MAY

WEEK 4

Date : 25.05.21

PCR OF 1&7 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	

Sample No.	Conc. (ng/uL)	A260/A280	A260/A230
1	408.4	1.82	2.16
7	391.3	1.81	2.18

Date : 25..05.21

INOCULATION FROM 4 SLANT PDA (C1,9,16,18,19) TO PDB

Protocol:

1.Cleaned all work surfaces with 70% ethanol solution.

2. Flame an inoculating loop.

3.Using the cooled loop pick up a small quantity of the culture from the slant PDA containing the sample and addit in a 25mL autoclaved PDB+ amp.

4. Warm the mouth of PDB containing conical flask and close it and keep it in incubator cum shaker at 28 degree & 150 rpm.

5..Wipe the laminar air flow with 70% ethanol solution

Result:
After 3-4 days mycelium mat/ beads are observed.

Date : 28.05.21

PCR CLEAN UP OF SAMPLE 1,3,4,7

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.

Date : 29.05.21

FILTRATION OF MYCELIUM (C1,9,16,18,19)

Protocol:

1.Autoclave beaker, funnel, conical flask, forceps, Eppendorf, beaker, Eppendorf stand,tissue paper one day before.

2.Keep everything in UV for 15 mins and wash hands and wear gloves and wipe hands & laminar airflow surface area with ethanol.

6.Label the 1.5ml Eppendorf tubes. And take Whatman filter paper No.1

8.Warm the neck of the funnel & conical flask.

9.Put filter paper on the funnel and put it on a conical flask (250mL)

10.Filter the mycelial beads from culture broth using Whatman No. 1 filter paper.

11.Dry the mycelium very properly using tissue paper press it hard, dry as much as you can

12.Let all the broth liquid pass through.

13.Wipe the forceps with ethanol and heat them and then let it cool down

15.Collect the mycelium mat from filter paper dry it as much as you can and transfer it to the Eppendorf.

16.Heat the neck of the conical flask and add ethanol to it and then autoclave it & then it can be discarded.

Result:

Dried mycelium is collected.

Date : 30.05.21

ISOLATION OF GENOMIC DNA: CTAB (C1,9,16,18,19)

Protocol:

1.Take amount of sample in a 1.5 ml microcentrifuge tube
2.Keep the sample in liquid nitrogen for a few seconds.
3.Grind well the sample in 200 µL CTAB buffer, using a micro pestle.
4.Incubate at 65^0C for 5- 8 minute in a water bath
5.Allow to cool. Add equal volume of 24:1 (Chloroform: Isoamyl alcohol)
6.Mix gently. Centrifuge at 13000 rpm for 10 minutes.
7.Transfer aqueous phase to a new Eppendorf tube.
8.Add 2/3 volume of ice cold Isopropanol.
9.Incubate at room temperature for 20 minute
10.Centrifuge at 13000 rpm for 20 minute at 4^0C
11.Wash the pellet with 70% Ethanol, air dry for 30 minutes
12.Re-suspend the pellet in 50 µL 0.1 X TE buffer and store in -20^0C for further analysis.

NANODROP RESULT

S.No.	Concentration	A260/A280	A260/A230
1	507.8	1.53	0.65
9	80.7	1.54	0.62
16	54.8	1.93	0.64
18	217.7	1.46	0.73
19	137.4	1.31	0.45

Can not do PCR with this as it has RNA contamination