Pho Lab Notebook

PhoA Cloning Workflow
PhoA and PhoB Characterization Workflow
PhoB original and PhoB Consensus Workflow

March:

Mon	Tue	Wed	Thurs	Fri
1	2	3	4	5
8	9	10	11	12
15	16	17	18	19 - Got the PhoA part
22	23 - Successful transformation of PhoA	24 - Inoculated colonies from the PhoA transformation	25 - Created a glycerol stock - Mini-prepped colonies from PhoA transformation - Failed sequencing of purified PhoA DNA	26
29 - Inoculated PhoA Transformation	30 - Mini-prepped colonies from PhoA Transformation	31		

April:

Mon	Tue	Wed	Thurs	Fri
			1	2
5	6	7	8	9
12	13 - Prepared for experimentation	14 - Prepared for experimentation - Made liquid culture of PhoA and PhoB glycerol stocks	15 - Inoculated liquid cultures of PhoA and PhoB into flasks - Created phosphate solutions	16 - Characterization of PhoB and PhoA
19 - Analyzed the	20	21	22	23 - Ran a colony

characterization data from PhoB and PhoA				PCR of colonies from PhoA transformation
26 - Successful gel of the PhoA colony PCR (Figure 1) - Prepared for characterization	27 - Prepared for characterization	28 - Characterization of PhoA	29	30

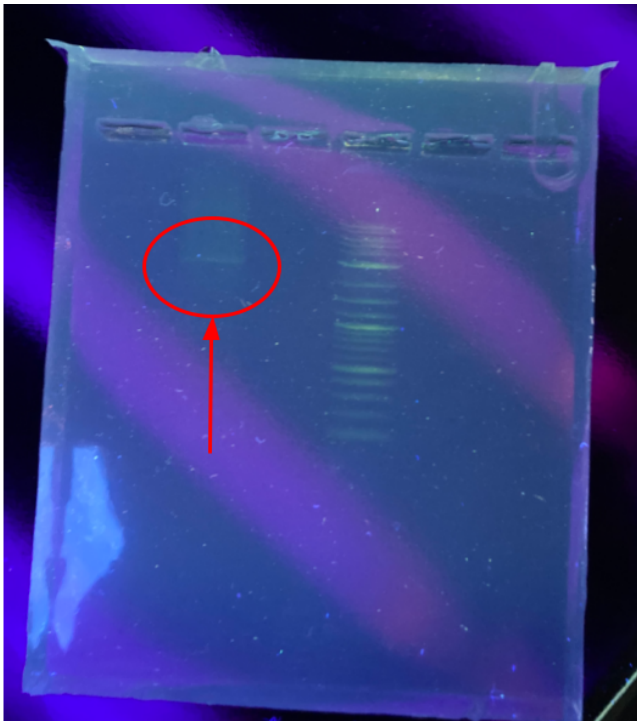

Figure 1. *Successful gel results of PhoA Colony PCR. Well 2 contain the PhoA DNA, and well 4 contains the standardized DNA ladder (4/26/21).*

May:

Mon	**Tue**	**Wed**	**Thurs**	**Fri**
3	4	5	6	7
10	11 - Streaked PhoA glycerol stock onto plates	12 - Inoculated colonies from PhoA streaking	13 - Inoculated colonies from PhoA streaking	14 - Mini-prepped PhoA glycerol stock - Successful sequencing of purified PhoA DNA

17	18	19	20	21
24	25	26	27	28
31				

July:

Mon	Tue	Wed	Thurs	Fri
			1	2
5	6 - Prepared for experimentation	7	8	9
12	13	14	15	16
19 - Inoculated PhoB from glycerol stock	20 - Grew PhoB cells from inoculation into flasks	21 - Made phosphate solutions	22	23 - Characterization of PhoB
26	27	28	29	30

August:

Mon	Tue	Wed	Thurs	Fri
2	3	4	5	6 - PhoB consensus part arrived
9 - Hydrated PhoB consensus DNA - Inoculated PhoB original from mini-prepped DNA - Failed transformation of PhoB consensus from hydration and PhoB original from glycerol stock (Figure 2)	10	11 - Successful transformation of PhoB consensus from hydration (Figure 3) - Ran colony PCR on colonies from PhoB transformation	12 - Mini-prepped PhoB original from the transformation - Ran colony PCR on PhoB consensus from the transformation - Successful sequencing of purified PhoB original DNA	13 - Failed sequencing of purified PhoB consensus DNA - Successful transformation of PhoB original from glycerol stock (Figure 4)
16 - Ran a colony	17	18 - Ran a Colony	19 - Successful gel	20 - Resuspended

PCR on colonies from PhoB original transformation - Failed gel of PhoB original and PhoB consensus from colony PCR (Figure 5)		PCR on PhoB original and PhoB consensus using new polymerase	on PhoB consensus and PhoB original from colony PCR - Inoculated PhoB original and PhoB consensus from the transformation	PhoB original in MOPS to check for fluorescence (successful) - Successful sequencing of purified PhoB original DNA - Made glycerol stock of PhoB consensus
23 - Inoculated PhoB original from the transformation	24 - Resuspended PhoB original and PhoB consensus in MOPS	25 - Prepared for characterization	26 - Prepared for characterization	27 - Characterization of PhoB original - Failed inoculation PhoB consensus (no cells grew)
30 - Streaked PhoB consensus on to plates from glycerol stock - Inoculated PhoB consensus from inoculation	31 - Resuspended PhoB consensus in MOPS			

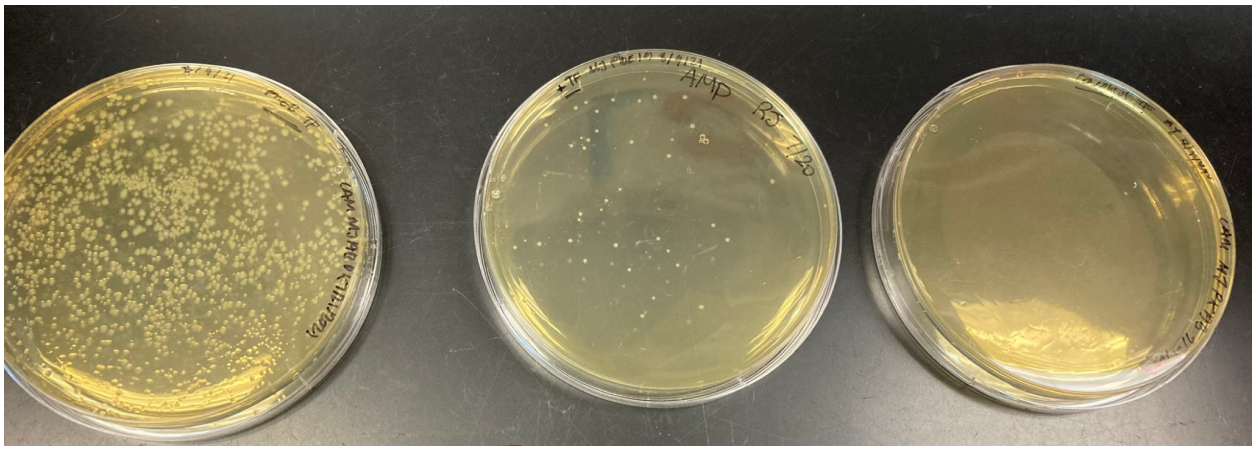

Figure 2. *Failed PhoB original (left), pUC19 control (middle), and PhoB consensus (right) transformation plates (8/9/21).*

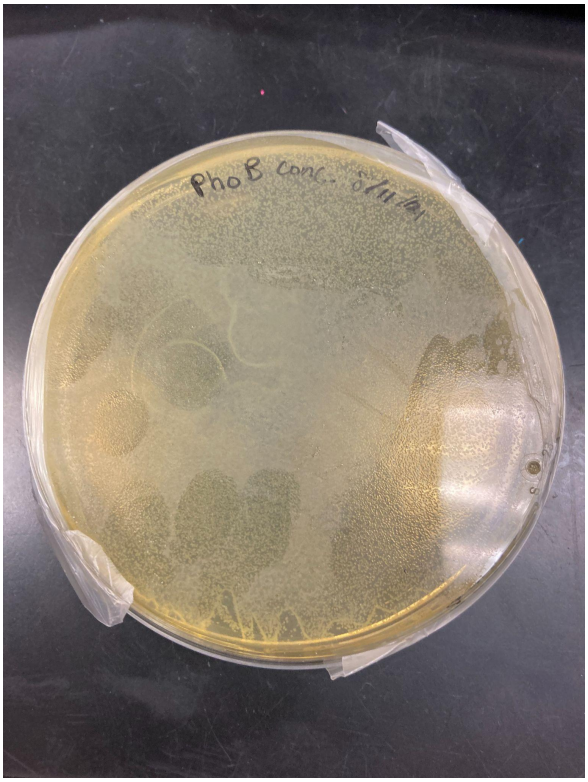

Figure 3. *Successful PhoB consensus transformation plate (8/11/21).*

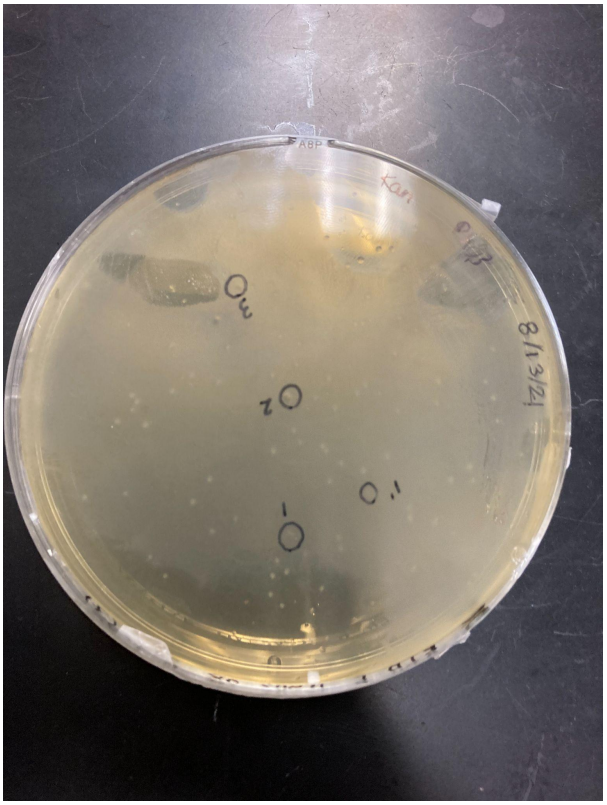

Figure 4. *Successful PhoB original transformation plate (8/13/21).*

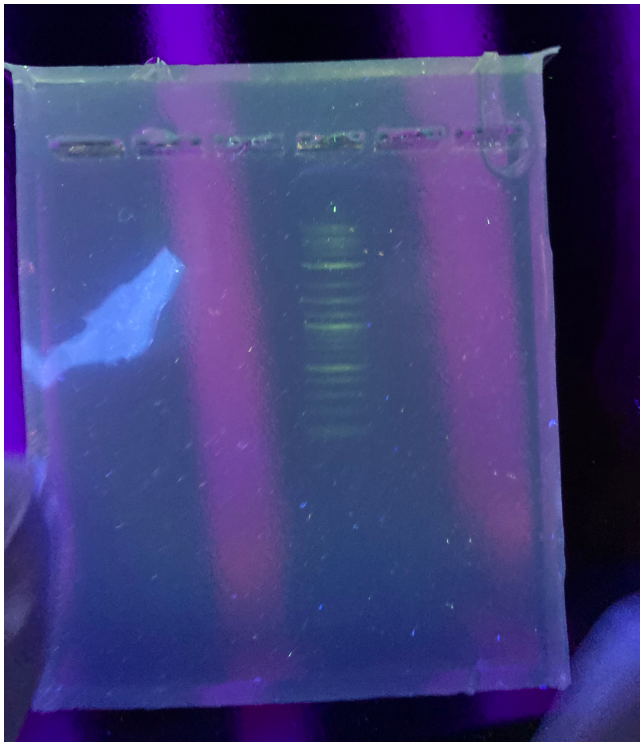

Figure 5. *Failed gel results of PhoB original and PhoB consensus. Well 4 contains the standardized DNA ladder (8/16/21).*

September:

Mon	Tue	Wed	Thurs	Fri
		1 - Failed transformation of PhoB consensus from glycerol stock (Figure 6) - Inoculated colonies from PhoB consensus inoculation into flasks	2 - Successful characterization of PhoB consensus using a modified protocol	3
6	7 - Prepared for experimentation - Inoculated PhoB original from glycerol stock	8 - Mini-prepped PhoB consensus - Grew PhoB original inoculation into flasks	9 - Characterization of PhoB original - Successful sequencing of purified PhoB consensus DNA	10 - Analyzed PhoB original characterization data
13 - Inoculated PhoB consensus and PhoB original from	14 - Hydrated PhoB consensus forward primer	15 - Checked for resuspension glow of PhoB original	16 - Analyzed vortex and plate variance experiment data	17

glycerol stock	- Successful sequencing of purified PhoB consensus DNA - Resuspended PhoB original and PhoB consensus into MOPS	and consensus - Ran vortex and plate variance experiments		
20	21	22	23	24
27 - Inoculated PhoB consensus from glycerol stock into flasks	28 - Ran sample testing of Lambert Hydroponics Sample	29 - Failed sequencing of purified PhoB consensus DNA	30 - Successful transformation of PhoB consensus from mini-prepped DNA (Figure 7)	

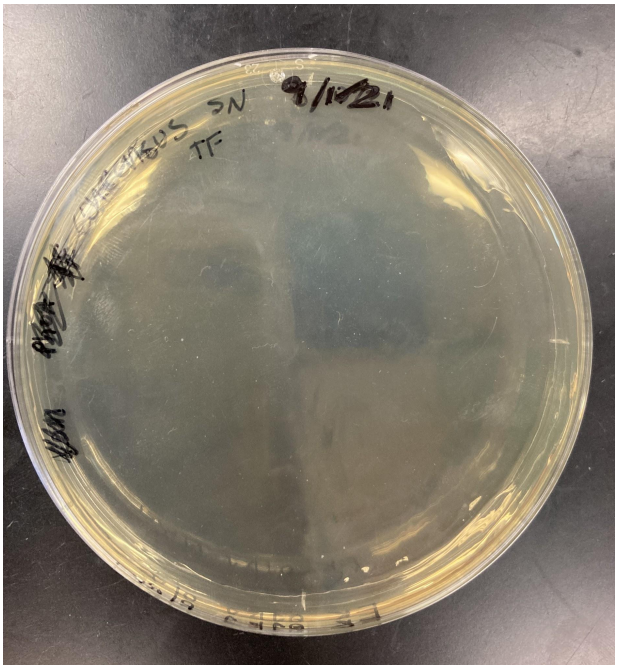

Figure 6. *Failed PhoB consensus transformation plate (9/1/21).*

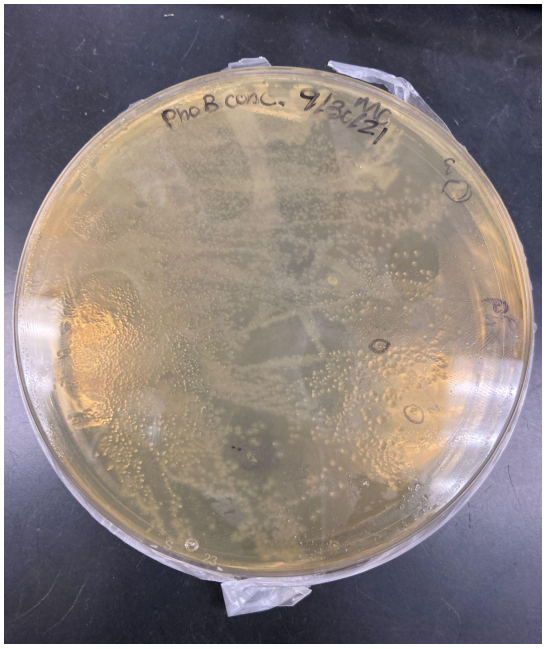

Figure 7. *Successful PhoB consensus transformation plate (9/30/21).*

October:

Mon	**Tue**	**Wed**	**Thurs**	**Fri**
				1 Successful transformation of PhoB (Figure 8)
4 - Inoculated PhoB consensus from transformation	5 - Ran sample testing on all samples (Lambert Hydroponics, Dick's Creek, Sweetwater Aeroponics, Chattahoochee Pointe Park)	6 - Mini-prepped PhoB consensus - Successful sequencing of purified PhoB consensus DNA	7 - Prepared for sample testing	8 - Prepared for sample testing
11 - Ran sample testing trial on Lambert Hydroponics, Dick's Creek, Sweetwater Aeroponics, and Chattahoochee Pointe Park	12	13	14	15
18	19 - Checked	20	21	22

	concentrations of water samples using Lamotte phosphate testing kit (Figure 9)			
25	26	27	28	29

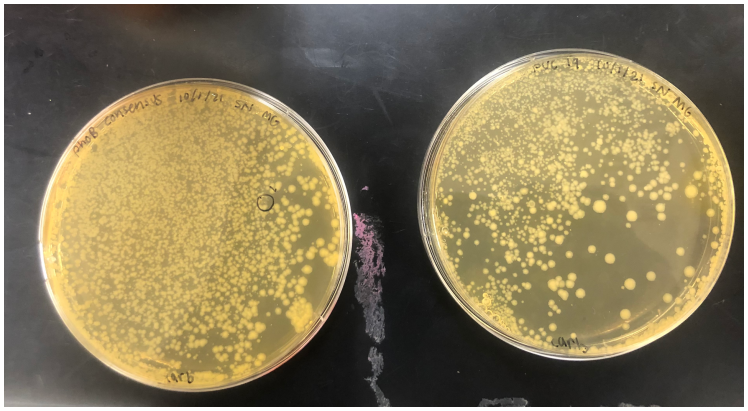

Figure 8. *Successful transformation of PhoB original plate (10/1/21).*

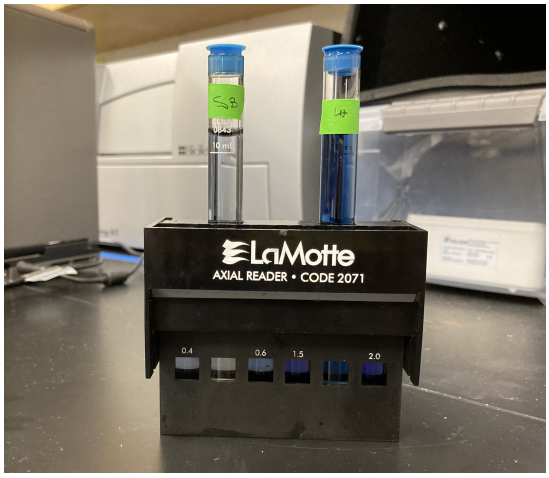

Figure 9. *Results of water samples using Lamotte phosphate testing kit (10/19/21).*