Workshop testing w/ Romie Tue 22 Jan 2013

Goal: run a successful PCR from QuickExtract-Isolated gDNA. Testing all old Genelaser primers.

EpiBio Quick Extract datasheet suggests the final QE solution contains 2-14 ng / uL gDNA and recommends 5 uL of solution in a 50 uL PCR reaction.

Bioneer AccuPower PCR PreMix datasheet suggests adding 5 - 50 ng template DNA in the PCR and 5 - 10 pMole primer to the 20 uL reaction volume.

PCR Mix

1 lyophilized Bioneer AccuPower 20 uL PCR PreMix
1 uL 20 uM Primer Mix
3 uL QuickExtract gDNA
15 uL ddH2O

Sample	Source	Primers set
1	QuickExtract Mac - 1	rs713598
2	QuickExtract Mac - 1	rs7903146
3	QuickExtract Mac - 1	rs3057
4	QuickExtract Mac - 1	rs4680
5	QuickExtract Mac - 2	rs713598
6	QuickExtract Mac - 2	rs6152
7	QuickExtract Mac - 6 uL QE DNA, 12 uL ddH20	rs713598
8	QuickExtract Mac - 2	none - control

PCR Thermocycling Protocol

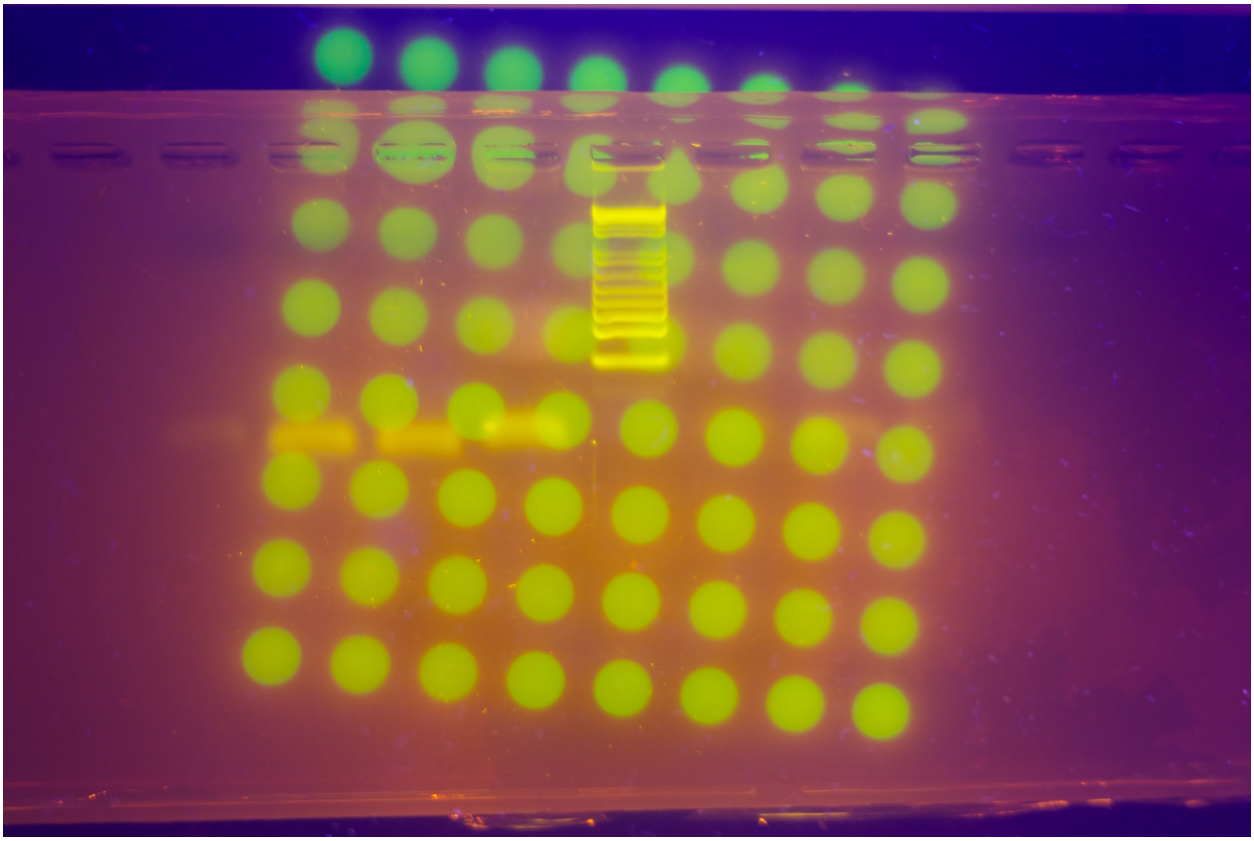
Initial Denature - 5 mins @ 95 C (Note: inserted tubes when block was at 90 C)
30 cycles:
    30s @ 95 C
    60s @ 55 C
    60s @ 72 C
Final extension - 5 mins @ 72 C
Lid: 110 C

2% Agarose Gel, 50 mL

50 mL 1X TBE
1g Agarose
- microwave until clarified

10 uL of 10,000x GelGreen - 2x normal ratio for extra staining!

Results



Sample 1 is visible to the left of the LED array, followed by samples 2-4. A 100 bp ladder is visible in the middle, followed by samples 5-8. There appear to be only primer bands at the middle of the gel, with no other PCR products visible. Interestingly, samples 5-8 present less intense bands than 1-4.