Molecular Biology

Aph 162 Winter 2009
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.**
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,…

![Diagram of pZE12-d plasmid]
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

double-stranded recombinant plasmid DNA introduced into bacterial cell

bacterial cell

cell culture produces hundreds of millions of new bacteria

many copies of purified plasmid isolated from lysed bacteria

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.
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)
This list shows the sites on the phage lambda genome that will be cut by various restriction enzymes.
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.

Figure 10-18  Essential Cell Biology, 2/e, (© 2004 Garland Science)
**Aph 162 Subcloning**

**Genotype**

*E. coli* expressing protein β-galactosidase

**Phenotype**

*E. coli* expressing fluorescent protein from jellyfish (YFP)
Subcloning

Cloning

plasmid purification / (double) restriction digest / gel purification

Insert

Ligation

Transformation
Subcloning

plasmid purification / (double) restriction digest / gel purification

PCR

Ligation

Transform (Electroporation)
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)
Polymerase Chain Reaction

1. **High temp (98°C)**
   - DNA denatures

2. **Lower temp (62°C)**
   - Primers anneal

3. **Forward Primer**
4. **Reverse Primer**
Polymerase Chain Reaction

Lower temp (62C) Primers anneal

Free nucleotides

Raise temp (72C) Polymerase extends DNA
Polymerase Chain Reaction

35 cycles = $2^{35}$ copies
PCR Revisited: Constructing the Insert

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

**KpnI**

5'...AAAGCTT...3'
3'...TTTCGAA...5'

**HindIII**

5'...GGTACC...3'
3'...CCATGG...5'

**pZE21-lacZ**

- Kanamycin resistance
- **P_LtetO1** (promoter)
- KpnI site
- lacZ (reporter gene)
- HindIII site
- **coliE1** (Origin of replication)

**Double digest**

- **P_LtetO1** (promoter)
- Kanamycin resistance
- **coliE1** (Origin of replication)
- lacZ

**Only HindIII**

- **P_LtetO1** (promoter)
- Kanamycin resistance
- **coliE1** (Origin of replication)
- lacZ

**Only KpnI**

- **P_LtetO1** (promoter)
- Kanamycin resistance
- **coliE1** (Origin of replication)
- lacZ

**No digestion!**

- **P_LtetO1** (promoter)
- Kanamycin resistance
- **coliE1** (Origin of replication)
- lacZ

- KpnI site
- HindIII site
Plasmid Structure

- **pZE21-LacZ**
  - $P_{\text{LtetO1}}$ (promoter)
  - KpnI site
  - lacZ (reporter gene)
  - HindIII site
  - Kanamycin resistance
  - coE1 (Origin of replication)

- **pZE21-Venus(YFP)**
  - $P_{\text{LtetO1}}$ (promoter)
  - KpnI site
  - YFP
  - HindIII site
  - Kanamycin resistance
  - coE1 (Origin of replication)
Vector / Insert Ligation

Vector + Insert + Ligase

fluorescent cells
blue cells
white cells
Polymerase Chain Reaction

FIRST CYCLE
(producing two double-stranded DNA molecules)

SECOND CYCLE
(producing four double-stranded DNA molecules)

THIRD CYCLE
(producing eight double-stranded DNA molecules)

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