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**Cloning of DNA Fragments**

Created: December 27, 1994
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A. Ligation

1. Purify vector (e.g., pUC19, pET11d) by commercial plasmid prep (e.g., Quiagen QUIAprep Spin Plasmid Kit) and run on an agarose gel to determine purity and concentration. Additionally, determine $OD_{260}$ and $OD_{280}$ of a 1:100 dilution in 250 µl cuvetts in UV spectrophotometer.

2. Digest 1 to 2 µg (or as much as possible) of plasmid in a final volume of 40 µl with the appropriate restriction enzymes. Purify plasmid DNA by phenol and chloroform extraction followed by ethanol precipitation with 1/10 volume NaCl. Dissolve pellet in 9 µl dest. water and check restriction digest and DNA concentration by running 2 µl on an agarose gel.

3. Digest 1 to 2 µg (or as much as possible) of the target DNA with the appropriate enzymes. Purify DNA after restriction digest. For PCR products, use Quiagen Kit and dry DNA afterwards in vacuum centrifuge (SpeedVac). Otherwise, purify by phenol and chloroform extraction followed by ethanol precipitation with 1/10 volume NaCl. Dissolve pellet in 9 µl dest. water and check restriction digest and DNA concentration by running 2 µl on an agarose gel.

4. Set up ligation in a 20 µl volume with a vector:insert ratio of cohesive ends between 1:1 and 3:1. Set up a negative control ligation with vector and no insert.

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| digested and purified vector DNA: | 100 ng |
| digested and purified insert DNA: | 1 to 3 times as many cohesive ends as vector |
| 10X ligase buffer: | 2 µl |
| T4 ligase: | approx. 0.1 Weiss Unit |
| Dest. water: | up to 100 µl |

Incubate @ 15°C overnight.

B. Transformation

1. Mix about 10 µl of the ligation and 200 µl competent cells (prepared as described in separate protocol), keep on ice for 45 min.

2. Transfer cells into tube preheated @ 42°C and incubate @ 42°C for **exactly** 90 sec.; chill in ice bath for 1 - 2 min.

3. Add 800 µl LB to cells and incubate @ 37°C (on shaker) for 45 min.

4. Plate cells on LB/X-gal/IPTG (for blue white screening) or on LB plates with appropriate antibiotic (e.g., 100 µg/ml ampicillin) and incubate @ 37°C for 12 to 24 h. Plate 10 µl, 50 µl and 200 µl of the transformation. Keep remainder of transformation at 4°C.

**References:**

Sambrook J., E. F. Fritsch, and T. Maniatis (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.