Species Identification Protocol for Sharks

Be sure to have the following supplies handy:
Forceps, razor blade, 5% bleach solution, distilled water, paper towels, 1X TBE, agarose, HotstarTaq Master Mix, PCR primers, tube strip filled with 200µL of 10% Chelex.

DNA Extraction:

1. Using forceps and razor blade cut a small (~1mm x 1mm) piece of fin and place it into a labeled tube containing 200µL of 10% chelex.
2. Place forceps in bleach solution to clean and then de-ionized water to remove bleach and then dry with paper towel.
3. Repeat steps 1-2 until complete with all samples.
4. Close strip tube lids and briefly centrifuge.
5. Place strip in a PCR machine or heat block and incubate at 60°C for 20 minutes then 103°C for 25 minutes.
6. Briefly centrifuge samples and store at 4°C

PCR:

*** note: Be sure to use the appropriate primer mix (e.g. Group ID, Hammerhead ID, Thresher ID, or Misc Shark ID)**

1. Make up a mastermix of Hotstar Master Mix, primers mix, and water. Per reaction add 5µL Hotstar Master Mix, 2.75µL distilled water, 1µL Coral Load buffer, and 0.25µL of the primers mix (10µM each primer).
2. Aliquot 9.5µL of this mix to each PCR tube.
3. Add 1.0µL of Chelex DNA extraction to the appropriately labeled tubes.
4. Close strip tube lids, vortex briefly and centrifuge briefly.
5. PCR cycle as follows: 95°(5:00),[94°(30 sec), 63°(60sec), 70°(90sec)]35 cycles
6. Briefly centrifuge samples and store at 4°C

Preparation of Agarose Gel:

1. Weigh 4g of agarose and add to glass jar
2. Measure 200ml of 1X TBE and add to glass jar
3. Mix briefly, remove orange cap, and then microwave at high power for 2 minutes. If the agarose is not completely melted it can be heated for 20 more seconds.
4. Secure plates on both ends of the gel plate and place combs in the gel
5. Pour the melted agarose into the gel tray until the gel is ~ 5mm thick.
6. After the gel has solidified (~15 min), remove the combs and remove the plates on the end of the gel plate

Mini-gel:

1. Load 6µL of the GelPilot DNA ladder into the first (left) well of the gel.
2. Load 5µL of PCR product into an empty well.
3. Run gel for 30 minutes at 120 volts.
4. Remove gel from the tray and place into the gel staining container.
5. Add 100ml of 1x TBE to the container and 4µL of Gel Red DNA stain and gently mix.
6. Leave the gel in the container with the stain for ~ 30 minutes
7. Remove gel from the container and place on transilluminator.
8. Place camera hood over the transilluminator, turn the power on, and take a picture of the gel.
9. Compare bands to the standard to infer species identification.

Preparation of 10% Chelex for DNA extraction

1. Weigh 5g of dry Chelex 100 Resin (Biorad www.biorad.com part # 142-1253)
2. Measure 45ml of distilled water
3. Combine chelex and distilled water in a clean container and add a small magnetic stir bar.
4. Place container of Chelex solution on a stir plate and stir at a slow to medium speed.
5. While the solution is stirring, pipette 200µL into 0.2ml strip tubes until you have a sufficient # of tubes for your DNA extractions.
6. When done remove the chelex solution from the stir plate and store at 4°C.

Preparation of 10X TBE

1. Open prepackaged 10X TBE package and pour into a 1L container.
2. Add distilled water to the 1L mark on the container and add a magnetic stir bar.
3. Close container, shake briefly, and stir on stir plate at a medium speed until the TBE powder is dissolved
4. Store at room temperature

Preparation of 1X TBE

1. Measure 50ml of 10X TBE and pour into a 500ml container
2. Add distilled water to the 500ml mark on the container, close, and shake briefly.
3. Store at room temperature

Chemicals and equipment used in this course (or similar):

PCR machine (FotoDyne) www.fotodyne.com Multigene Mini 48-Well Thermal Cycler E8-7003 (24 0.2ml tubes) $2895

Chelex 100 resin 50g (Biorad www.biorad.com part # 142-1253) $180 (~$0.07/sample)

Equipment for gels (Digital Camera, 2 mini centrifuges, transilluminator, gel box, power supply, 8 pipettors) (FotoDyne – E2-17) www.fotodyne.com $6340

HotstarTaq Plus Master Mix 2500 (Qiagen part#203645) www.qiagen.com $633 (~$0.03/sample)

PCR Primers (Integrated DNA Technologies) www.idtdna.com ~$70 for all primers ($0.01/sample)

GelPilot 100bp plus ladder – 100 lanes (Qiagen part# 239045) www.qiagen.com $146 ($1.27/ gel)

TBE Buffer 10X Ready Pack (ISC Bioexpress part# 0478) www.bioexpress.com $33.50 (1.68/ liter 1X TBE)

GelRed DNA stain 10000x in water 0.5ml (Phenix Research part# RGB-4103) www.phenixresearch.com $104.90 (~$0.17/ gel)

*note: this is a non-toxic DNA Stain, Ethidium Bromide is more commonly used because it is much less expensive but it is also toxic.

PCR strip tubes – 125 strips of 8 tubes (ISC Bioexpress part# T-3013-1) www.bioexpress.com $159.67 ($0.16/tube)

GenePure LE Quick Dissolve Agarose (125g) – (ISC Bioexpress part# E-3119-125) www.bioexpress.com $163.75 (~$0.94/ gel)

1-200µL Pipette Tip 10 wafers of 96 (ISC Bioexpress part# P-4146-5) www.bioexpress.com $51.59 (~$0.05/ tip)

Approximate cost per sample for consumables ~$0.60 USD