JUNE WEEK 3

PCR CLEAN UP OF SAMPLE 1,2*,3,4

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 μ I 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.

NANODROP RESULT

S.No.	Concentration	A260/A280	A260/A230
1	6.2	1.72	1.28
2*	13.9	1.75	1.70
3	25	1.61	1.26
4	29.2	1.68	0.99

OBSERVED C4 UNDER BRIGHT FIELD MICROSCOPE



OBSERVED C8 UNDER BRIGHT FIELD MICROSCOPE





OBSERVED C19 UNDER BRIGHT FIELD MICROSCOPE





OBSERVED C1 UNDER BRIGHT FIELD MICROSCOPE









OBSERVED C4 UNDER CONFOCAL MICROSCOPE



OBSERVED C8 UNDER CONFOCAL MICROSCOPE











OBSERVED C1 UNDER CONFOCAL MICROSCOPE



