Cloning Tips from the **T**errible **C**loner

PURPOSE OF MOLECULAR CLONING

1). Efficient manipulation of a chosen piece of DNA
2). Study on gene function/regulation

WHY IS IMPORTANT TO BE A PREFECT CLONER??

1). Completion of human genome project will mark a new era of functional genomics!
2). Descriptive research is not enough!
3). Molecular medicine will become reality.

REQUIREMENTS FOR CLONING VECTORS (PLASMIDS)

1) A replication Ori
2) An antibiotic selection marker: amp, kan, tet, strep, & zeo etc
3) Multiple cloning sites (MCS) or polylinker
4) A circular molecule

CHOICE OF VECTORS

1) High copy number vectors

a. high yield of plasmid DNA
b. pUC18 or pUC19 Ori-based, >20 copies/cell
c. good for small fragment cloning (<5kb)
d. good for single-step cloning

2) Low copy number vectors

a. lower yield on plasmid DNA preps
b. BR322 Ori-based, <20 copies/cell
c. good for any cloning (esp >5kb)
d. good for multiple-step cloning

TYPES OF CLONING VECTORS (PLASMIDS)

1) Prokaryotic cloning vectors: *general cloning*
2) Bacteriophage vectors: *ssDNA, phage library*
3) Yeast vectors: *expression or library*
4) Prokaryotic expression vectors: *fusion proteins (GST or His)*
5) Eukaryotic expression vectors: *transgene expression*
6) Recombinant adenoviral vectors: *making adenoviruses*

LIGATION REACTIONS AND TRANSFORMATION

1) Vector Preparation

a. use 0.5-1.0 ug DNA (>1ug leading to high background)
b. do stepwise digestions (in 100ul reaction) whenever possible
c. avoid gel purification (reducing ligation efficiency) whenever possible

2) Insert Preparation

a. use 5-10ug DNA (or 15-20ul miniprep DNA)
b. do stepwise digestions whenever possible
c. usually need gel purification
d. may PCR amplify inserts (always digest PCR DNA first, then gel purify; any PCR amplified fragments need to be sequenced)

3) Ligation Setup

a. inserts are always in excess
   (e.g., vector =10-20ng/Rx, insert = 50-200ng/Rx)
b. always include a vector control ligation
   (insert control may be desirable in some cases).
c. incubation at 16^{o}C x 1-4 hrs (O/N may only be necessary when extremely large and/or blunt-ended inserts are involved)

4) Electroporation/Transformation

a. require ethanol precipitation/wash to remove salts
b. cool cuvettes on fresh ice
c. zap at 1.8KV/mm gap (bigger plasmids use lower voltage, 1.2-1.3KV/mm)
d. direct plating is good for low background, but 10-20 min incubation at 37^{o}C is needed for inoculating to liquid culture. If you do not which way is suitable for your ligation/transformation, you can plate 100-200ul of the E. coli mix immediately after transformation, and plate the rest after a 20-40min incubation).

SCREENING FOR AND CONFIRMATION OF POTENTIALLY CORRECT CLONES

1) Direct miniprep/diagnostic digestions (low background)
2) PCR screening (moderate background)
3) Colony-hybridization (high background /challenging cloning)

TROUBLE-SHOOTING GUIDELINES

1) No Colonies

a. check insert and vector on gel
b. check proper antibiotic selection
c. check electrocompetent cells
d. check electroporation procedure
e. check cloning strategies
f. re-do ligation with more vector and/or insert
g. re-do vector and insert preparation

2) High Background

a. direct plating after electroporation
b. use less vector for digestion and/or ligation
c. treat vector with CIAP (calf intestinal alkaline phosphotase) (but not on both insert & vector; not on vectors when unkinased oligo cassettes are used as inserts)
d. cut ligation mix with a linker enzyme that is not present in the final construct.
e. use inserts from different antibiotic selection marker.