Cloning vectors

• Propagation of DNA
  – gene of interest
  – cDNA or genomic library

• Manipulation of DNA
  – nucleotide sequencing
  – site-directed mutagenesis

• Delivery of DNA
  – expression of large quantities of protein
  – functional expression
Types of cloning vectors

- plasmids
- filamentous phages
- lambda phages
- cosmids
- bacterial artificial chromosomes (BACs)
- yeast artificial chromosomes (YACs)
Plasmids

Plasmids are double-stranded, circular, self-replicating, extrachromosomal DNA molecules.
Plasmids (continued)

• a replicator
  – ColE1, pMB1
  – copy-number

• a selectable marker
  – dominant (e.g. Amp, Tet, Kan)
  – recessive (e.g. LeuB)

• a multiple cloning site (MCS)
  – restriction sites
Plasmids (continued)

- 8-bp-cutter sites for insert excision
- oligonucleotide complementary sequences
- RNA promoters for transcription of cRNA
- LacZ gene for insert selection
- Trc promoter for regulated expression in E. coli
- propagation, selection and expression outside E. coli
- transcriptional / translational fusion products
pBluescript II KS +/- phagemid
2.96 kb

BssH II

T7 ↓
Sac I
BstX I
Sac II
Eag I
Not I
Xba I
Sph I
BamHI
Sma I
Pst I
EcoR I
EcoR V
Hind III
Bsp106 I (Cia I)
Sal I/Hinc II/Acc I
Xho I
EcoO109 I (Dra II)
Apa I
Kpn I

T3 ↑
BssH II
Philamentous phages

Philamentous phages are single-stranded DNA phages that infect F-factor containing E. coli, and can be recovered in both single-stranded (phage) and double-stranded (plasmid) form.
Philamentous phages (continued)

• examples: f1, M13, fd

• advantages: very useful for sequencing and mutagenesis

• some plasmids can propagate as “phagemids” when co-transfected with helper phages
Lambda phages

Lambda phages are ~50 kb double-stranded DNA phages that replicate in E. coli either in the lytic or in the lysogenic mode.
Lambda phages  (continued)

• examples: EMBL3, λgt10, λZAP

• suitable for either small (0-5 kb) or large (10-20 kb) inserts

• efficient cloning and easy maintenance (cDNA and genomic libraries)

• easy screening of large amounts of recombinant clones (libraries)
wild-type lambda DNA
λ ZAP
Cosmids, BACs and YACs

These vectors can host extremely large inserts (upto 50 kb for cosmids, and 100-500 kb for BACs and YACs), and are primarily used in genome sequencing projects.
SuperCos
cosmid vector
7.9 kb

ampicillin

neo T

SV40 ori

cos

Xba I

MCS

T7

Not I

BamHI

T3

Not I

EcoRI

EcoRI
What determines the choice vector?

• insert size
• vector size
• restriction sites
• copy number
• cloning efficiency
• ability to screen for inserts
• what down-stream experiments do you plan?