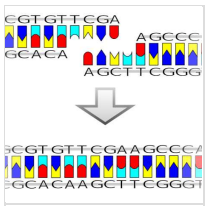

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# Ligation (Instructor Protocol)

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Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for the student Ligation protocol.

The abstract for the student protocol explains the basics. I pre-digest the L2-01 backbone for my students, but I generally do not gel-purify it. I find that the ligation is generally pretty robust, with three caveats:

- Because I don't gel-purify, we need to bias the ligation towards ligating the annealed oligos (not the GFP that was digested out). To do so, I dilute the backbone to 10 fmol/ul and instruct the students to dilute their annealed oligos to 200 fmol/ul (which is [M]**200 nanomolar (nM)** ).
- Ligase is EXPENSIVE, and this kind of ligation does not need much -- so I dilute 1:5 into small tubes. Thus, if a beginning student screws something up (throws the tube away, or contaminates it, etc), the expensive master reagent tube isn't lost.
- The ligase buffer contains ATP, which is heat-labile. (When a ligation fails, it's often because the buffer is bad, not the enzyme!) Thawing and re-freezing is not good for it. So, I aliquot the ligase buffer into single-use 5 ul aliquots in PCR tubes.

PROTOCOL CITATION

Brian Teague 2022. Ligation (Instructor Protocol). **protocols.io** https://protocols.io/view/ligation-instructor-protocol-cev9te96

KEYWORDS

ligase, ligation, oligonucleotides

IMAGE ATTRIBUTION

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MATERIALS TEXT

- E. coli freezer stock transformed with the L2-01 plasmid
- LB agar **Contributed by users** + 50 µg/ml

  Kanamycin **Research Products International (rpi) Catalog #K22000-25.0**
- LB Broth **Contributed by users** + 50 µg/ml

  Kanamycin **Research Products International (rpi) Catalog #K22000-25.0**

  Monarch Plasmid Miniprep
- Kit **NEB Catalog #T1010** Step 3
- Nuclease-free Water **Contributed by users** In 2 steps

  Esp3I **New England**
- **Biolabs Catalog # R0734S** Step 5

  CutSmart® Buffer **New England**
- **Biolabs Catalog #B7204S** Step 5
- Monarch DNA Elution Buffer - 25 ml **New England Biolabs Catalog #T1016L** Step 8

  (optional)
- 10X NEB T4 DNA ligase buffer **New England Biolabs** Step 10

  T4 DNA Ligase - 20,000 units **New England**
- **Biolabs Catalog #M0202S** Step 11

  Diluent A - 5.0 ml **New England**
- **Biolabs Catalog #B8001S** Step 11

SAFETY WARNINGS

Some components of the miniprep kit are hazardous; wear appropriate PPE.

TE, oligos, ligase & buffer, etc. are not hazardous. HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

## Grow & miniprep L2-01

1 At least 48 hours before the lab, strike out the L2-01 E. coli strain from a frozen stock on an LB+Kan plate.

Sanity check -- because the L2-01 plasmid has a GFP cassette, the colonies should be bright green!

2 At least 24 hours before the lab: pick a colony of L2-01 into **5 mL** LB+Kan liquid media. Grow in a round-bottomed test-tube overnight on a shaker, **200 rpm, 37°C, 16:00:00**

This is a pretty big plasmid, so the culture may not be as turbid as you're used to.

Sanity check -- the culture should be bright green!

3 Miniprep the culture using the Monarch Plasmid Miniprep Kit **NEB Catalog #T1010** or another suitable miniprep kit.

4 Analyze the eluate on a Nanodrop for DNA concentration and purity.

Typical elution: 50 ul @ 130 ng/ul (20 fmol/ul)

## Linearize L2-01 3h 20m

5 We're going to linearize the ENTIRE miniprep. To a 200 ul PCR tube, add:

- **50 µL** miniprep
- **38 µL** Nuclease-free Water **Contributed by users**
  CutSmart® Buffer **New England**
- **10 µL** **Biolabs Catalog #B7204S**
  Esp3I **New England**
- **2 µL** **Biolabs Catalog # R0734S**

Flick several times to mix well, then pulse down in a microcentrifuge.

6 Incubate **37 °C** for **03:00:00** , then inactivate the reaction at **65 °C** for **00:20:00** 3h 20m

7 Optional: gel-purify the backbone. Run the entire miniprep on a preparative agarose gel, then use a kit such as the Monarch DNA Gel Extraction Kit **New England Biolabs Catalog #T1020S** to purify the *larger* band.

8 Optional: dilute with Monarch DNA Elution Buffer - 25 ml **New England Biolabs Catalog #T1016L** to a final concentration of 10 fmol/µl.

Aliquot / dilute reagents

9 Aliquot **1 mL** of Nuclease-free Water **Contributed by users** per 4 students. Store at **Room temperature** .

10 Aliquot 10X NEB T4 DNA ligase buffer **New England Biolabs** into **5 µL** single-use aliquots in 200 ul PCR strip tubes. Store at **-20 °C** .

I like to store these in a 200 µl tip box.

11 Dilute T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S** 1:5 in Diluent A - 5.0 ml **New England Biolabs Catalog #B8001S** . I make tubes containing **2 µL** of ligase and **8 µL** of buffer, one per 4 students. Store at **-20 °C** .

Instructor Tips & Common Student Errors

## 12 Instructor Tips

- The first step of the student protocol requires another dilution. Again, I'm hands-off for this one. A common student question is "what is the concentration of my annealed oligos?" and I suggest that they might be able to figure it out by reviewing the annealing protocol.
- I usually instruct students to run this protocol and the URA3 PCR protocol at the same time: set up the ligation, then while the ligation is incubating, set up their PCR.

## 13 Common Student Errors

- Omitting the dilution step (particularly if they need to retry the protocol.)
- Small-volume pipetting.