**Created by Yongtao Zhu (Spring 2021)**

**Protocol for DNA Ligation**

Run the digested plasmid/PCR product (2 µl) on an Agarose gel before setup the ligation. If a bright single band is observed for each sample and the brightness is similar between the two samples, follow the steps below:

1. Label a microcentrifuge tube with: plasmid name + primer names
2. Set up the following reaction in the microcentrifuge tube on ice

* 7 µl digested / purified plasmid
* 10 µl digested / purified PCR product
* 2 µl 10 X T4 DNA Ligase Buffer
* 1 µl T4 DNA Ligase

Total volume should be 20 µl. You can adjust the volumes of the digested plasmid and PCR products based on their brightness.

**Alternatively**, you can determine their concentrations and use the NEBioCalculator (<https://nebiocalculator.neb.com/#!/ligation>) to calculate the amounts needed for 1:1 – 7:1 molar ratios (insert: vector).

1. Flick the tubes a few times (don't vortex). Spin down tube for 5 seconds in the micro-centrifuge.
2. Incubate at room temperature overnight (can be longer).
3. 65oC, 10 min for deactivation of the ligase and release of the ligase from DNA (for better efficiency in transformation).
4. Freeze the heated ligation product until next step.