

Improving Student Outcomes with an Adaptable Molecular Cloning Course-Based Undergraduate Research Experience

Experiment B Planning Worksheet

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Example data shown in red, corresponding to sequence files provided in **Supplemental File 8**, and purified amplicon concentrations reported by a student in Spring 2022.

Complete this worksheet to prepare for your Gibson Assembly.

1. Convert the concentration of your purified amplicons from ng/ μ L to molar concentration (M)

An [online calculator](#) may be helpful.

You will need the molecular weight of your amplicon(s) (and your vector backbone if it was provided separately), which should be recorded on your Experiment A Planning Worksheet.

Primer Pair	Amplicon Size (base pairs)	Concentration (ng/ μ L)	Concentration (M)
1	2684	41.3	23.7×10^{-9}
2	1752	31.9	28.1×10^{-9}
3			
4			

2. Calculate the volume of each DNA component needed for the Gibson Assembly

Homemade MasterMix

- Calculate the volumes of the vector and each amplicon such that **17.0 femtomoles** (fmol) (17.0×10^{-15} mol) are in the solution. Note that this is an **amount**, NOT a concentration.
- For ease of pipetting when putting together the Gibson Assembly, each amplicon must have a **minimum volume of 1 μ L** pipetted into the Assembly. **

Reagent	[Stock]	Volume to Pipet (μ L)	[Final]/Amount
Sterile H ₂ O	---	1.00	---
PCR-Purified Insert(s)	17.0×10^{-9} M	1.00	17.0 fmol
Linearized Vector	17.0×10^{-9} M	1.00	17.0 fmol
2X Gibson Assembly Master Mix	2X	3.00 μ L	1X
Total Volume	---	6.00 μ L	---

** If your amplicons required dilutions to reach this 1 μ L volume, note that below, or mark N/A:

Experiment B Planning Worksheet

NEBuilder® HiFi DNA Assembly Master Mix

- Calculate the volumes of the vector and each amplicon such that **50 femtomoles (fmol)** (50×10^{-15} mol) are in the solution. Note that this is an **amount**, NOT a concentration.
- For ease of pipetting when putting together the Gibson Assembly, each amplicon must have a **minimum volume of 1 μ L** pipetted into the Assembly. **

Reagent	[Stock]	Volume to Pipet (μ L)	[Final]/Amount
Sterile H ₂ O	---	4.12	---
PCR-Purified Insert(s)	17.0×10^{-9} M	2.94	50.0 fmol
Linearized Vector	17.0×10^{-9} M	2.94	50.0 fmol
2X Gibson Assembly Master Mix	2X	10.0 μ L	1X
Total Volume	---	20.0 μ L	---

** If your amplicons required dilutions to reach this 1 μ L volume, note that below, or mark N/A:

Summarize your dilutions below, as necessary.

Linear Fragment 1: 3.00 μ L amplicon (23.7×10^{-9} M) + 1.18 μ L sterile H₂O.

Diluted Linear Fragment 1 Concentration = 17.0×10^{-9} M

Linear Fragment 2: 2.00 μ L amplicon (28.1×10^{-9} M) + 1.31 μ L sterile H₂O.

Diluted Linear Fragment 2 Concentration = 17.0×10^{-9} M

Linear Fragment 3: _____ μ L amplicon (_____ M) + _____ μ L sterile H₂O.

Diluted Linear Fragment 3 Concentration = _____

Linear Fragment 4: _____ μ L amplicon (_____ M) + _____ μ L sterile H₂O.

Diluted Linear Fragment 4 Concentration = _____