Gene Cloning(subcloning)

2013. 12. 31

Il Shin Kim
UNIST Central Research Facilities (UCRF)

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- President
- Vice President for Research Affairs
- Head of UNIST Multidisciplinary Institute
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- Director

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- Pre-treatment Sampling
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In Vivo Research Center
- TG Mouse Zone
- Small Animal Zone
- BSL-2 Zone
- in vivo Imaging
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- Histopathology

Radiation Safty Control

Application of Technical Support

1. UCRF 홈페이지 접속

- UCRF homepage : http://ucrf-eng.unist.ac.kr/main/main.php
- 자료마당 → 자료실 → 실험기기 사용 신청서 다운로드

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자료실
Q & A
관련사이트
홈페이지 개선사항

연구지원을 위한 고가의 첨단 장비와 교육지원

NANOFABRICATION LAB Packaging
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공지사항
- Solid NMR 사용으로 인한 예약 불.. 2012.12.10
- Normal TEM , Bio TEM 교육 0000.00.00
- FT-NMR 전기 공사 (12월 6일) 2012.12.06

자료실
- 연구지원본부 팀원 연락처 2012.12.05
- 연구지원본부(분석실) 출입신.. 2012.12.05
- 연구지원본부 장비 이용료(신.. 2012.11.13
- xps 샘플의뢰서(xps sample 2012.10.25

바로가기
장비예약신청 | 견학신청 | 나의장비예약현황
보유장비 | IACUC | IRB

Q & A
- OuxXzPXcNYxbs 2012.12.07
- OuxXzPXcNYxbs 2012.12.07
- OuxXzPXcNYxbs 2012.12.07
- OuxXzPXcNYxbs 2012.11.28

교육 및 세미나
- SEM 자율사용자 교육 2012.11.23
- AFM 교육 2012.11.14
- BET Technical Seminar 2012.08.29
- CATIA V5 기본교육 2012.08.09

홈 > 자료마당 > 자료실

자료실 | 연구지원본부 자료실입니다.

Total 62 Articles, Now 4/6 Pages

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	기기분석실	HR TEM 분석 의뢰서 (Written Request for HR-TEM Analysis)	박수현	2013-11-06	332
	기기분석실	DSC, TGA, SDT, DMA, Rheometer 분석 의뢰서 (Analysis Request Form)	이경애	2013-07-19	475
	전체	연구그룹 기자재 목록	이경선	2013-04-30	474
	기기분석실	xps 샘플의뢰서(xps sample submit form)	ggarbi73	2012-10-25	1250
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Application of Technical Support

2. 기술 지원 신청서 작성(UNIST IVRC ANI #4)

UNIST IVRC ANI #4

No. ____________

Experimental Analysis(Technical Support)

1. Applicant

Name		Office Tel./ C.P.	/
		E-mail	
Affiliation (Department/Lab.)		Position (Mark V)	☐ Professor ☐ Researcher ☐ Graduate student ☐ Undergraduate student
Principal Investigator (Professor in charge)	(Sign)	Office Tel./ C.P.	/
		E-mail	
Project Title (IACUC)		IACUC Approval Number	
Date	YYYY. MM. DD.	Site	
Species/Strains	/	Age/ Sex/ Number	weeks/ /
비고			

2. List of Technical Support

Contents			Price(won)	Mark
Basic Experiment	Inj.	Oral	1,500	
		Intraperitoneal	1,000	
		Subcutaneous	1,000	
		Intravenous	2,000	
	Blood Collection	Whole	2,000	
		Orbital venous plexus	2,000	
	Tissue Collection	Biopsy(High)	5,000	
		Biopsy(Middle)	3,000	
		Biopsy(Low)	2,000	
	Anesthesia	Euthanasia	2000	
		Isofluran (100ml)	50,000/ea	
		Zoletile	35,000/ea	
		Roumpun	20,000/ea	

항목 (건)			수가 (원)	Mark
Basic Experiment	Others	Handling	500	
		Body Weight	500	
		Tumor model	30,000/hr	
		Other Experimental Support	30,000/hr	
		Anatomy Training	50,000	
Embryo Freezing &Transfer	Embryo Cryopreservation	Storage (Embryo 200ea, 1strains)	10,000/Mon	
		Supply(basic 2pairs)	50,000/Heads	
	Embryo transfer	with Microbial monitoring examination, without shipping costs	700,000 /strains	
	Animal Clearing	Providing of mice(four weeks) after transplantation of surrogate mother (with Microbial monitoring	800,000 /strains	
Gene Cloning Support		production of cell based expression vector	70,000	
		Production of transgenic mouse vector	5,000,000	
Microbiological monitoring Examination (KRIBB)		Virus	15,000	
		Bacteria & Fungi	15,000	
		Parasites	5,000	

* Embryo Freezing &Transfer - Additional Form . UNIST IVRC ANI #4-1

** Gene Cloning Support - Additional Form . UNIST IVRC ANI #4-2

※ Reception & Inquiries . Sogah Park(sapark@unist.ac.kr/ T. 052-217-5212)

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Our center collect personal information such as e-mail, contacts to forward the notice. After achieving the purpose of the collection and use of personal information, in principle, this information will be destroyed without delay. You can refuse to consent to provide personal information and this can be withdrawn at any time after consent. However, when you do not agree to provide personal information, the information provided may be limited.

I was aware of the above and agree to use personal information.

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UNIST *In Vivo* Research Center IVRC

- 의뢰자 정보기입
  의뢰자의 정보와 IACUC Number 작성

- 유전자 정보 기재
- 유전자 Sample 상태 기재

- 기술지원 신청일 및 의뢰자 sign

Application of Technical Support

3. 기술 지원 신청서 작성(UNIST IVRC ANI #4-2) 및 담당자와 조율

- 신청서 작성
- 기술지원에 관한 세부사항은 미리 담당자와 조율할것

유전자 클로닝 지원

UNIST IVRC ANI #4-2

No.

A Letter of Request

Approval	Client	Manager

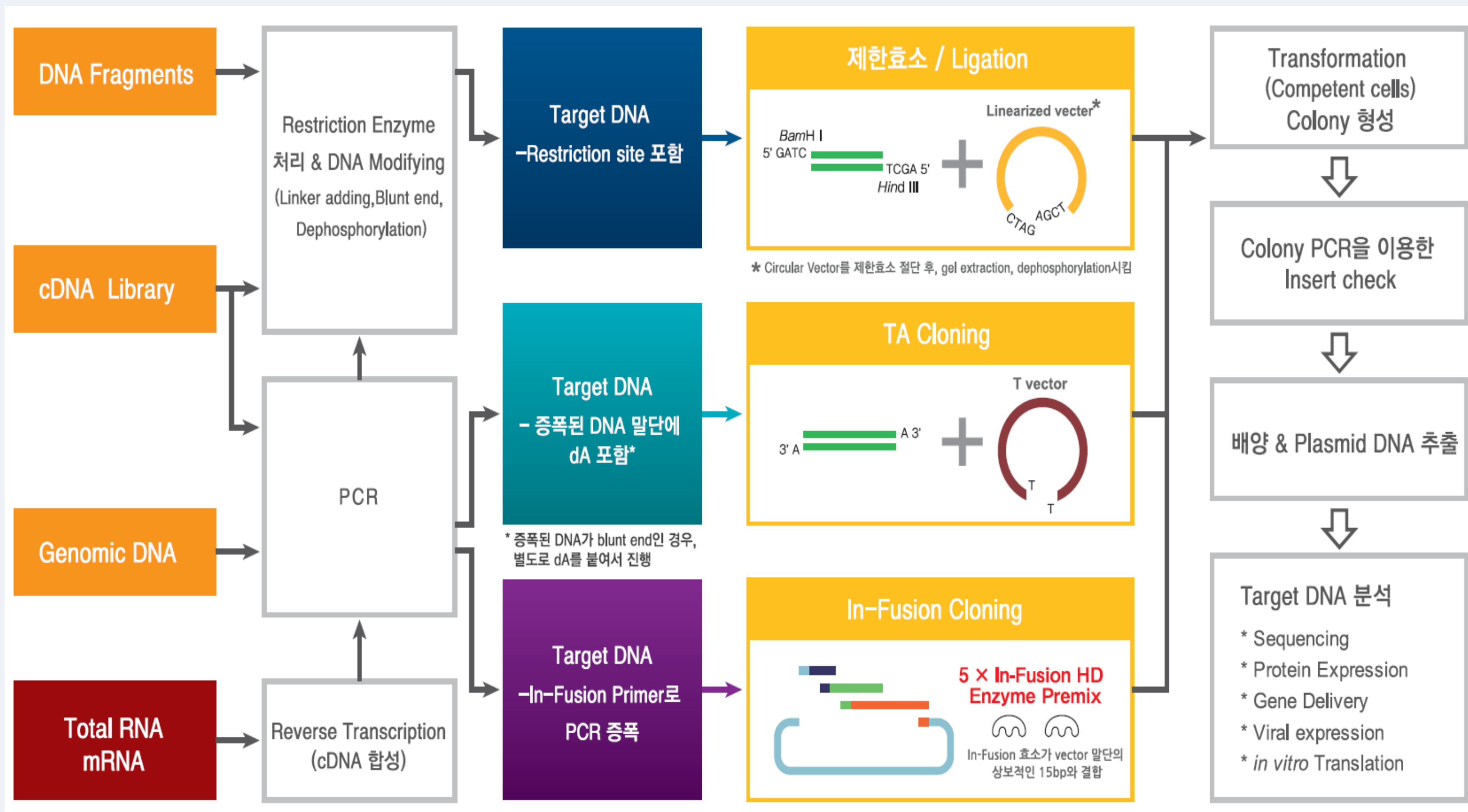
Ref No.		Data No.	
Type of cloning	☐ Sub cloning ☐ Knock-out vector cloning	☐ Mutagenesis ☐ Etc ( )	
University / Company			
Client	Laboratory (Supervisor)	Position	
	Name (Signature)	Contact number	
	E-mail		
Gene species (Accession No.)	☐ Human ☐ Rat ☐ Xenopus	☐ Mouse ☐ Bovine ☐ Etc ( )	
Kind of Vector	☐ Expression vector( ) ☐ Knock-out vector( ) ☐ T-Vector ☐ Etc ( )	No. of samples	
Sample Condition	☐ plasmid(ug/ul) ☐ E.coli ☐ Etc ( )		
Misc. Information			

UNIST *In Vivo* Research Center. IVRC

- 서명
- Type of Cloning 기재
- 기술지원 신청자의 정보를 기재
- 유전자 정보 기재
- 유전자 Sample 상태 기재

Gene Cloning

Gene Cloning의 기본적 흐름

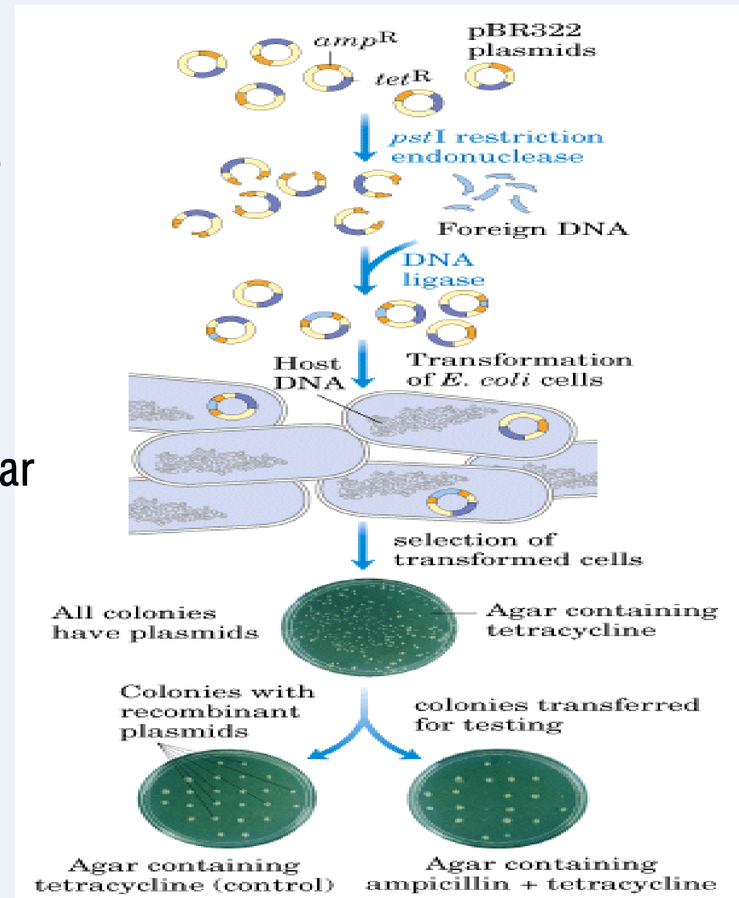


Defining Cloning

◆ "Cloning" is a loaded term that can be used to mean very different things.

- Cutting a piece of DNA from one organism and inserting it into a vector where it can be replicated by a host organism. (Sometimes called subcloning, because only part of the organism's DNA is being cloned.)

- Using nuclear DNA from one organism to create a second organism with the same nuclear DNA



DNA Cloning

DNA cloning is a technique for reproducing DNA fragments.
It can be achieved by two different approaches:

- cell based
- using polymerase chain reaction (PCR).

a vector is required to carry the DNA fragment of interest into the host cell.

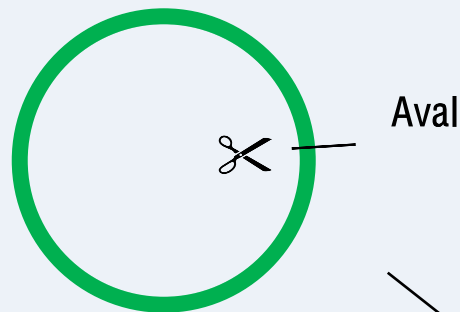
DNA cloning allows a copy of any specific part of a DNA (or RNA) sequence to be selected among many others and produced in an unlimited amount.
This technique is the first stage of most of the genetic engineering experiments:

- production of DNA libraries
- PCR
- DNA sequencing

Massive amplification of DNA sequences
Stable propagation of DNA sequences
A single DNA molecule can be amplified allowing it to be:

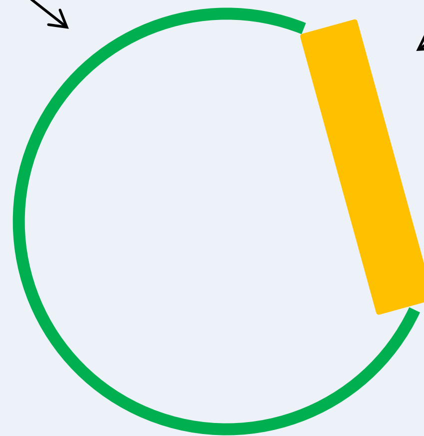
- Studied - Sequenced
- Manipulated - Mutagenised or Engineered
- Expressed - Generation of Protein

Cloning a Piece of DNA



Cut plasmid vector with EcoR I

Excise DNA insert of interest from source using EcoR I



Ligate the insert of interest into the cut plasmid

Plasmid Cloning Strategy

◆ **Involves five steps:**

1. **Enzyme restriction** digest of **DNA sample.**
2. **Enzyme restriction** digest of **DNA plasmid vector.**
3. **Ligation of DNA** sample products and plasmid vector.
4. **Transformation** with the ligation products.
5. **Growth on agar** plates with selection for **antibiotic resistance**.

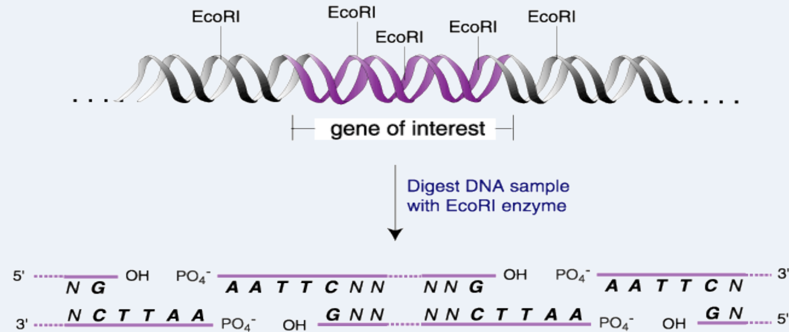
※ Strategy depends on the starting information and desired endpoint.

Starting Information or Resources:

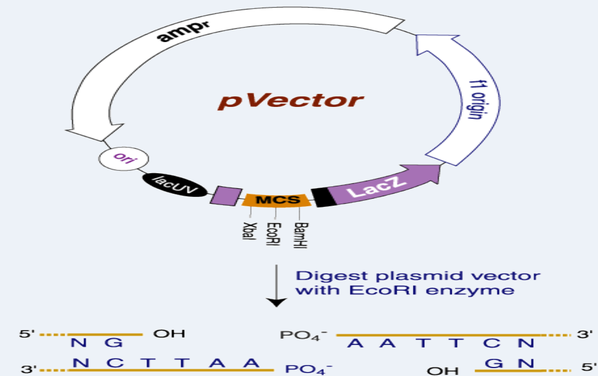
- Protein sequence
- Positional cloning information
- mRNA species / sequence
- cDNA libraries
- DNA sequence known or unknown
- Genomic DNA libraries
- PCR product

Plasmid Cloning Strategy

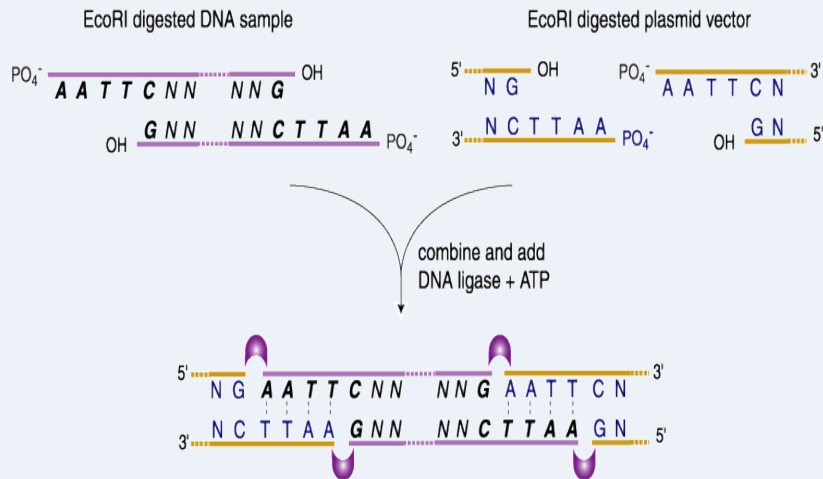
Step 1. Re Digestion of DNA Sample



Step 2. Re Digestion of Plasmid DNA



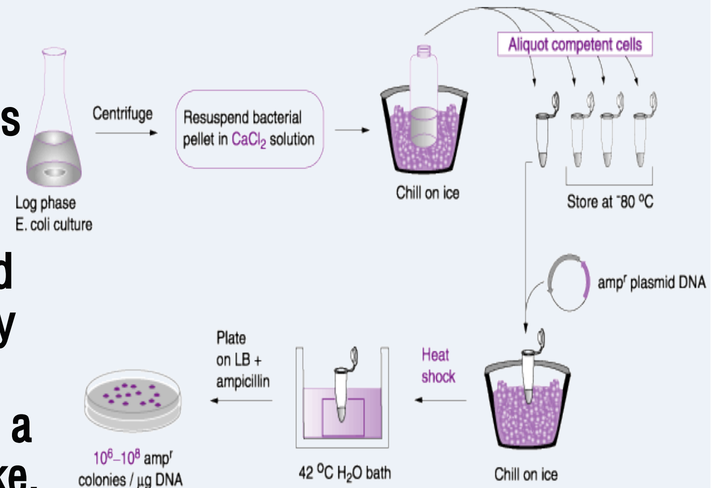
Step 3. Ligation of DNA Sample and Plasmid DNA



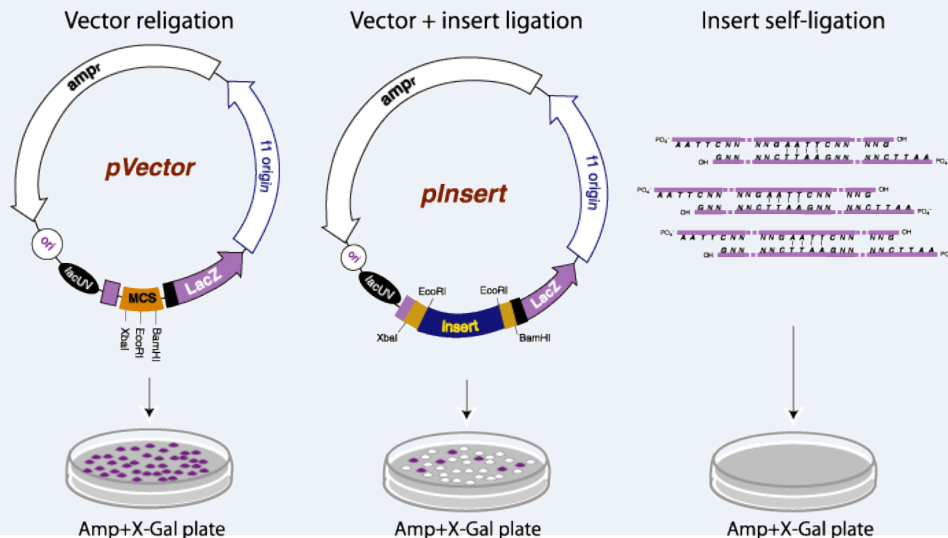
Plasmid Cloning Strategy

Step 4. Transformation of Ligation Products

The process of transferring exogenous DNA into cells is call “transformation”
There are basically two general methods for transforming bacteria. The first is a chemical method utilizing CaCl2 and heat shock to promote DNA entry into cells.
A second method is called electroporation based on a short pulse of electric charge to facilitate DNA uptake.

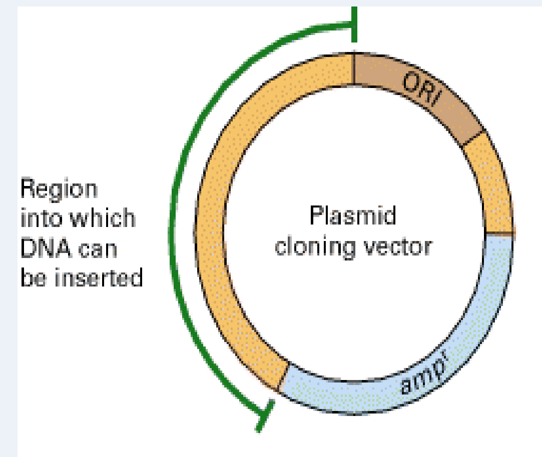


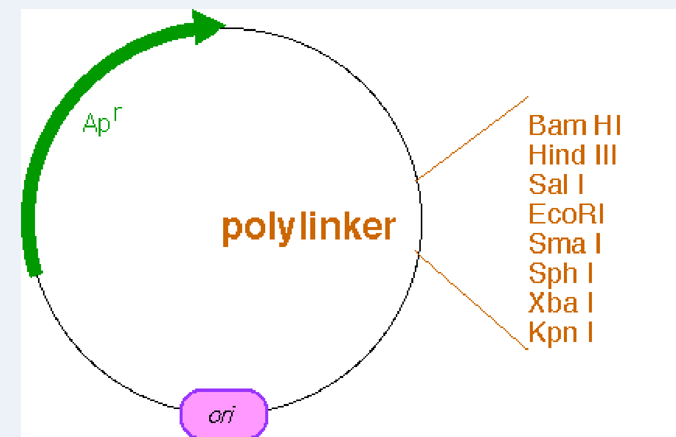
Step 5. Growth on Agar Plates



Plasmid Vector

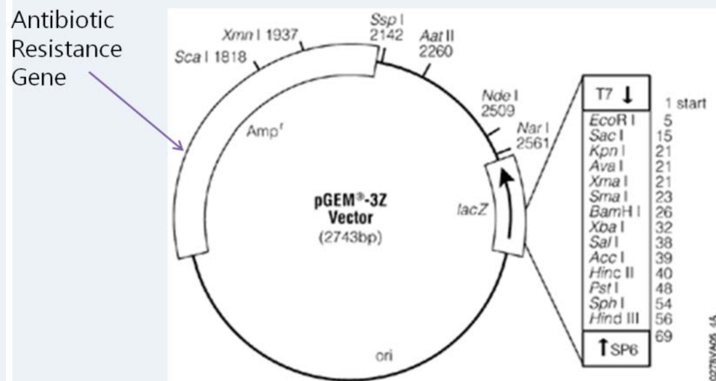
- Plasmid vectors are ≈1.2–3kb and contain:
- replication origin (ORI) sequence
- a gene that permits selection,
- Here the selective gene is *ampr*; it encodes the enzyme b-lactamase, which inactivates ampicillin.
- Exogenous DNA can be inserted into the bracketed region .
- Many cloning vectors contain a **multiple cloning site** or **polylinker**: a DNA segment with several unique sites for restriction endo- nucleases located next to each other
- Restriction sites of the polylinker are not present anywhere else in the plasmid.
- Cutting plasmids with one of the restriction enzymes that recognize a site in the polylinker does not disrupt any of the essential features of the vector

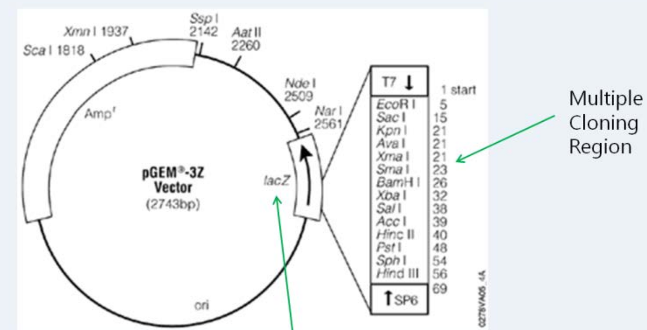




Plasmid Vectors

- Plasmids are circular pieces of DNA found naturally in bacteria.
- Plasmids can carry antibiotic resistance genes, genes for receptors, toxins or other proteins.
- Plasmids replicate separately from the genome of the organism.
- Plasmids can be engineered to be useful cloning vectors.
- Plasmid vectors can be designed with a variety of features:
  - Antibiotic resistance
  - Colorimetric "markers"
  - Strong or weak promoters for driving expression of a protein



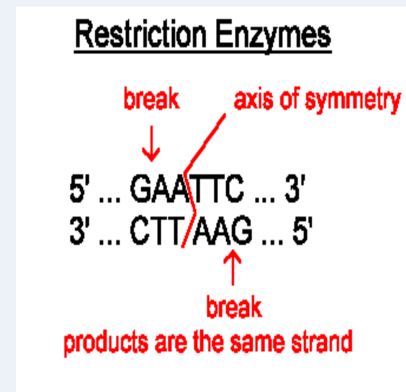


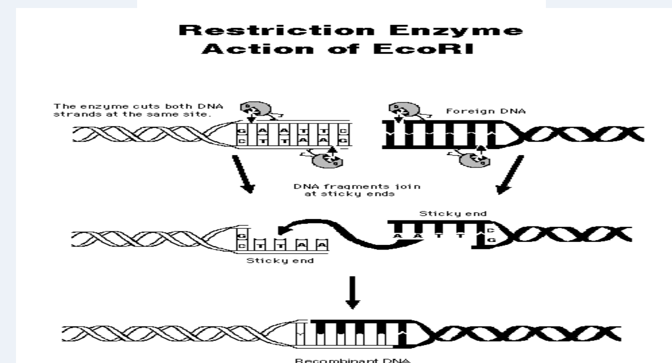
Restriction Enzymes

Restriction Enzymes (also called Restriction Endonucleases) are proteins that cleave DNA molecules at specific sites, producing discrete fragments of DNA.

Generally restriction enzymes are thought to protect bacterial cells from phage (bacterial virus) infection. Bacterial cells that contain restriction enzymes can "cut up" invasive viral DNA without damaging their own DNA.

- Restriction enzymes: enzymes that cut DNA in specific places function:
- Inactivate foreign DNA
- Breaks only palindrome sequences, i.e. those exhibiting two-fold symmetry
- Important in DNA research, i.e. sequencing, hybridization
- Companies purify and market restriction enzymes



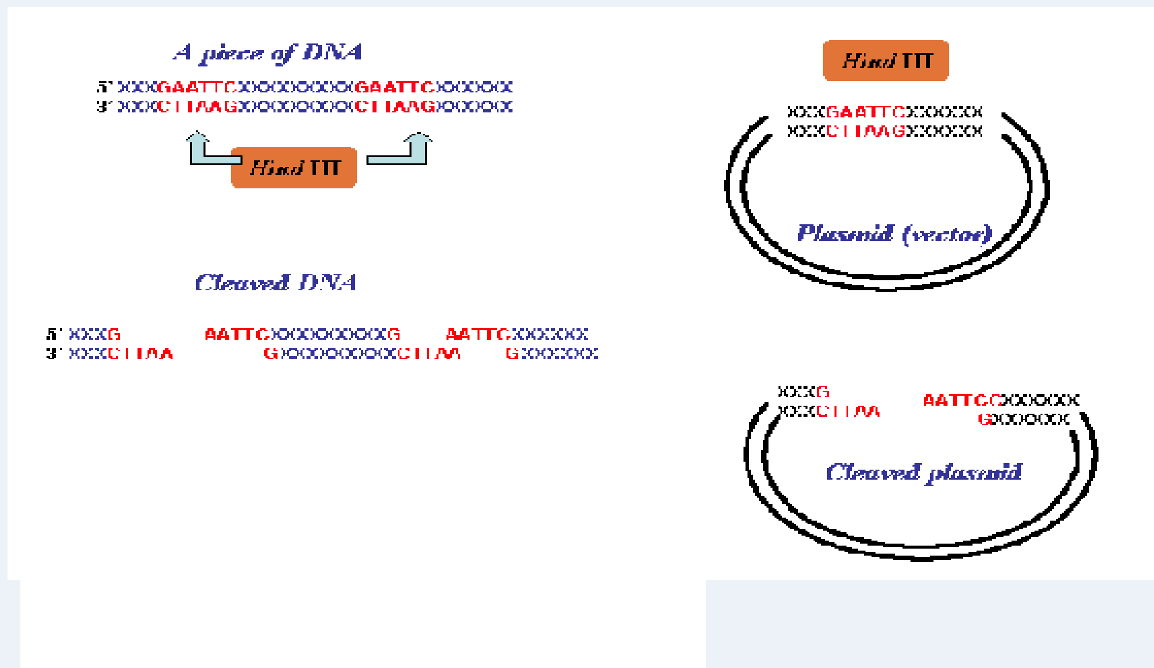


Isolating Genes

- Herbert Boyer and Stanley Cohen built on the work of Berg, Nathans and Smith to use restriction enzymes to isolate a single gene, place it into a plasmid vector.
- Bacterial cells were then transformed with the recombinant plasmid.
- The bacteria host cells replicated the plasmid, producing many copies of the gene, thus amplifying it.
- The practical application was that expensive human protein products, like insulin, which were used to treat disease, could eventually be produced from recombinant molecules in the laboratory using bacteria or another host.
- Human protein products like insulin could be used in very large quantities from the recombinant molecule. Patients no longer had to use insulin isolated from pigs or cows.

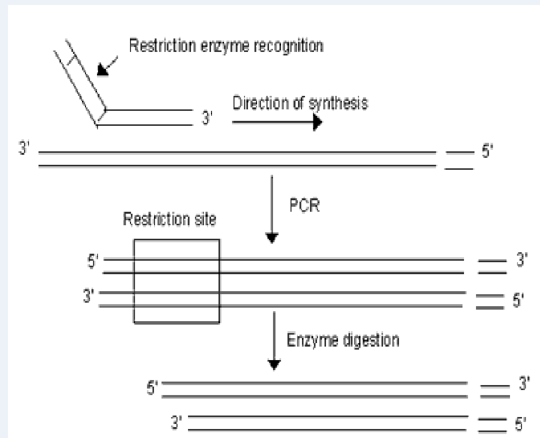
Performing the Restriction Digests

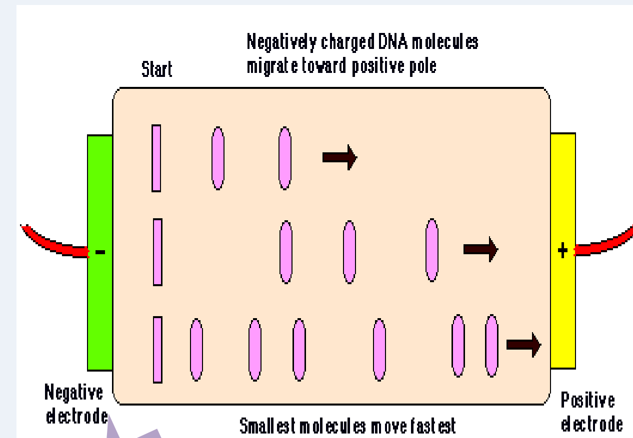
- You will need to set up a restriction digest of your plasmid vector and your DNA of interest
- Restriction enzymes all have specific conditions under which they work best. Some of the conditions that must be considered when performing restriction digest are: temperature, salt concentration, and the purity of the DNA



Purify your DNA Fragments

- The insert of interest that you want to clone into your plasmid needs to be separated from the other DNA
- You can separate your fragment using Gel Electrophoresis
- You can purify the DNA from the gel by cutting the band out of the gel and then using a variety of techniques to separate the DNA from the gel matrix

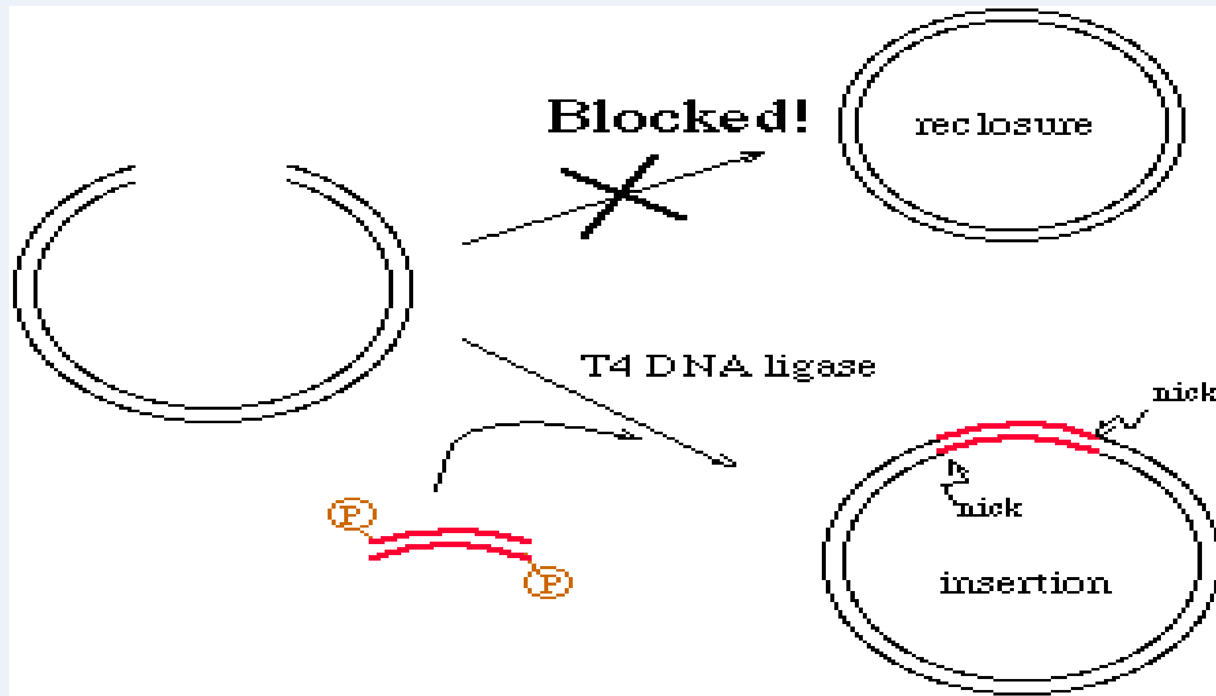




Gel Elution

Ligation

- Ligation is the process of joining two pieces of DNA from different sources together through the formation of a covalent bond.
- DNA ligase is the enzyme used to catalyze this reaction.
- DNA ligation requires ATP.



Transformation in Bacteria cells

1. Transforming Bacteria

- After you create your new plasmid construct that contains your insert of interest , you will need to insert it into a bacterial host cell so that it can be replicated.
- The process of introducing the foreign DNA into the bacterial cell is called transformation.

2. Competent Host Cells

- Not every bacterial cell is able to take up plasmid DNA.
- Bacterial cells that can take up DNA from the environment are said to be competent.
- Can treat cells (electrical current/divalent cations) to increase the likelihood that DNA will be taken up
- Two methods for transforming: heat shock and electroporation

3. Selecting for Transformants

- The transformed bacteria cells are grown on selective media (containing antibiotic) to select for cells that took up plasmid.
- For blue/white selection to determine if the plasmid contains an insert, the transformants are grown on plates containing X-Gal and IPTG.

Expressing your cloned gene

- Even if your plasmid contains insert, it may not be able to generate functional protein from your cloned DNA.
  - The gene may not be intact, or mutations could have been introduced that disrupt it.
  - The protein encoded by the gene may require post-translational modifications (i.e., glycosylation or cleavage) to function.
  - Also, some enzymes are a complex of peptides expressed from separate genes.

- Expression of a cloned gene can be accomplished by:
  - The *E. coli* host
  - Mammalian cells (if the plasmid used is designed for expression in mammalian cells)
  - Using an in vitro using a cell-free system.

Cloning into a Plasmid