

# Culture Inoculation Protocol

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## Introduction

This is a protocol for Inoculating a larger culture from a 5 mL overnight. Used for protein expression. Will take one day in lab.

## Materials

- › 5 mL overnight culture of desired bacteria
  - › This should contain the plasmid coding for the protein you want to express
  - › Make sure the strain is suitable for expression (e.g. BS21)
- › 150 mL baffled flask
  - › This flask will provide more surface area for aeration of bacteria (which means better growth!)
- › Rich Media
  - › Typically use 2xYT, the protocol for which should be listed in the protocols section

## Procedure

### Inoculation of a Larger culture

1. Measure OD of the overnight culture, using a 1:10 dilution in media on your nano drop.
2. Perform the following calculation:  
$$OD_i \cdot V_i = OD_f \cdot V_f$$
to calculate  $V_i$  the inoculation volume that you will pull from the overnight to the larger flask.  
Where  $OD_i$  is the OD of the overnight culture  
 $V_f$  is the volume of the culture that you are starting (e.g. 30mL)  
 $OD_f$  is 0.08; this is standard inoculation OD  
  
Some ballpark values might look like:  
$$OD_{0.08} \cdot 30\text{mL} / 8 = V_i = 0.3 \text{ mL}$$
This means that you (under flame) put 0.3 mL into your 30 mL starter culture.
3. Inoculate your culture
4. Take OD every ~1 hr (figure out doubling time)
5. Induce at ~OD .5
6. Grow to OD 3
7. Spin down cells
8. Remove supernatant
9. Store in -20 for a few weeks, -80 for longer