with complementary sticky ends than blunt ends. T4 DNA ligase is the most commonly used DNA ligase for molecular biology techniques and can ligate 'sticky' or blunt ends.

The two components of the DNA in the ligation reaction should be equimolar and around 100µg/ml. Most commonly, one wants to ligate an insert DNA molecule into a plasmid, ready for bacterial transformation. Typically, DNA and plasmid vector are individually cut to yield complementary ends, then both are added to a ligation reaction to be circularised by DNA ligase. If the plasmid backbone to insert DNA ratio is too high then excess 'empty' mono and polymeric plasmids will be generated. If the ratio is too low then the result may be an excess of linear and circular homo- and heteropolymers.

Materials

Reagents

- T4 DNA ligase
- 10x T4 DNA Ligase Buffer
- Deionized, sterile H_2O
- Purified, linearized vector (likely in H_2O or EB)
- Purified, linearized insert (likely in H_2O or EB)

Equipment

Vortex

Procedure

10µL Ligation Mix

Larger ligation mixes are also commonly used

- 1.0 µL 10X T4 ligase buffer
- 6:1 molar ratio of insert to vector (~10ng vector)
- Add (8.5 - vector and insert volume)µl ddH_2O
- 0.5 µL T4 Ligase

Calculating Insert Amount

$$\text{Insert Mass in ng} = 6 \times \left[\frac{\text{Insert Length in bp}}{\text{Vector Length in bp}}\right] \times \text{Vector Mass in ng}$$

The insert to vector molar ratio can have a significant effect on the outcome of a ligation and subsequent transformation step. Molar ratios can vary from a 1:1 insert to vector molar ratio to 10:1. It may be necessary to try several ratios in parallel for best results.

Method

1. Add appropriate amount of deionized H_2O to sterile 0.6 mL tube
2. Add 1 µL ligation buffer to the tube.
   Vortex buffer before pipetting to ensure that it is well-mixed.
   Remember that the buffer contains ATP so repeated freeze, thaw cycles can degrade the ATP thereby decreasing the efficiency of ligation.
3. Add appropriate amount of insert to the tube.
4. Add appropriate amount of vector to the tube.
5. Add 0.5 µL ligase.
   Vortex ligase before pipetting to ensure that it is well-mixed.
   Also, the ligase, like most enzymes, is in some percentage of glycerol which tends to stick to the sides of your tip. To ensure you add only 0.5 µL, just touch your tip to the surface of the liquid when pipetting.
6. Let the 10 µL solution sit at 22.5°C for 30 mins
7. Denature the ligase at 65°C for 10min
8. Dialyze for 20 minutes if electroporating
9. Use disks shiny side up
10. Store at -20°C

Critical steps

Troubleshooting

Factors affecting efficiency

From Tom Ellis

A protocol analysis experiment for a typical DNA ligation (7.2 kb vector + 0.6 kb insert, sticky ends) gave optimal ligation efficiency when 50 ng of vector was ligated overnight at 16°C with a 2:1 insert:vector molar ratio and standard T4 ligase. Ligase was heat inactivated at 65°C for 20 mins before 2 µL (of 20 µL) was used to transform commercial heat-shock competent cells.

Ligation efficiency was **marginally decreased** by

1. Doing a 1 hr ligation at room temperature
2. Using 100 ng vector
3. Using insert:vector molar ratios of 5:1 and 1:1

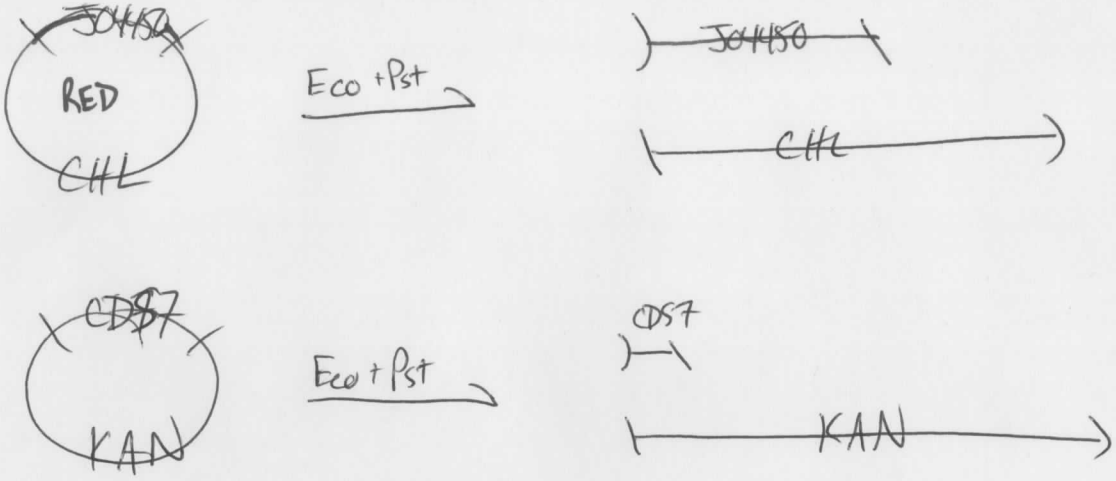
Ligation efficiency was **noticably decreased** (x100) by

1. Sticky end ligation with a larger insert (5.2 kb vector + 2.6 kb insert)
2. Blunt end ligation

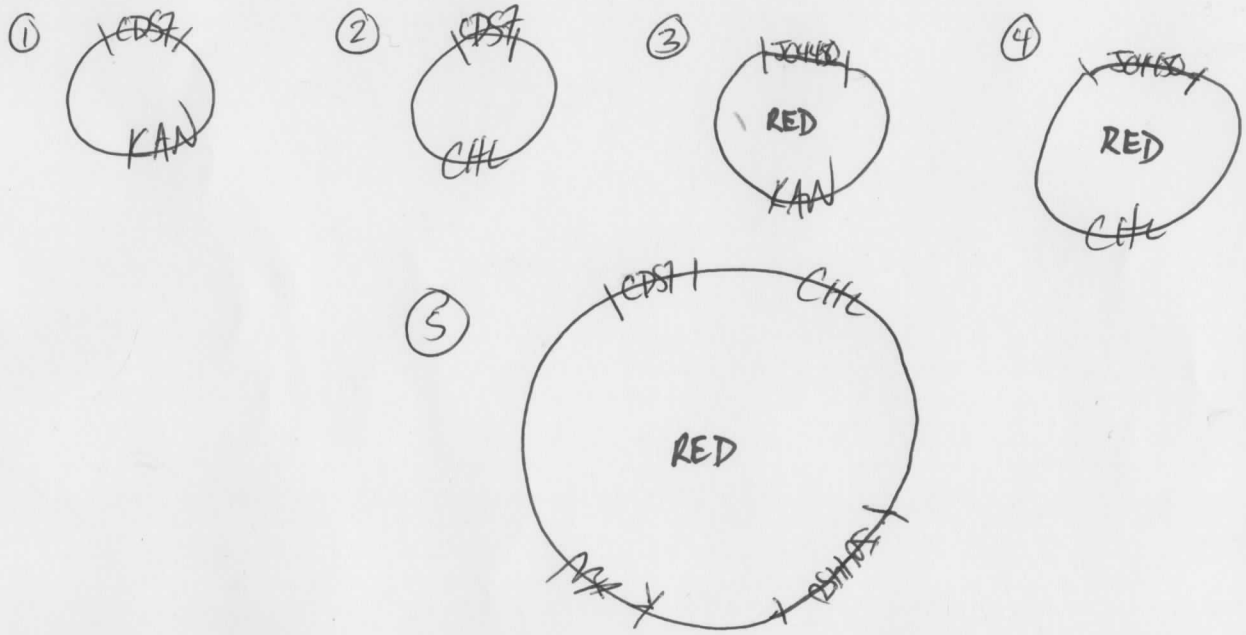
Ligation efficiency was **severely decreased** (x10000) by

1. Using DNA fragments that have been exposed to UV during the gel extraction procedure (*can avoid by blind excision, or by using a black-light or 365nm UV transilluminator instead of the usual 312nm type*)

Trial 1



LIGATE ALL PIECES TOGETHER

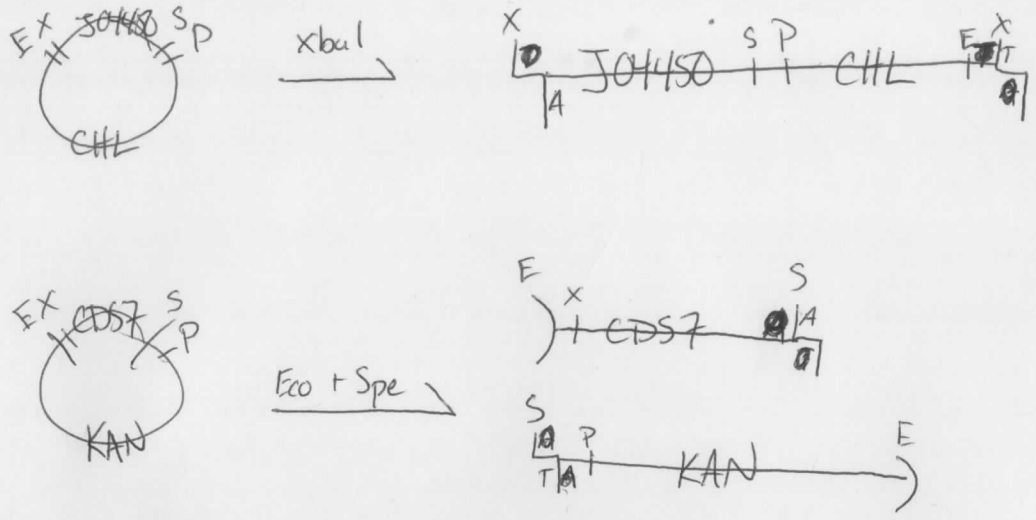


SELECTION

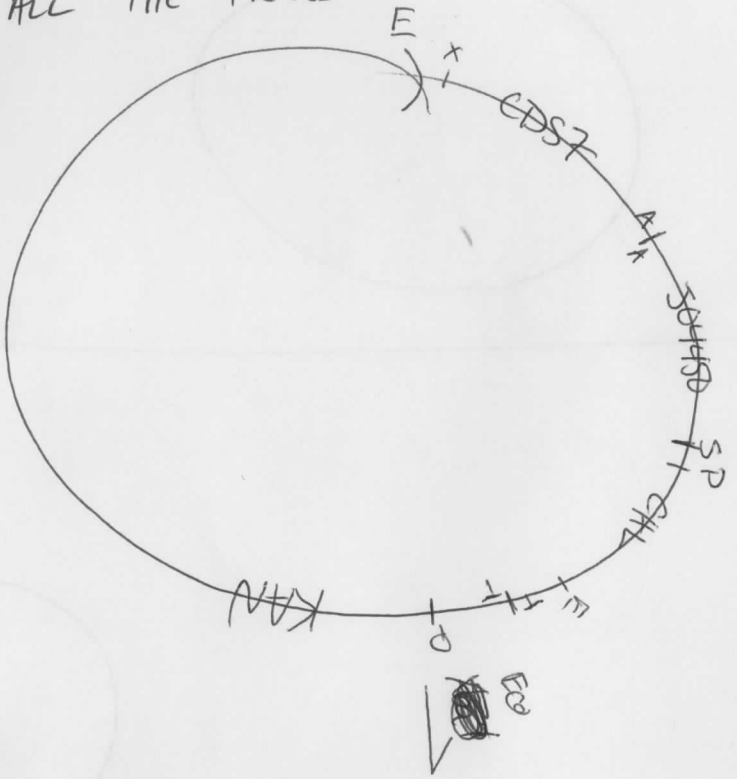
① CHL only plates → only 2, 4 & 5

② Select only WHITE colonies → only 2

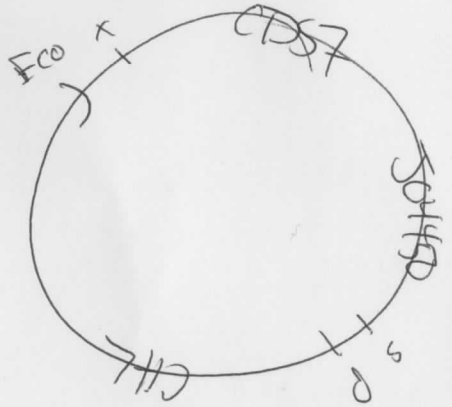
Trial 2

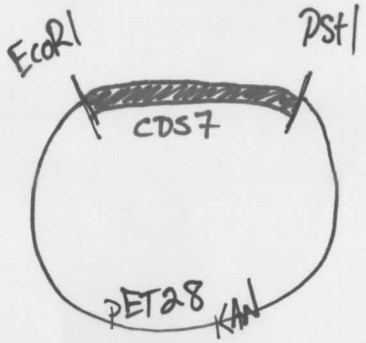


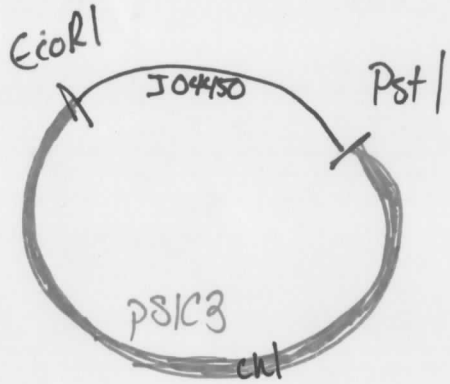
LIGATE ALL THE PIECES



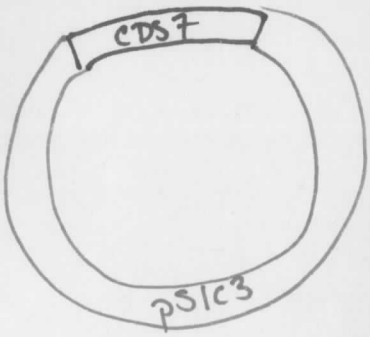
GEL EXTRACT & LIGATE.




EcoRI
PstI
CDS7
pET28
KAN


EcoRI
J04450
PstI
pSB1C3
CM

Goal:


CDS7
pSB1C3