

## **DNA CLONING**

Last updated: December 10, 2010

### **Notes:**

- Order appropriate primers the week before
- ALWAYS keep stocks at each step! (For backtracking)

### **STEP 1 – PCR, digest and ligation (~9hr)**

#### **INSERT:**

- PCR (~3hr)
- Gel: PCR check, gel purification (1.5hr)
- PCR product (insert) digest (~3hr)
- Gel: PCR product (insert) isolation and purification (~1.5hr)

#### **VECTOR**

- Vector digest (~3hr)
- Gel: vector purification (~1.5hr)
- Overnight vector/insert ligation (~0.5hr)

### **STEP 2 – transformation and cPCR check**

- DAY 1: bacterial transformation (~2.5hr, pref. early afternoon)
- DAY 2: colony PCR check, streak plates 37C overnight (~4hr, pref. late morning)

### **STEP 3: overnight cultures and mini-prep**

- DAY 1: 37C overnight cultures of successful colonies (0.5hr, late afternoon)
- DAY 2: mini-prep cultures to extract DNA (1.5hr, morning)

### **STEP 4: electroporation and microscopy**

- DAY 1: DNA electroporation
- Day 2: fluorescence microscope measurements