**PCR Protocol Supplies for Forensic PCR**

**Materials needed per class (assumes 4 student per group, 7-8 groups per class)**

**8 groups x 5 PCRs/group = 40 PCR reactions. Make 50 PCR reactions/class for safety.**

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| **If you make your own master mix and primer mix: Use 25 µL master mix, 15 µL primer mix and 10 µL DNA in each reaction. Each PCR reaction volume is 50 µL.**Make a master mix for 300 samples (should be plenty for 6 classes) as follows:1.5 ml 10X Reaction Buffer300 ul 10mM dNTPs900 ul 50mM MgCl275 ul Taq Polymerase4.8 mL sterile DH2OAliquote mix into 6-12 tubesMake a primer mix for 300 samples (should be plenty for 6 classes) of the two primers:300 ul 10uM pUC19 Left Primer300 ul 10uM pUC19 Right Primer3.9 ml H2OAliquot mix into 6 to 12 tubes |

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| **If you purchase pre-made master mix, it comes in 2x concentration. Use 25 µL master mix, 15 µL primer mix and 10 µL DNA in each reaction. Each PCR reaction volume is 50 µL.** Aliquot 2X master mix, 1 mL or 0.5 mL in each of 6 to 12 tubesMake a primer mix for 300 samples (should be plenty for 6 classes)300 µL of 10 µM pUC19 Left Primer300 µL of 10 µM pUC19 Right Primer4.5 mL sterile distilled H2OAliquot mix into 6 to 12 tubes. |

**To prep PCR forensics DNA samples add the following:**

Make final concentration of pUC19 DNA to be 0.1pg/µL (.1 ng/mL)

+ control: 100 µL DNA

-control: 100 µL distilled water

Food 1: 100 µL distilled water

Food 2: 100 µL DNA

Food 3: 100 µL distilled water

NOTE: You can create any desired pattern of contamination

For each PCR, add the following in a 0.2 ml PCR tube:

25 ul Master Mix

15 ul Primer Mix

10 ul DNA sample (0.1 pg/ul pUC19 or H2O)

Thermal cycler conditions:

94C 3 minutes

94C 30 seconds

65C 30 seconds x 35 cycles

72C 30 seconds

72C 7 minutes

4C 

The pUC19 product is 511 bp.

Supplies per class (enough for 10 lab groups)

50 sterile PCR tubes, 10 of each color

10 PCR tube racks

10 ice buckets

10 sharpies

Gloves

Eye protection

p-20 micropipets (number depends upon lab setup)

p-200 micropipets (number depends upon lab setup)

2 0.5 mL tubes Master mix (if using stations. If set up in individual kits, 10 50µL tubes)

2 0.5 mL tubes PCR mix (if using stations. If set up in individual kits, 10 50µL tubes)

1 0.2 mL tubes + control DNA (if using stations. If individual kits, 10 20µL tubes)

1 0.2 mL tubes - control DNA (if using stations. If individual kits, 10 20µL tubes)

1 0.2 mL tubes Sample 1 DNA (if using stations. If individual kits, 10 20µL tubes)

1 0.2 mL tubes Sample2 DNA (if using stations. If individual kits, 10 20µL tubes)

1 0.2 mL tubes Sample3 DNA (if using stations. If individual kits, 10 20µL tubes)

PCR machine

PCR machine grid

10 electrophoresis kits (power supply, gel tray, gel box, comb, loading dye)

1 tube 100 bp ladder (if teacher pipets the ladder. If not, one tube per lab kit)

DNA Stain (ethidium or Carolina blue or other)

Gel camera (suitable for the type of DNA stain used)