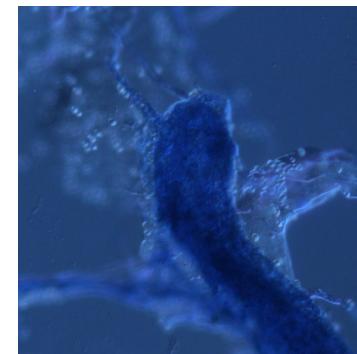
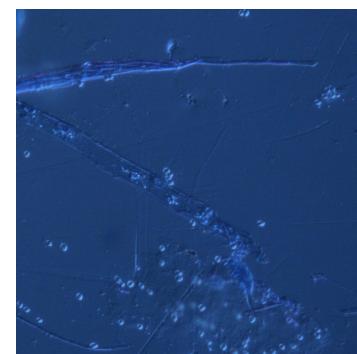
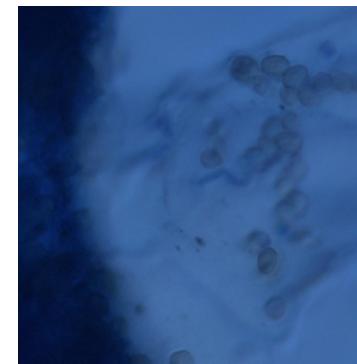
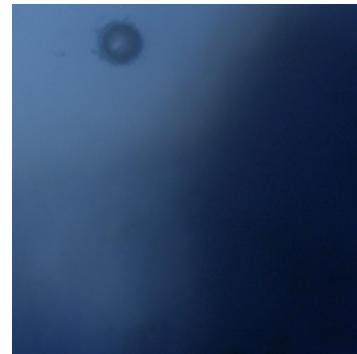
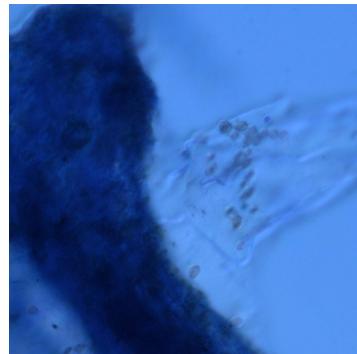


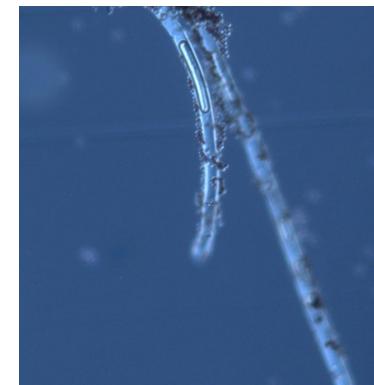
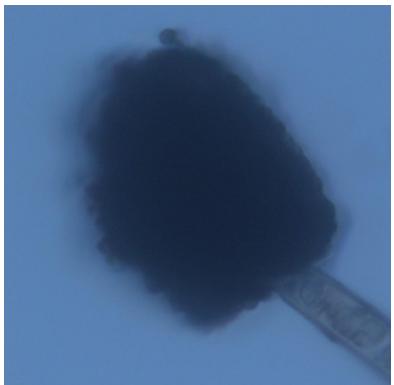
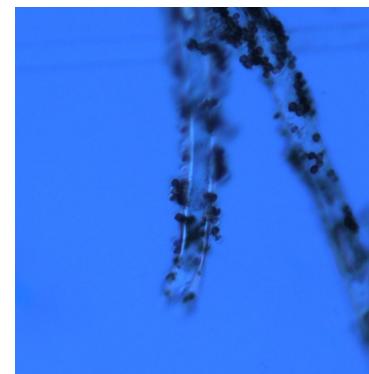
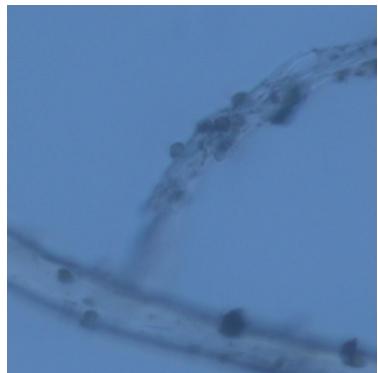
JUNE

WEEK 4

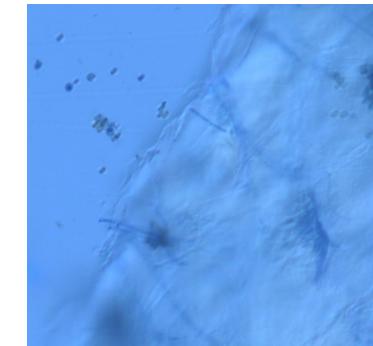
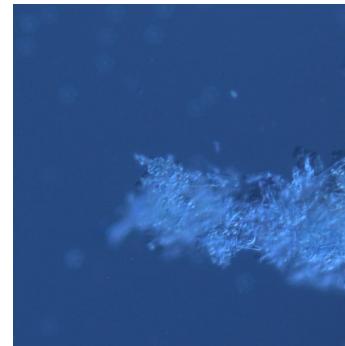
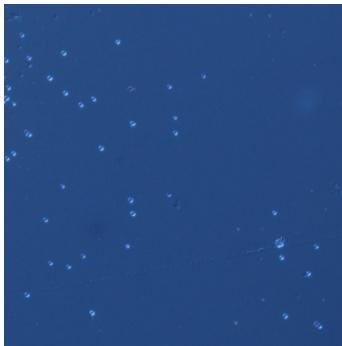
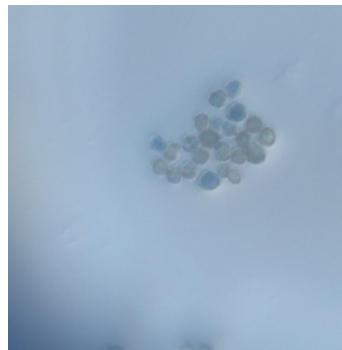
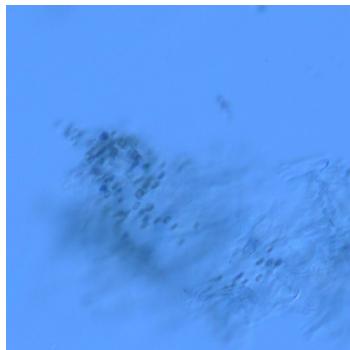
OBSERVED C18 UNDER DIC MICROSCOPE



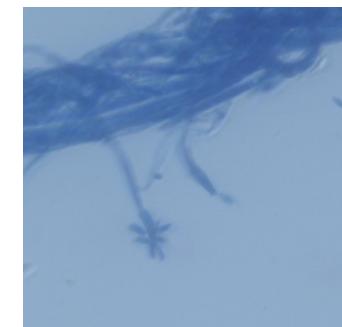
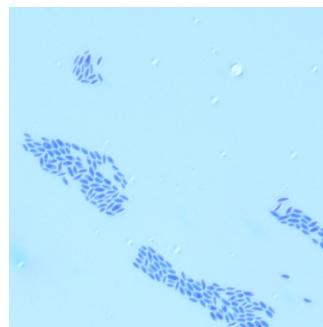
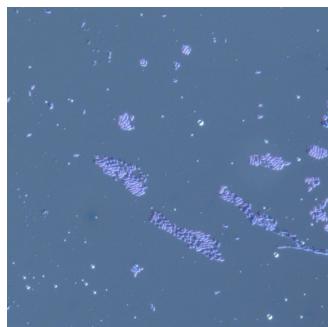
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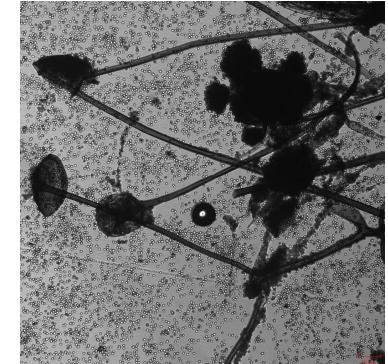
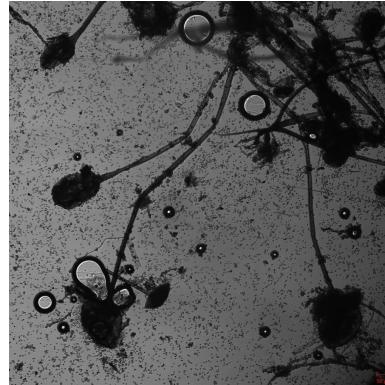
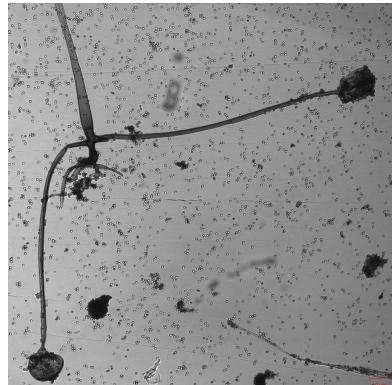
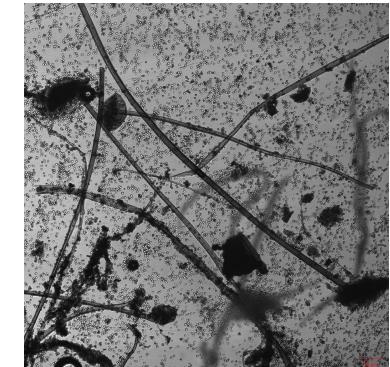
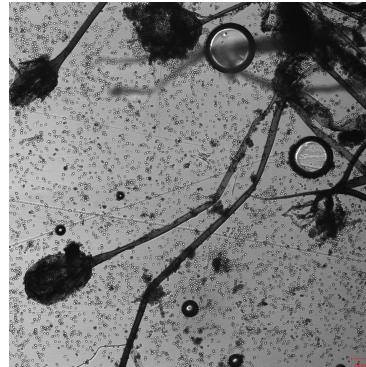
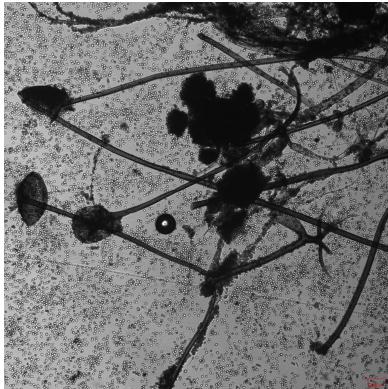
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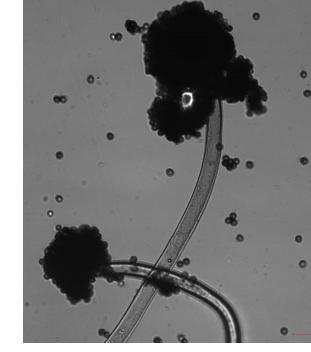
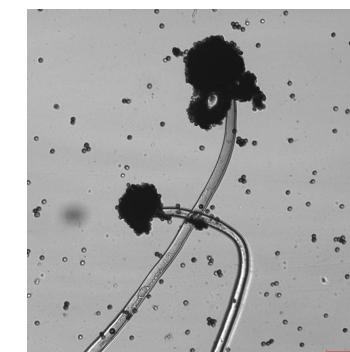
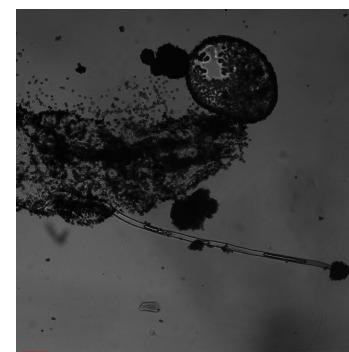
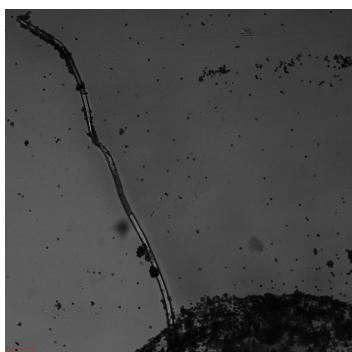
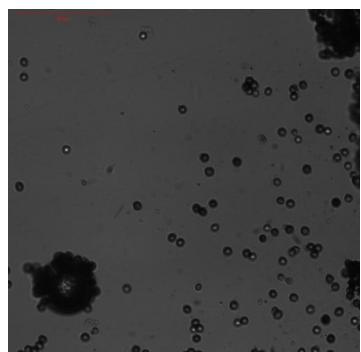
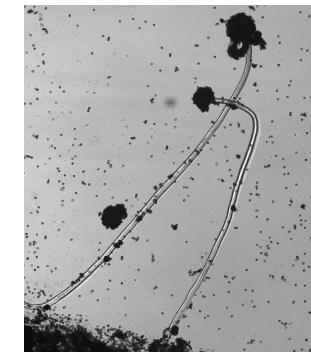
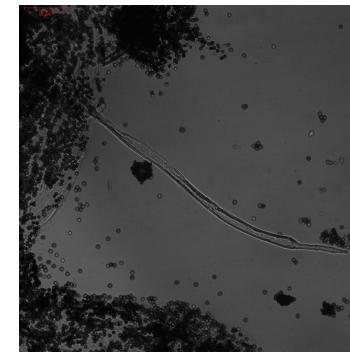
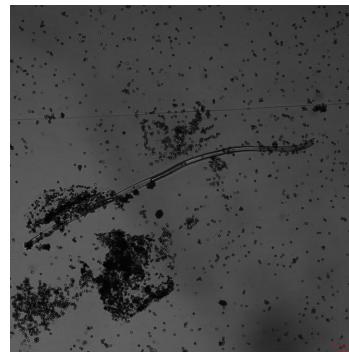
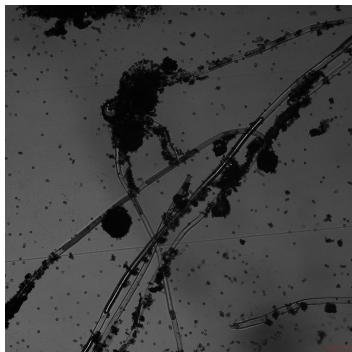
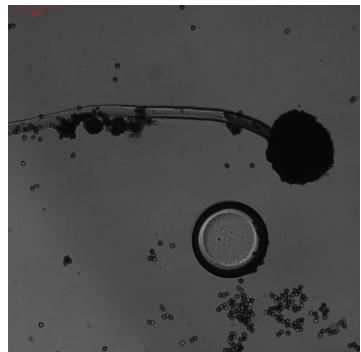
OBSERVED C5 UNDER DIC MICROSCOPE



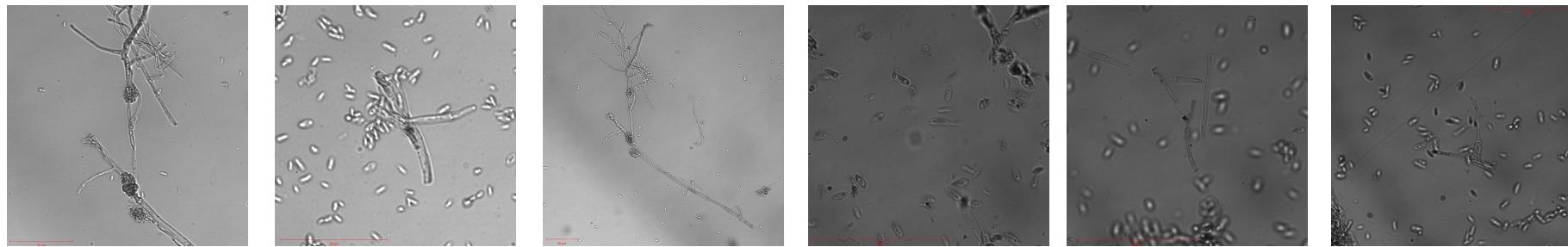
OBSERVED C18 UNDER CONFOCAL MICROSCOPE



OBSERVED C16 UNDER CONFOCAL MICROSCOPE



OBSERVED C5 UNDER CONFOCAL MICROSCOPE



SANGER PCR

2.2. BigDye V3.1 - Reaction Setup

Sequence mix	
Volume Per Reaction (µL)	1X
deionized (MQ) H ₂ O	4.75 (adjust to final volume)
Sequence buffer	1.75
Sequence mix Big dye	0.5
Sequencing primer 10mM	1
Template (purified)	2 (adjusted to conc.)
Total	10

2.3. BigDye Cycling Condition

Parameter	Stage/step				
	Incubate	25 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time [mm:ss]	01:00	00:10	00:05	04:00 ⁽¹⁾	Hold until ready to purify.

⁽¹⁾ Shorter extension times can be used for short templates.

SEQUENCING REACTION CLEAN UP

Ethanol/EDTA Precipitation

To precipitate 20 μ L sequencing reactions in 96-well reaction plates: Note: 10 μ L of nuclease free water can be added to the PCR mixture for making the volume to 20 μ L.

- 1.Remove the 96-well reaction plate from the thermal cycler and briefly spin.
- 2.Add 5 μ L of 125 mM EDTA to each well. Note: Make sure the EDTA reaches the bottom of the wells.
- 3.Add 60 μ L of 100% ethanol to each well.
- 4.Seal the plate with aluminium tape and mix by inverting 4 times.
- 5.Incubate at room temperature for 15 min.
- 6.Spin in a plate centrifuge for 30 min at 3000g. (Alternatively, in case of limiting maximum speed, spin for 45 minutes at 2200 g)
- 7.Invert the plate and spin up to 185g for 1 minute, then remove from the centrifuge. (Provide a cushion of three-four tissue layers in the plate holder for absorbing the decanted ethanol) Note: Start timing when the rotor starts moving.
- 8.Add 60 μ L of 70% ethanol to each well
- 9.With the centrifuge set to 4°C, spin at 1650 g for 15 min.
- 10.Invert the plate and spin up to 185 × g for 1 min, then remove from the centrifuge. Note: Start timing when the rotor starts moving.
- 11.To continue, resuspend the samples in the injection buffer (10 μ L HiDi Formamide), cover with septa, denature, snapchill and proceed for electrophoresis. To store, cover with aluminium foil, and store at 4 °C.

SANGER SEQUENCING

Summary

Sample Files	Sample Files With QV	Low QV	Med QV	High QV
8	8	< 15	>= 15 and < 20	>=20

Legend: Success Success with warnings Failed Analysis System Error

Length of Read (LOR): AverageQV of 20 bases >= 20

Low LOR = 0-300	Medium LOR = 301-500	High LOR = 500+
Samples with low LOR = 5	Samples with medium LOR = 0	Samples with high LOR = 3

Sample Details

Sample File Name	BC Status	PP Sta tus	W ell	C ap #	Pea k 1	Bas e Sp aci ng	# Lo w QV	# Me d QV	# Hig h QV	S am ple S core	L O R	'A'	'C'	'G'	'T'	Av g S/ N	C R St ar t	CR Stop
A11_1_01		N/A	A1	1	1631	-13.2	5	0	0	0	0	4	5	4	4	4	N/A	N/A
B11_2_02		N/A	B1	2	1631	-13.2	5	0	0	0	0	6	8	6	5	6	N/A	N/A
C11_3_03		N/A	C1	3	1243	11.55	51	15	579	52	645	36	33	30	37	34	N/A	N/A
D11_4_04		N/A	D1	4	1631	-13.2	5	0	0	0	0	5	6	5	5	5	N/A	N/A
E11_5_05		N/A	E1	5	1309	13.25	74	24	481	43	567	14	17	14	12	14	N/A	N/A
F11_6_06		N/A	F11	6	1631	-13.2	5	0	0	0	0	6	10	6	6	7	N/A	N/A
G11_7_07		N/A	G1	7	1819	13.25	109	6	0	8	0	613	132	419	461	704	N/A	N/A
H11_8_08		N/A	H1	8	1981	13.25	118	35	435	34	562	9	15	8	9	10	N/A	N/A

Errors

Sample File Name.	Step Name.	Description.
A11_1_01	BaseCalling	Analysis failed due to poor data quality : (20323)Failed estimation of spacing curve: : (20327)Failed estimation of width curve: : (30335)Signal strength too low for analysis:
B11_2_02	BaseCalling	Analysis failed due to poor data quality : (30335)Signal strength too low for analysis:
D11_4_04	BaseCalling	Analysis failed due to poor data quality : (30335)Signal strength too low for analysis:
E11_5_05	BaseCalling	Analysis succeeded with warnings : (20327)Failed estimation of width curve:
F11_6_06	BaseCalling	Analysis failed due to poor data quality : (30335)Signal strength too low for analysis:
G11_7_07	BaseCalling	Analysis succeeded with warnings : (20323)Failed estimation of spacing curve: : (20327)Failed estimation of width curve: : (20337)Analysis stopped due to low signal condition: : (near raw scan 5716) : (20337)Analysis stopped due to low signal condition: : (near raw scan 3543)
H11_8_08	BaseCalling	Analysis succeeded with warnings : (20323)Failed estimation of spacing curve: : (20327)Failed estimation of width curve:

RESULT

SAMPLE	IDENTIFIED AS
1	
2	
3	Rhizopus oryzae
4	
5	Fusarium solani
6	
7	
8	Aspergillus versicolor