PCR Sewing

You will need the following ingredients for the Sewing PCR mix:

Fragment #1	5.0ul
Fragment #2	5.0ul
dNTPs	1.0ul
DMSO (optional)	1.0ul
5x Phusion buffer	10.0ul
ddH_2O	27.5ul (or enough to 50ul total)
Phusion polymerase	0.5ul
	50.0ul

1. Always start a PCR mix by writing the recipe above in your notebook, making sure to write specific names for the fragments.
2. Locate all of the ingredients except for the polymerase and **place them on ice** to let them thaw.
3. If you are doing multiple PCR's, prepare a **Master Mix** by adding the common ingredients for all reactions to one pot. **Add the polymerase LAST.** For example, if I were doing a PCR on six **different** DNA fragments, my notebook might look like this:

			Master Mix
Fragment #1	5.0ul		
Fragment #2	5.0ul		
dNTPs	1.0ul		6.0ul
DMSO (optional)	1.0ul		6.0ul
5x Phusion buffer	10.0ul	X6	60.0ul
ddH_2O	0.5ul		3.0ul
Phusion polymerase	27.5ul		165.0ul
Total Volume	**50.0ul**		**240.0ul**

4. Aliquot the **Master Mix** into each PCR tube.
5. Add the remaining ingredients not included in the Master Mix.
6. Label the tube and record the labeling in your notebook.

Running the Reaction

1. Take your tubes to an available thermocycler.
2. The PCR sewing reaction takes place in two parts. In the first part, you will run the usual PCR cycle for only 5 cycles in order to let the fragments prime each other and synthesize a small amount of full length DNA product.

Step 1 (Denaturation):	98˚C	0:30 min	
Step 2 (Denaturation loop):	98˚C	0:15 min	X5 cycles
Step 3 (Annealing):	50˚C – 60˚C	0:25 min	
Step 4 (Elongation):	72˚C	30 sec/kb	
Step 5 (Final Elongation):	72˚C	5:00 min	
Step 6 (Storage):	4˚C	forever	

3. After the first 5 cycles are over, add **1.0ul of 10uM end primers** to each tube. **Also add 0.5ul of Phusion polymerase.** Run the PCR for an additional 20-25 cycles, as usual.

Step 1 (Denaturation):	98˚C	0:30 min	
Step 2 (Denaturation loop):	98˚C	0:15 min	X20-25 cycles
Step 3 (Annealing):	50˚C – 60˚C	0:25 min	
Step 4 (Elongation):	72˚C	30 sec/kb	
Step 5 (Final Elongation):	72˚C	5:00 min	
Step 6 (Storage):	4˚C	forever	

4. When your PCR run is over, you will have to purify the DNA using **Gel Purification/Extraction**. Look for a band corresponding to the sum of the two fragments you attempted to sew together.