HHMI Laboratory: Genomic DNA/PCR Lab

YOU NEED TO CENTRIFUGE YOUR GENOMIC DNA, RUN A GEL TO DEFINE THE QUALITY, MEASURE THE QUANTITY USING A SPECTROPHOTOMETER AND SET UP A PCR REACTION.

A: POPULATION GENETICS: QC DNA AND PCR

1) Start centrifuging your DNA in isopropanol and salt before presentations.

2) After centrifugation, pour of isopropanol.

3) To wash DNA, add, 500ul of 70% Ethanol,

4) remove all Ethanol

6) add 100 ul of T. E. to DNA. Vortex to make sure DNA is in solution. If the solution is cloudy, centrifuge again for 3 minutes, max speed to sediment any non-soluble material.

B: Divide and Conquer

Get a 96-well plate. Divided it in fourths (3 rows of 12 wells: 1, 2, …12).
for each sample you want

10 ul for Spectrophotometery

10 ul for gel electrophoresis

You will use 5ul for the PCR reaction

IMPORTANT: you need to know the order of your samples. Please take good notes, so you know which samples are in which well

C: SPECTROPHOTOMETRY Two individuals per group
Two persons from each group, go use the spectrophotometer (NanoDrop).
Save one "Scan (310-230nm) for your presentation.

Record DNA 1) concentration, and the absorbance at 2) 260 and 3) 280.

How much DNA do you have? What is the 260 to 280 ratio? Why is it important?

D: GEL ELECTROPHORESIS Two individuals per group.
Each sample of DNA will be electrophoresed

Add 10ul of Red loading dye for each sample

Put 10 ul of DNA into "plate-wells" (part of 96 well plate).

Load sample into gel. DNA runs to positive (Black to Red)

After electrophoresis
Two different people from you group, take picture and
Print and save TIF image (you need the image for your presentation, for your final written report.

E: PCR.

1 no DNA (negative control) 2: Your DNA samples
Put your samples in "plate wells". 1, 2, 3, … 10 in ROW with letter A OR B OR C ….

1. 20 ul of Master mix with TAQ, nucleotides (dATP, dGTP, dCTP, dTTP), Mg++ and buffer.
2. 5 ul of DNA (?? How much DNA??)
3. 2 ul of Primer Mix (either "Vertebrate" or "Invertebrate" primers)
4. Water to equal total volume of 50 ul (i.e. add 23 ul).
5. Keep on ice until we are ready to put into thermal cycler.

Thermal cycler

1cycle 94°C, 20 sec, 42°C, 20 sec, 72°C for 1 minute: repeat 35 times.