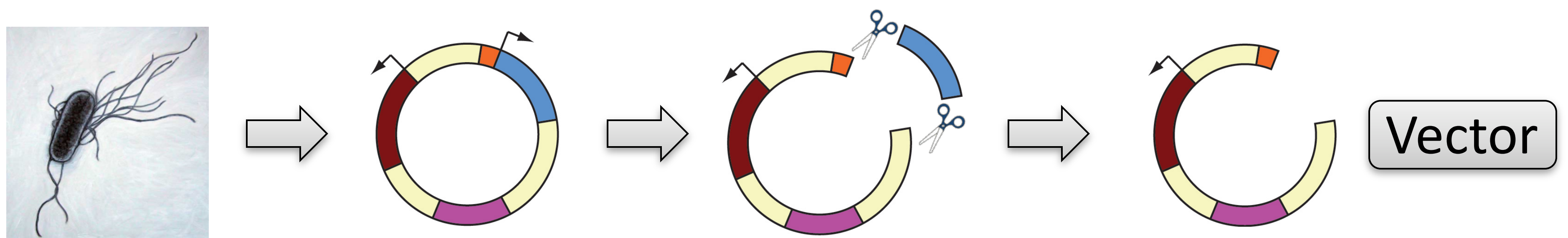


plasmid purification / (double) restriction digest / gel purification

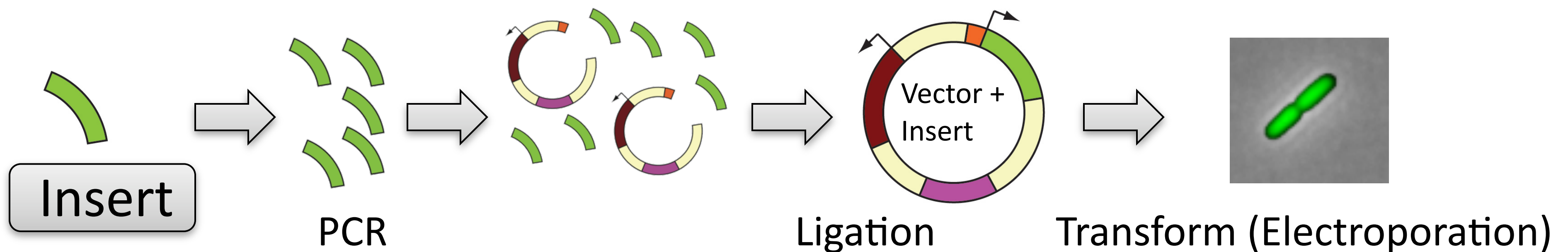


Subcloning





plasmid purification / (double) restriction digest / gel purification



The First Cycle of PCR

- ***Once we have some copies of our DNA fragments of interest, we need to generate a huge number of copies of these fragments for the purposes of actually generating large quantities of the insert.***

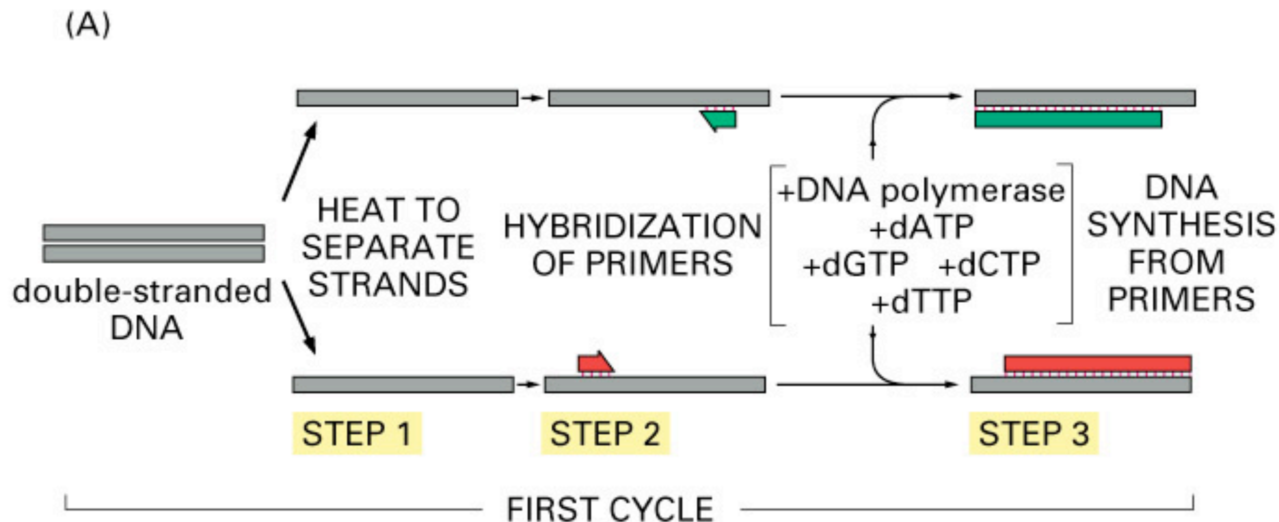
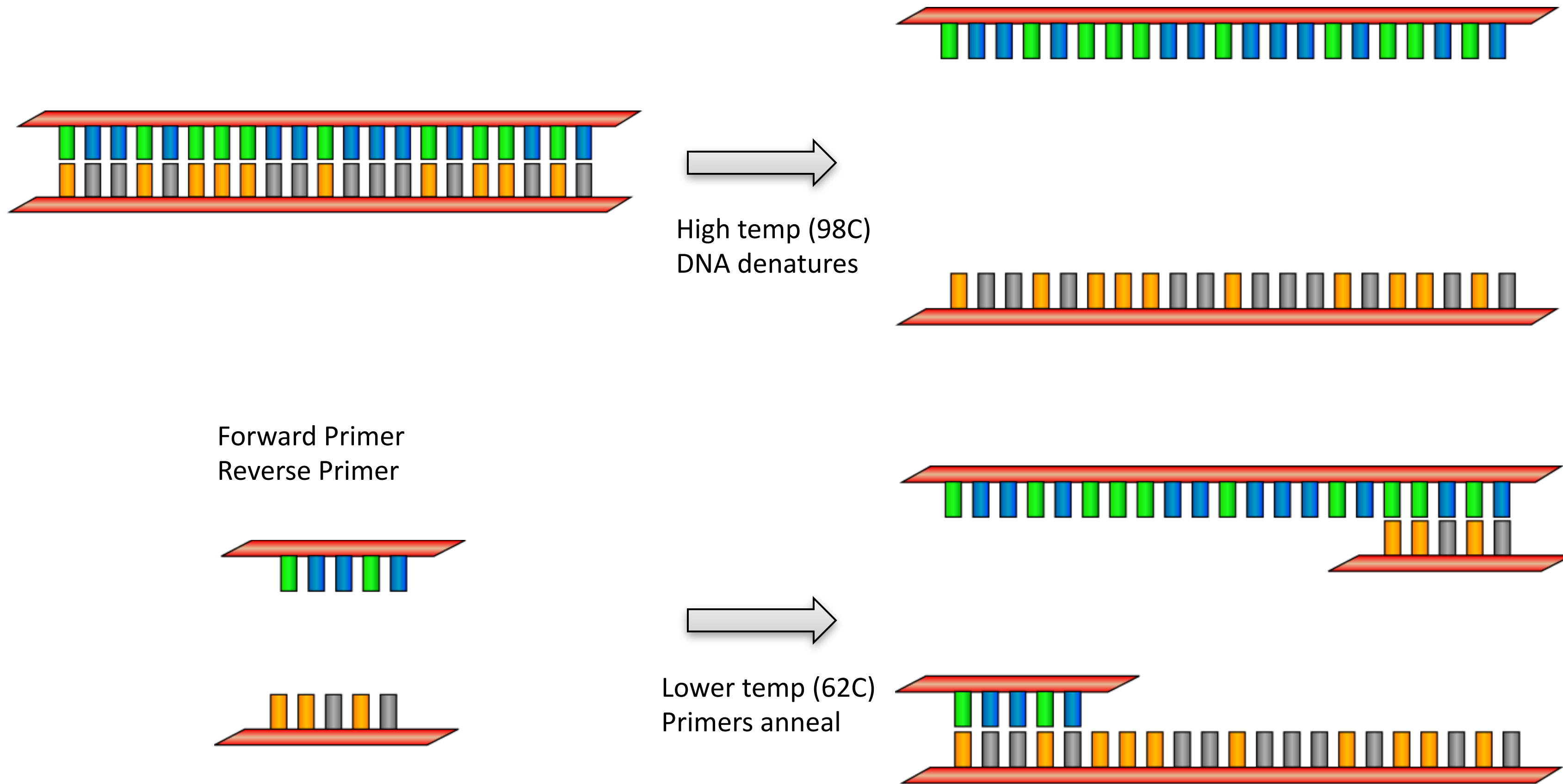


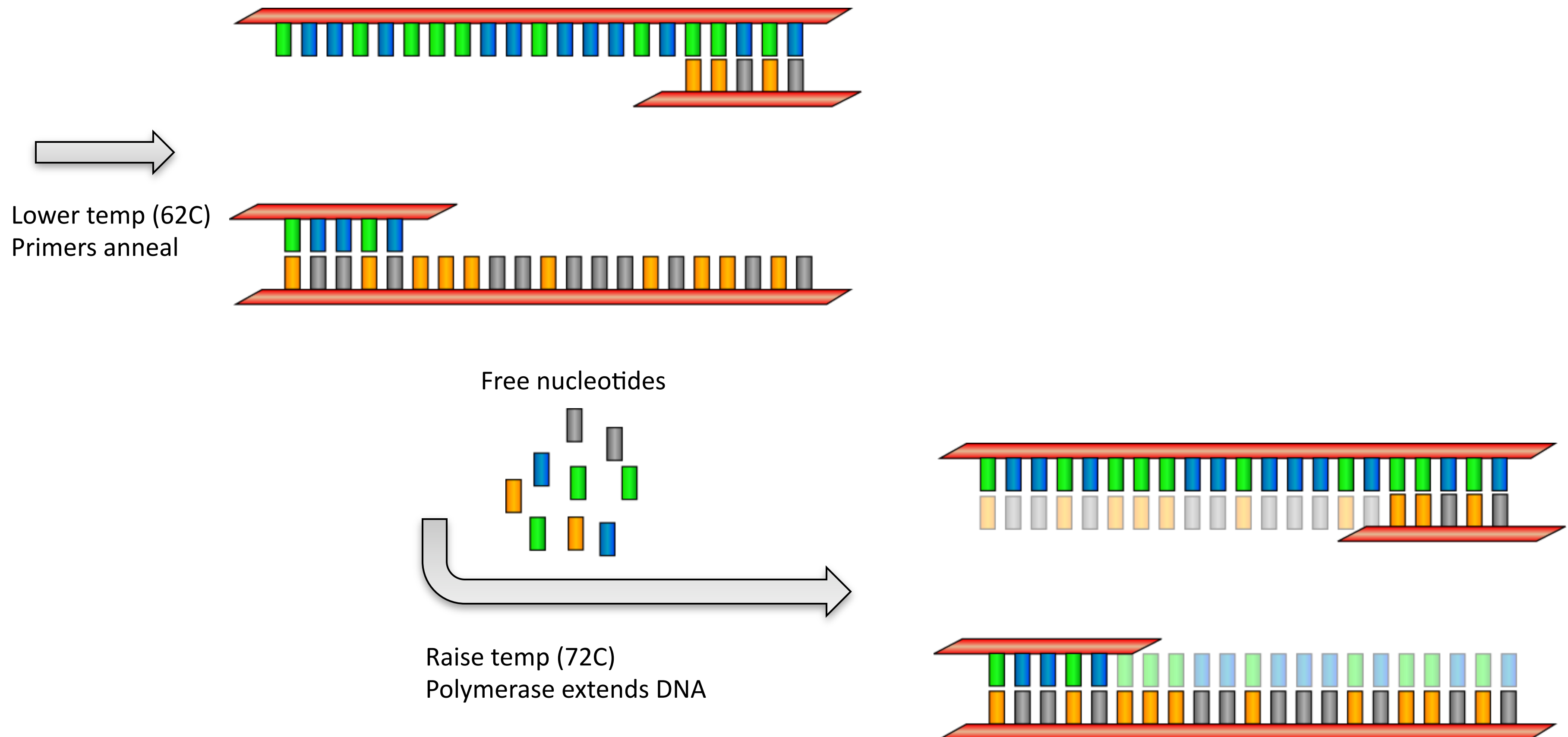
Figure 10-27 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Video

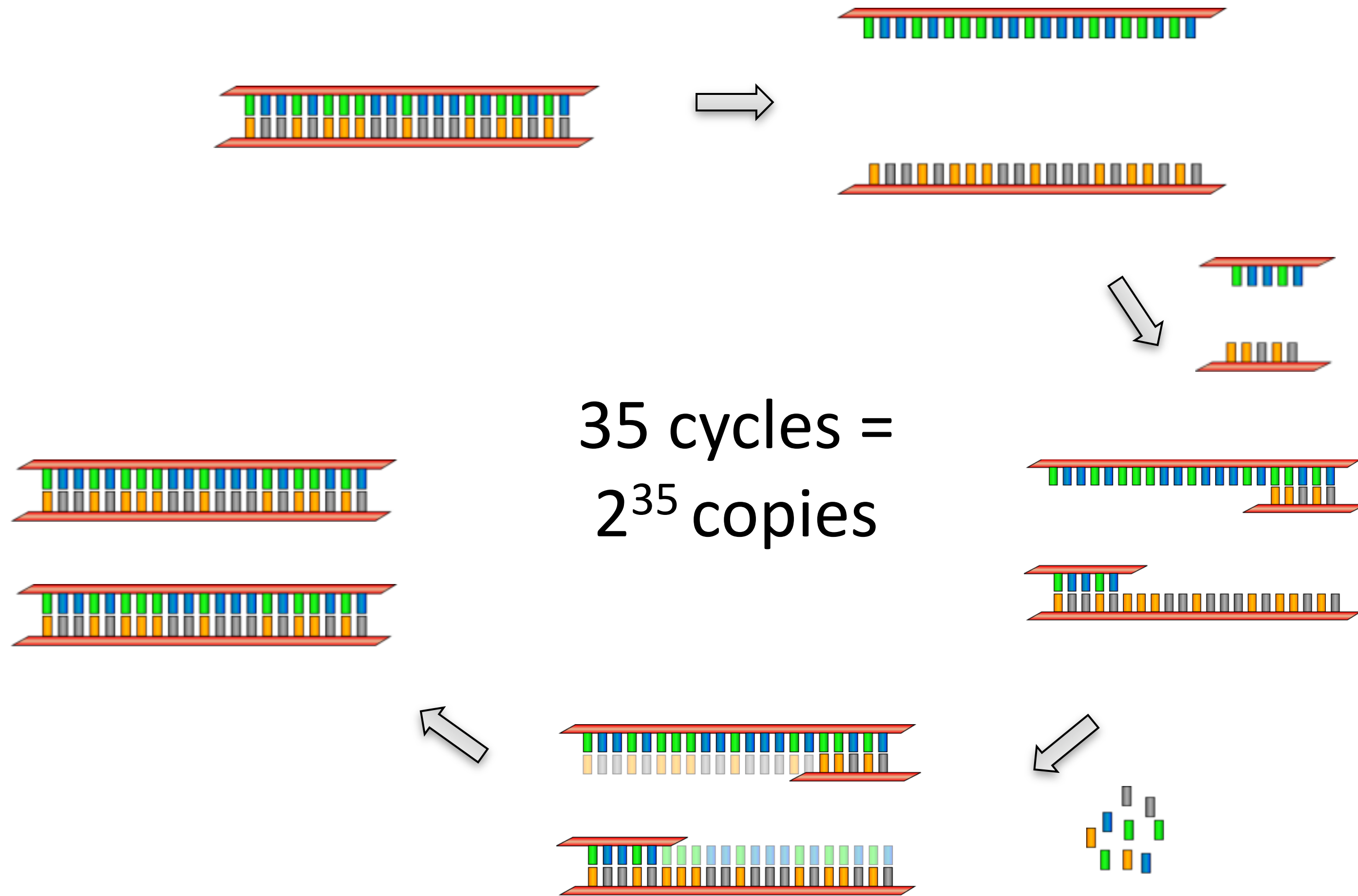
Polymerase Chain Reaction



Polymerase Chain Reaction



Polymerase Chain Reaction



PCR Revisited: Constructing the Insert

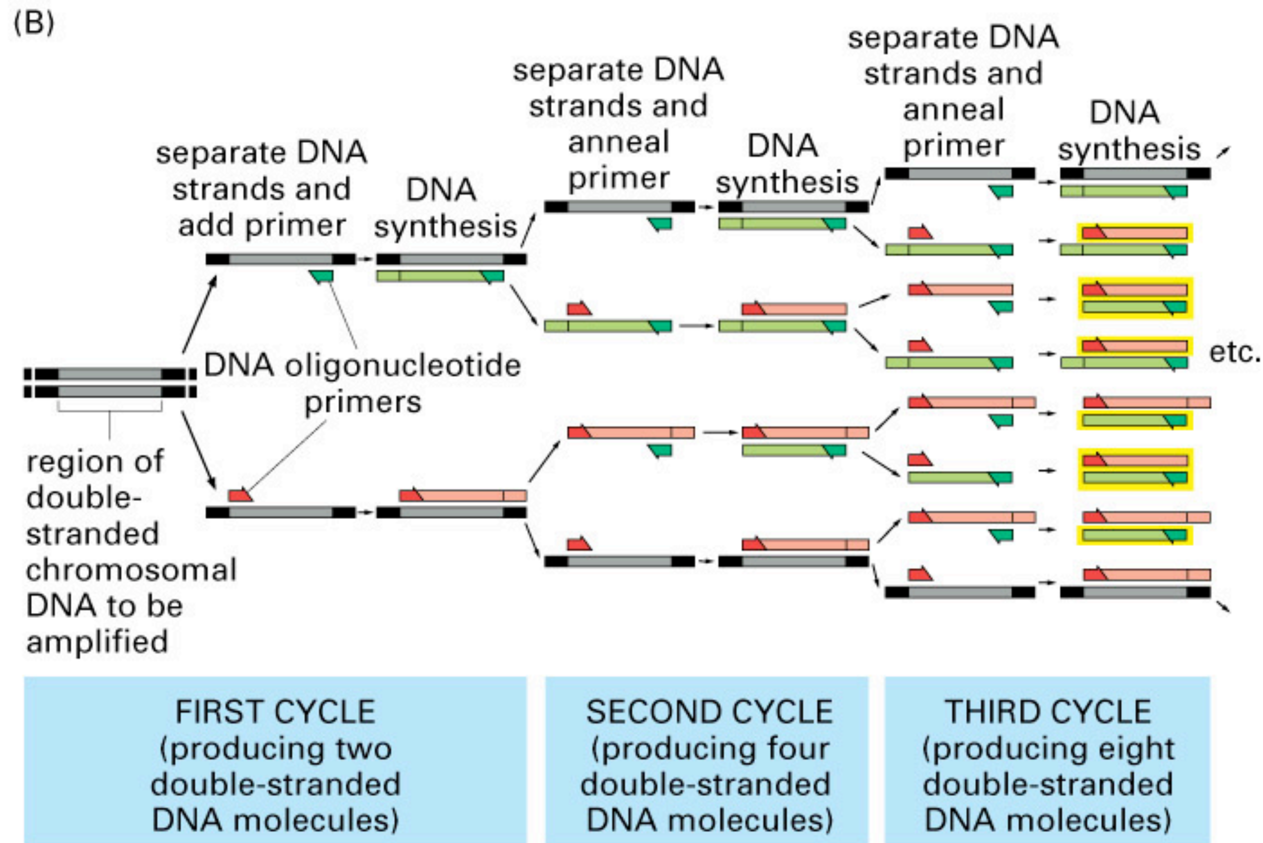
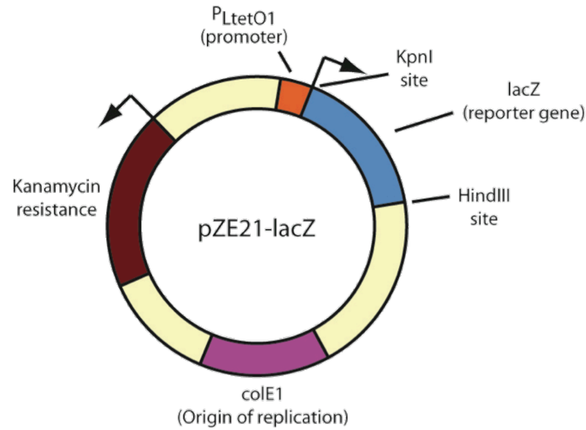


Figure 10-27 part 2 of 2 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

Plasmid Restriction

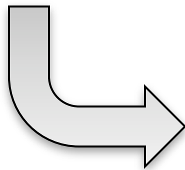


5′... A▼AGCTT ...3′
3′... TTCGA▲A ...5′

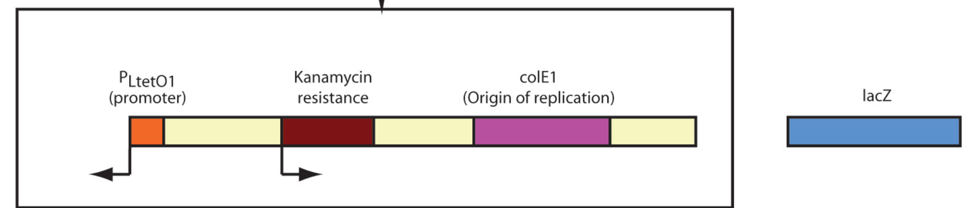
HindIII

5′... GGTAC▼C ...3′
3′... C▲CATGG ...5′

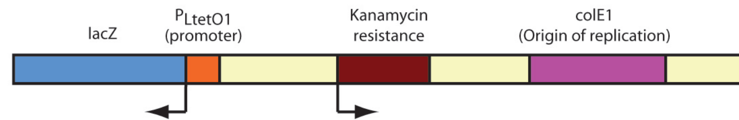
KpnI



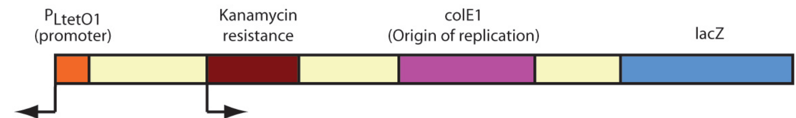
Double digest



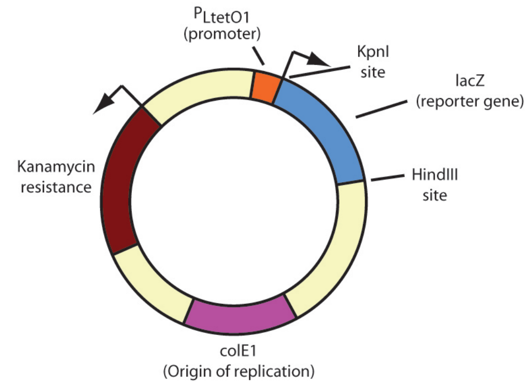
Only HindIII



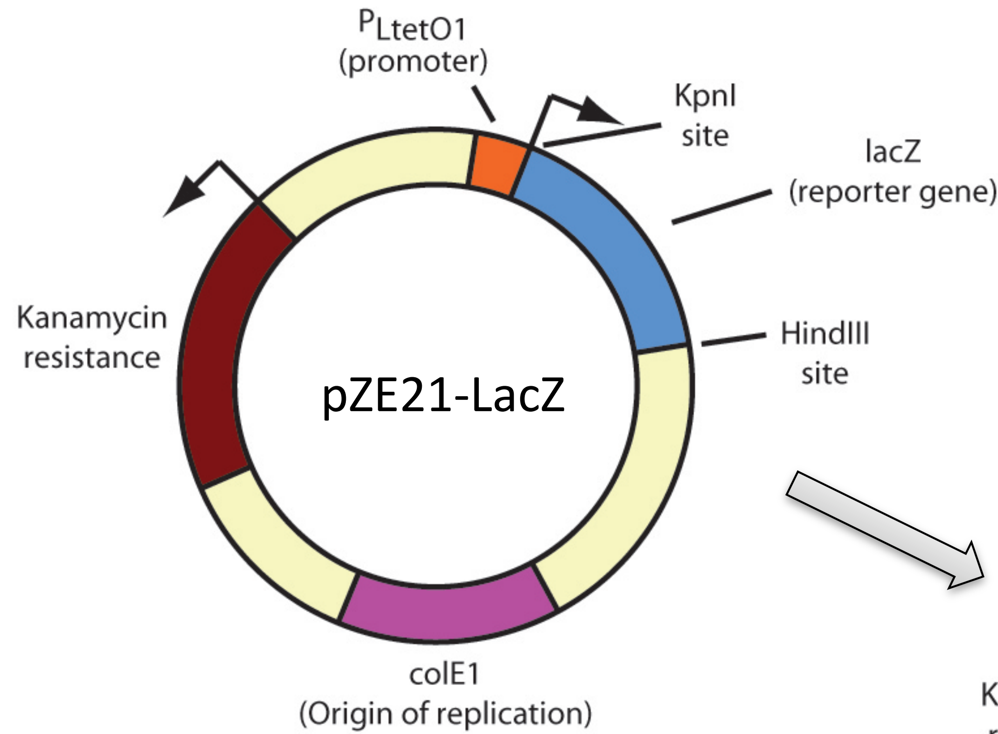
Only KpnI

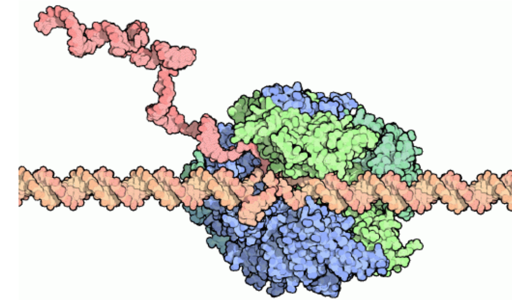


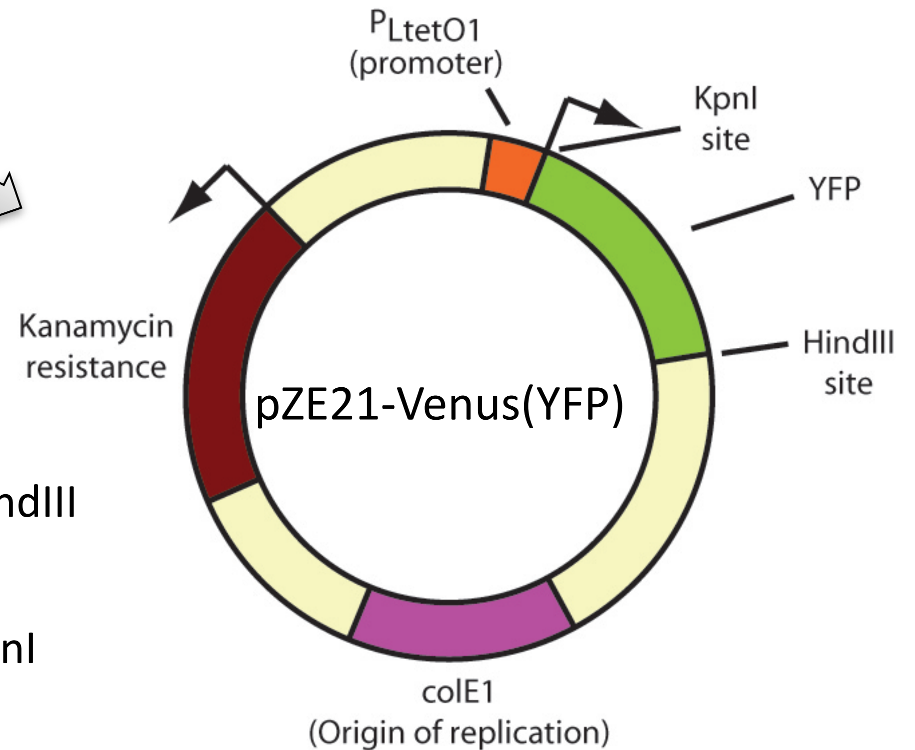
No digestion!

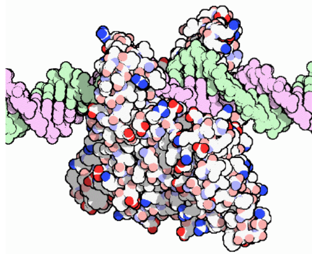


Plasmid Structure









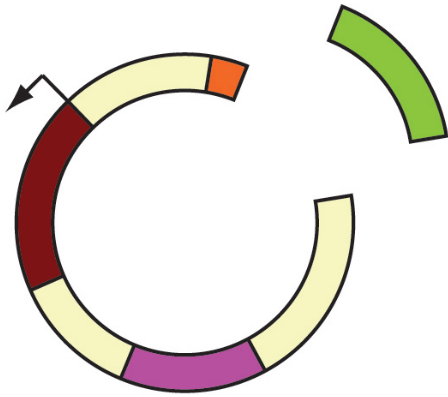
5′. . . A▼AGCTT . . . 3′
3′. . . TTCGA▲A . . . 5′

HindIII

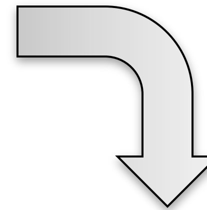
5′. . . GGTAC▼C . . . 3′
3′. . . C▲CATGG . . . 5′

KpnI

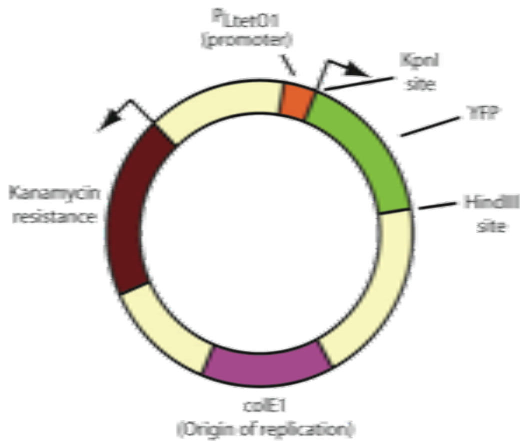
Vector / Insert Ligation



Vector + Insert + Ligase

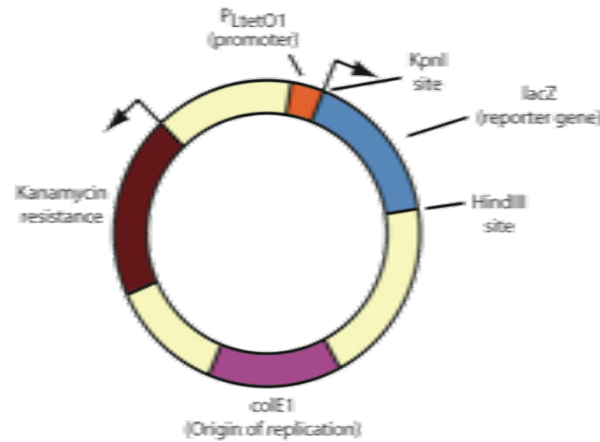


Success!



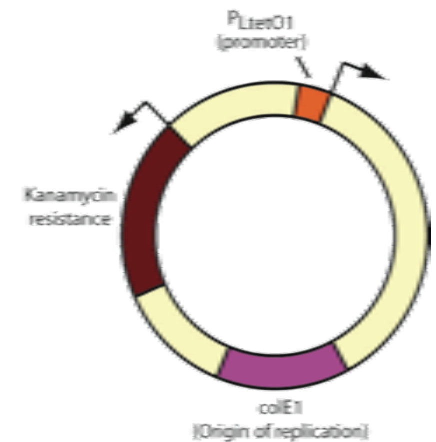
fluorescent cells

No digestion or
re-ligation



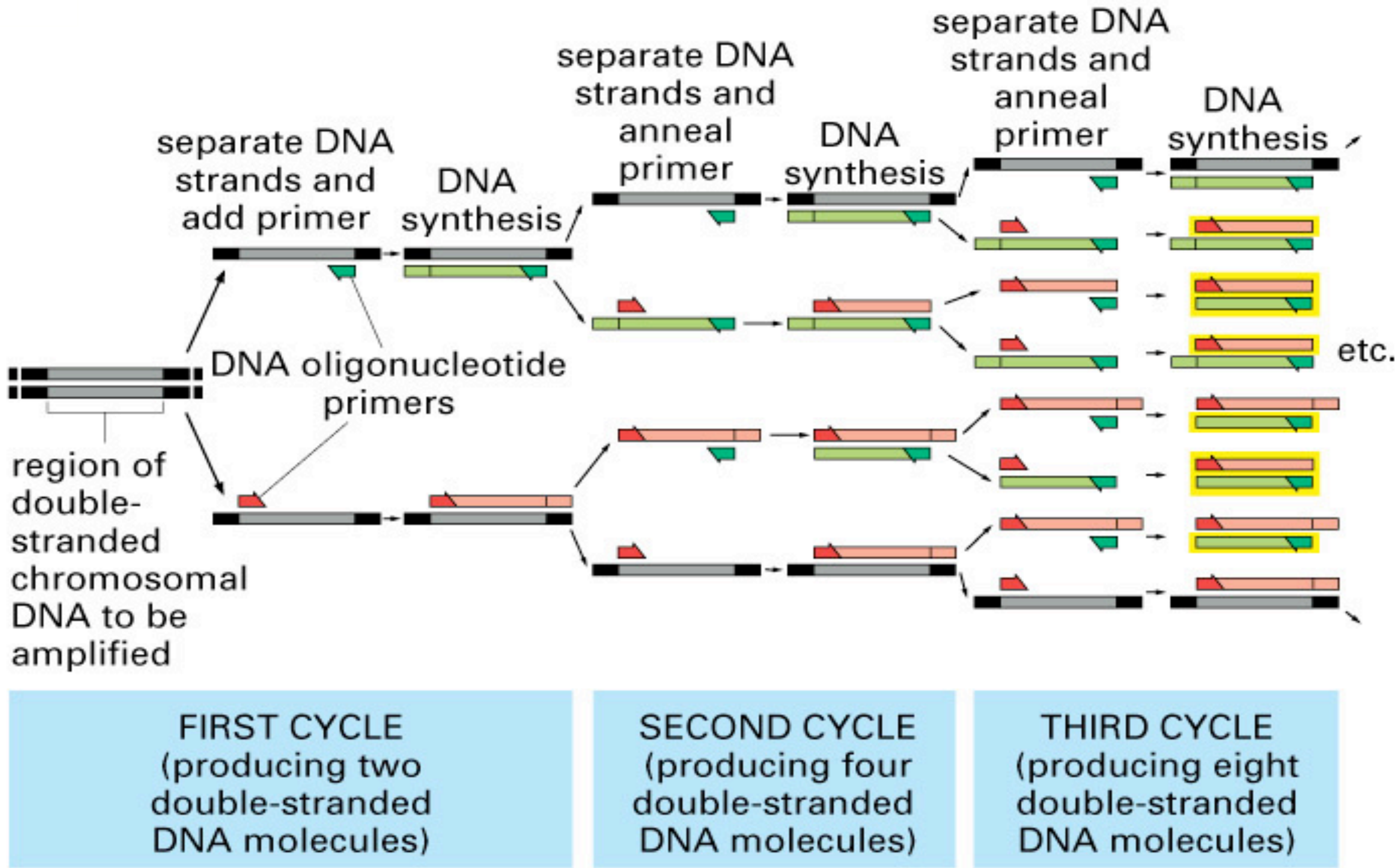
blue cells

Empty vector
(not to scale!)



white cells

Polymerase Chain Reaction



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