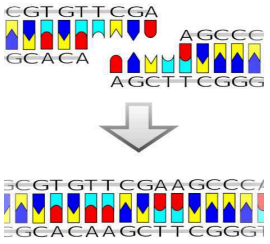
Aug 08, 2022 Version 3

Ligation V.3



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Yeast ORFans CURE

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OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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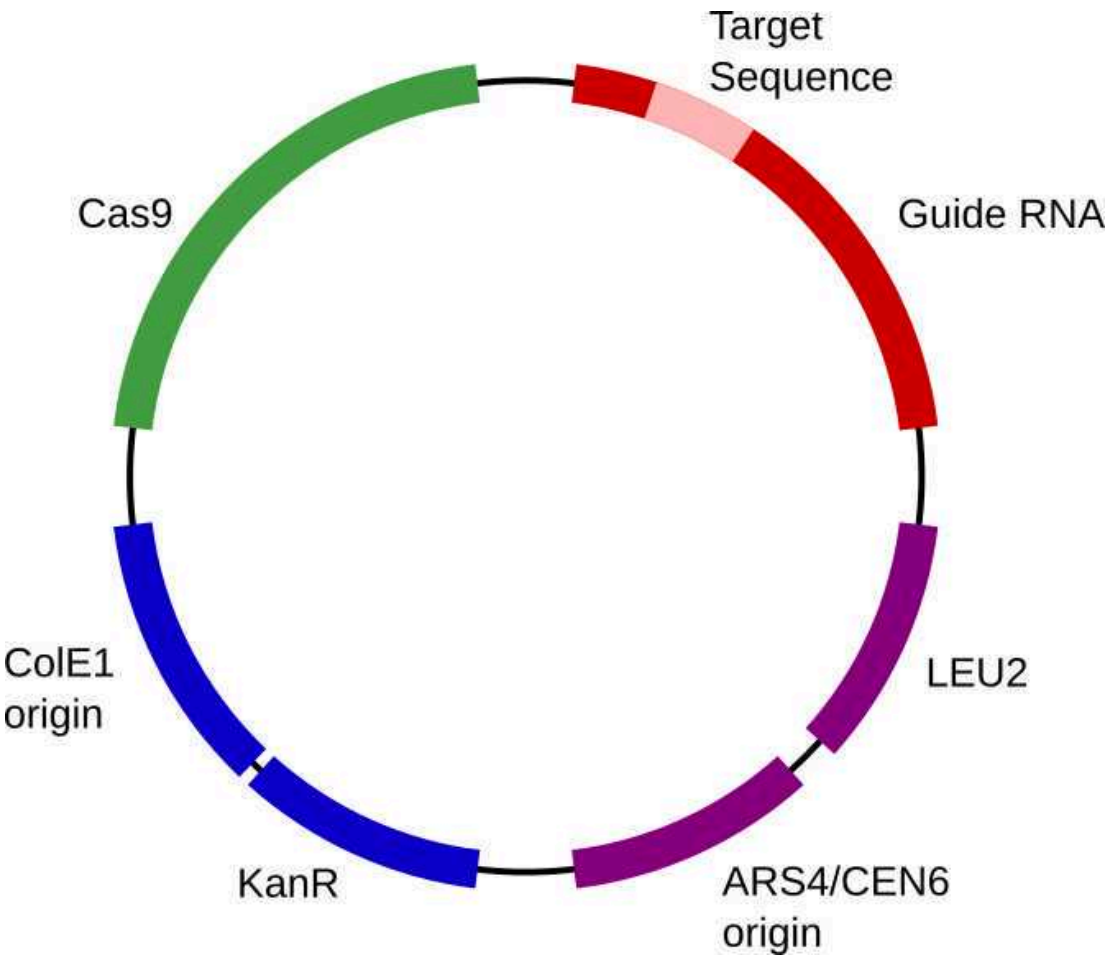
Abstract

A DNA ligase is an enzyme that forms phosphodiester bonds -- it can "glue" together two pieces of DNA. The ligase we're using comes from the T4 bacteriophage virus.

Why do we need to do a ligation anyway? Remember, we're going to use a Cas9 protein to "cut" your target gene in a yeast cell. We can program the Cas9 to cut a particular place using a "guide RNA." However, does *Saccharomyces cerevisiae* -- brewers' yeast -- make Cas9 or a guide RNA on its own? No! So we need to give the yeast cell a set of instructions for making the Cas9 protein and the guide RNA.

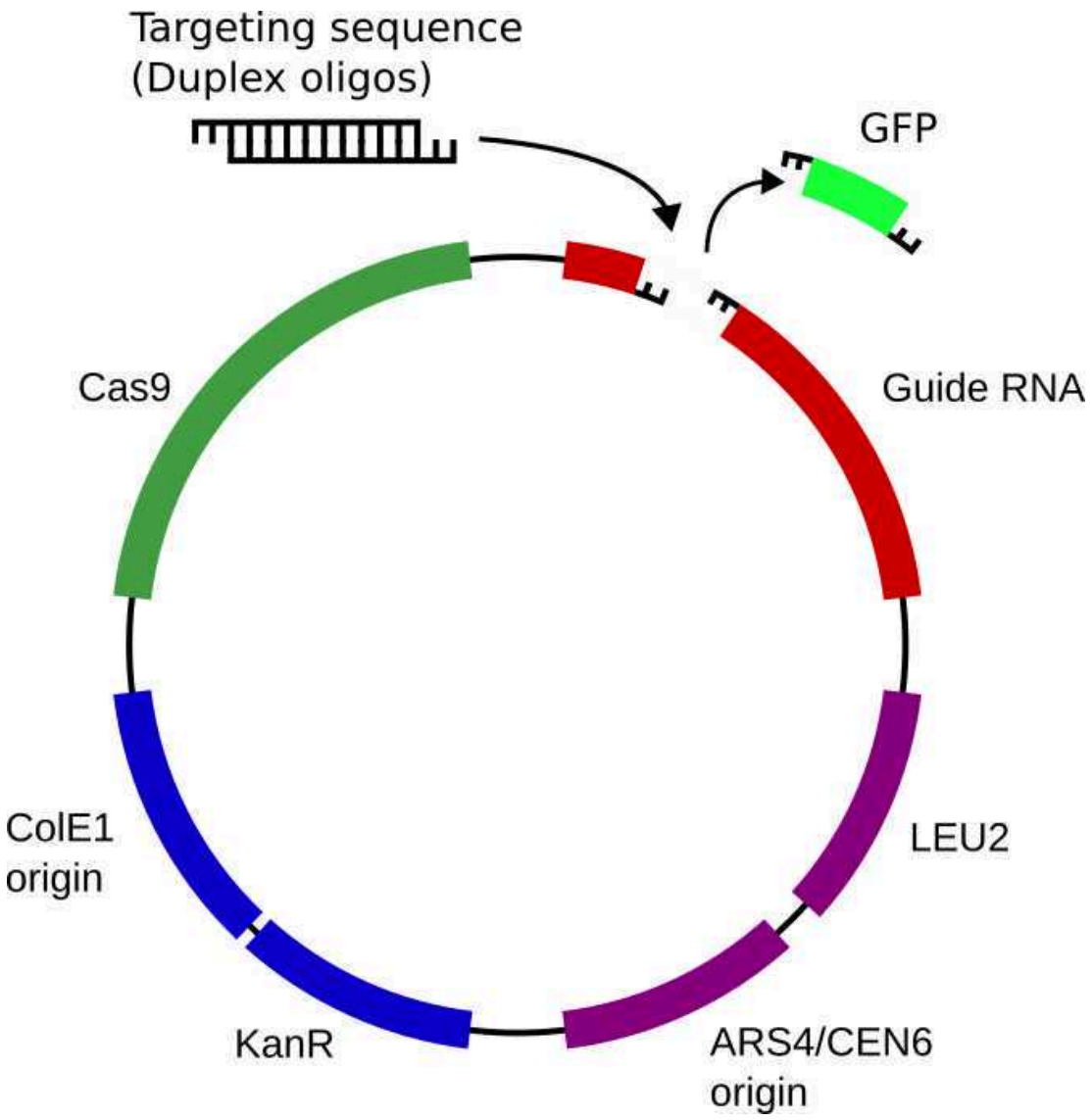
We do so by encoding those instructions on a *plasmid* -- a small circular piece of DNA -- and then putting that plasmid into the yeast cell. Then, the yeast cell "follows" the instructions to make the Cas9 protein and guide RNA. The plasmid we're making has several functional DNA sequences on it -- a diagram is below.



The functional sequences on the plasmid are denoted by colored boxes -- you can ignore most of them for now. Note, however, the boxes labelled Cas9 and Guide RNA -- these are the instructions for making Cas9 and the

guide RNA. Remember, though, that each group is knocking out a different gene -- so each group needs a different plasmid!

The part that needs to be customized for each group is the "target sequence" in the above diagram. Your instructor started with a plasmid similar to the one above, but where the target sequence is instead replaced by gene for a green fluorescent protein (GFP) -- cells that express it turn bright green! They have cut out the GFP, and now you need to replace it with a targeting sequence for your gene, as in the diagram below.



One last thing. Where is your targeting sequence coming from? It's the annealed oligonucleotides from last time. You'll mix the plasmid backbone, your annealed oligos, the ligase enzyme, and a ligase buffer (which contains all

of the chemicals the ligase needs to operate), and then the ligation will incubate at room temperature while the enzyme does its thing.

Image Attribution

By Madprime - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2161789

Guidelines

This protocol involves pipetting very small quantities of liquid. Pipette carefully!

Materials

- A 200 µl PCR tube
- L2-01 backbone plasmid, 10 fmol/ul
- Annealed oligonucleotides
- T4 DNA Ligase **New England Biolabs Catalog #**M0202
- 10X NEB T4 DNA ligase buffer **New England Biolabs**

Note

The DNA ligase buffer is kept in single-use aliquots -- little tubes with an "L" on top. Take one for your group, use as much as you need, then discard the rest.

- Nuclease-free Water
- TE Buffer

Protocol materials

10X NEB T4 DNA ligase buffer **New England Biolabs**
Nuclease-free Water
TE Buffer
T4 DNA Ligase **New England Biolabs Catalog #**M0202
TE Buffer
Nuclease-free Water
10X NEB T4 DNA ligase buffer **New England Biolabs**
T4 DNA Ligase **New England Biolabs Catalog #**M0202

Safety warnings

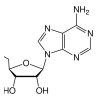
None of the materials in this lab are hazardous.

HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

Before start

This protocol depends on the successful completion of the Annealing Oligonucleotides protocol, below:

Protocol



NAME

Annealing Oligonucleotides

CREATED BY

Brian Teague

PREVIEW

Ligation

2h 30m

1 Dilute the annealed oligonucleotides in TE Buffer to make 100 µL of working stock at a final concentration of [M] 200 nanomolar (nM)

2 In the PCR tube, mix *in order*:

- 4 µL Nuclease-free Water
- 2 µL of the L2-01 DNA plasmid backbone
- 2 µL of the **diluted** annealed oligos
- 1 µL 10X NEB T4 DNA ligase buffer **New England Biolabs**
- 1 µL T4 DNA Ligase **New England Biolabs Catalog #**M0202

3 Flick the tube several times to mix the components, then spin briefly in the microcentrifuge to collect everything at the bottom of the tube.

4 Incubate at room temperature at least 00:30:00, and up to 02:00:00 (longer is better). 2h 30m

5 Store at -20 °C