Month : July

FUNGUS IDENTIFICATION

WEEK 2

Date : 07.07.21

PCR OF 1 SAMPLE

Protocol:

Taq buffer	5 uL
MgCl2	1 uL
dNTP	1uL
Taq Polymerase	1 uL
10uM FP	1 uL
10uM RP	1 uL
Template(120 ng/uL)	3 uL
Water	8.5 uL
Total	50 uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	165.5	1.66	1.24

NO BAND OBSERVED

Date : 10.07.21

PCR CLEAN UP OF SAMPLE 1

PROTOCOL :

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
2. Place a QIAquick column in a provided 2 ml collection.
3. 3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60s.
4. Discard flow-through and place the QIAquick column back in the same tube.
5. To wash, add 600 µl Buffer PE to the QIAquick column & centrifuge for 30–60 s.
6. Discard flow-through and place the QIAquick column back into the same tube.
7. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
8. Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
9. To elute DNA, add 25 µl Buffer EB (10 mM Tris·Cl, pH 8.5) at 70 degree Celsius or water (pH 7.0–8.5) to the center of the QIAquick membrane let the column stand for 1 min in water bath at 70 degree and centrifuge the column for 1 min. For increased DNA concentration, add 25 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min and then centrifuge.

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	24.8	1.29	0.89

Date : 12.07..21

PCR OF 1 SAMPLE

Protocol:

Master Mix	10 uL
10mM FP	0.5 uL
10mM RP	0.5uL
Template(100-150) ng/uL	1 uL
Water	8.5 uL
Total	20 uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	431.6	1.81	2.13

NO BAND OBSERVED

Date : 12.07.21

PCR CLEAN UP OF SAMPLE 1

PROTOCOL :

1.Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
2.Place a QIAquick column in a provided 2 ml collection.
3.3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60s.
4.Discard flow-through and place the QIAquick column back in the same tube.
5.To wash, add 600 µl Buffer PE to the QIAquick column & centrifuge for 30–60 s.
6.Discard flow-through and place the QIAquick column back into the same tube.
7.Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
8.Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
9.To elute DNA, add 25 µl Buffer EB (10 mM Tris·Cl, pH 8.5) at 70 degree Celsius or water (pH 7.0–8.5) to the center of the QIAquick membrane let the column stand for 1 min in water bath at 70 degree and centrifuge the column for 1 min. For increased DNA concentration, add 25 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min and then centrifuge.

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	15.7	1.58	1.68

Date : 12.07.21

PCR OF 6&7 SAMPLE

Protocol:

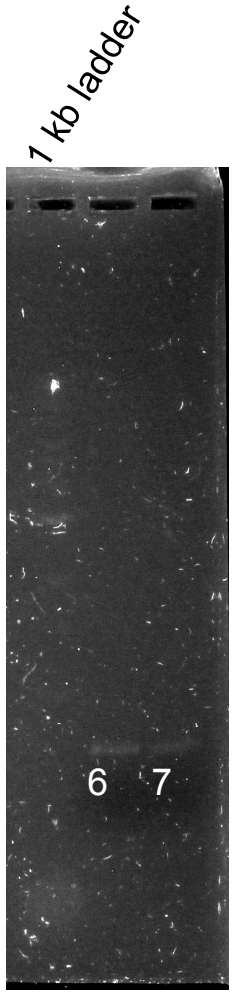
Master Mix	12.5 uL
10uM FP	0.625 uL
10uM RP	0.625uL
Template(250 ng/uL)	3 → 1.86 uL 4 → 0.86 uL
Water	3 → 9.39 uLuL
Total	25uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
6	375.5	1.82	2.18
7	379.4	1.83	2.08



Date : 13.07..21

PCR OF 6&7 SAMPLE

Protocol:

Master Mix	10 uL
10uM FP	0.5 uL
10uM RP	0.5uL
Template(100-150) ng/uL	1 uL
Water	8.5 uL
Total	20 uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

Date : 13.07..21

PCR OF 2&4 SAMPLE

Protocol:

Master Mix	12.5 uL
10uM FP	0.625 uL
10uM RP	0.625uL
Template(250 ng/uL)	2 → 2.2ul 4 → 0.97uL
Water	2→ 9.05 uL 4 → 10.28 uL
Total	25 uL

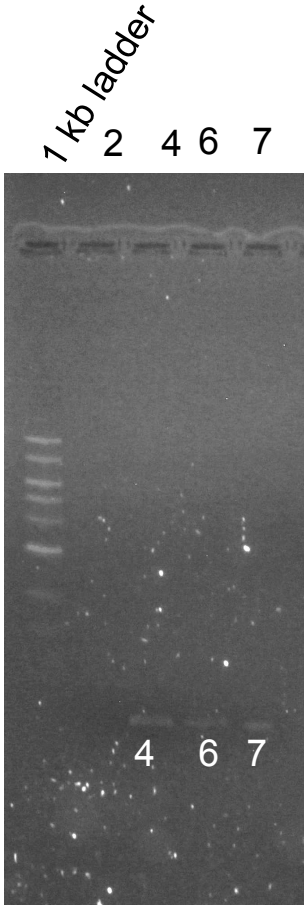
Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
6	366.4	1.83	2.12
4	425.2	1.83	2.12
2	374	1.84	2.12
7	390.3	1.84	2.08

BAND FOR 2ND SAMPLE IS MISSING



Date : 14.07..21

PCR OF 2&4 SAMPLE

Protocol:

Master Mix	10 uL
10uM FP	0.5 uL
10uM RP	0.5uL
Template(100-150) ng/uL	1 uL
Water	8.5 uL
Total	20 uL

Reaction:

95℃	5 min	
94℃	30 s	X 35
50℃	30 s	
72℃	1:30 min	
72℃	10 min	
4℃	hold	

RESULT

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
2	375.5	1.82	2.18
4	379.4	1.83	2.08