Cortisol Analysis and Receptor Genotyping in the Oswego Student Meditation Study

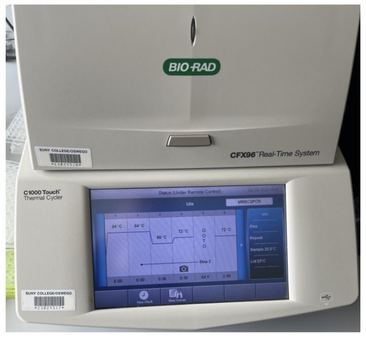
Devon Seale, Drs. Bendinskas, Reid, Wolford
Departments of Chemistry & Psychology, SUNY Oswego, NY

Introduction

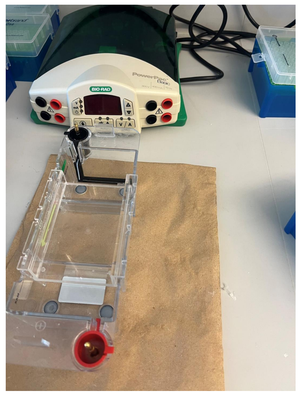
The information about SNP and hair cortisol concentration measurements will help assess the physiological stress in the meditation study participants. A type of nucleotide present (T/C) human genome at SNP rs1360780 atFKBP5 gene can affect how cortisol is "perceived" by its receptor. This study focused on developing methods that would allow researchers to determine the nucleotide (T/C) present at SNP rs1360780. We started by amplifying the sequence and checking the melting temperature of the amplicon. Then we changed course, began purifying and amplifying DNA, and sent samples to the Cornell Sequencing Core to find the present SNP.

Methods

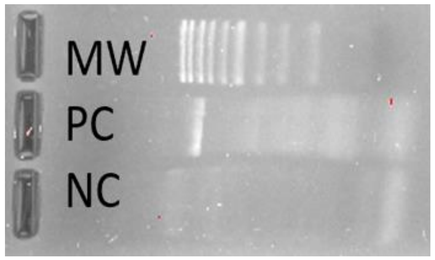
A forward primer, a reverse primer, the DNA template, and nuclease-free water were used to make each sample run through PCR. The PCR settings used for most of the work done included the following cycles: start cycle of 95 ºC for 3:00, then a three-step cycle of 95 ºC for 0:30, then 50 ºC for 0:30, then 72 ºC for 1 minute. After that, the step cycle was repeated 30 times. Samples were run through electrophoresis using a 2.0% agarose gel in 1xTBE for 90 minutes.



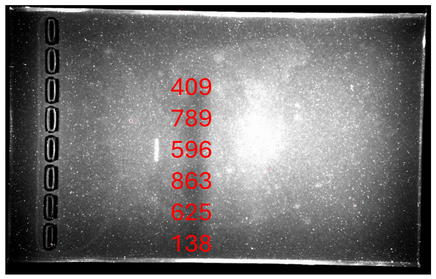
CFX96 real-time system C-1000 thermal cycler: used to gather real-time amplification data as well as the melting curve.



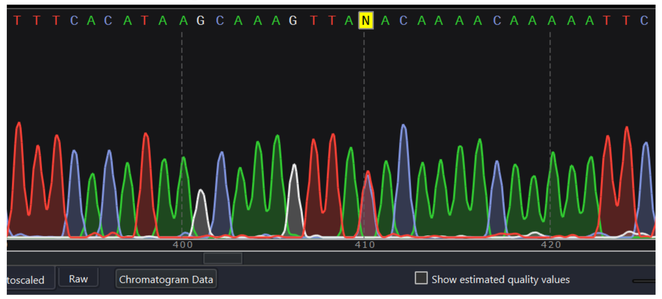
Electrophoresis chamber and power supply. After the gel has been set, it can be placed in the chamber and submerged in 1x TAE buffer before running.



First successful target amplification:
From top to bottom: 25-1000 weight ladder,
F2R2 positive control (760 bp),
F2R2 negative control (no DNA added).

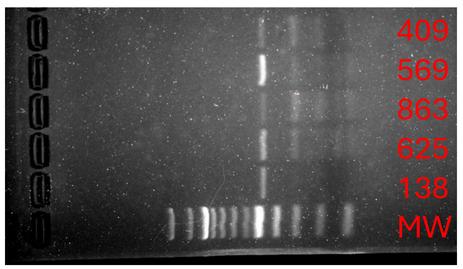


First attempt at amplifying meditation samples:
Bottom to top: 138, 625, 863, 596, 789, 409
The only lane with clear banding is the lane with 596 DNA

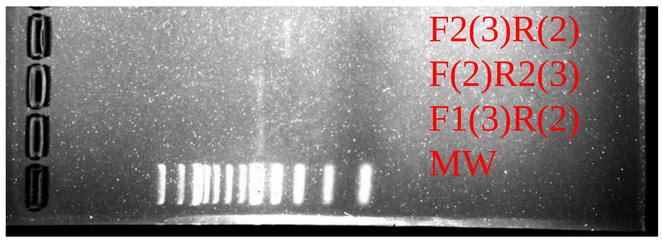


F primer chromatogram:
N is the target allele in subject X DNA. It is 50% C: 50%T; C:T and C:C are common and TT is more rare.

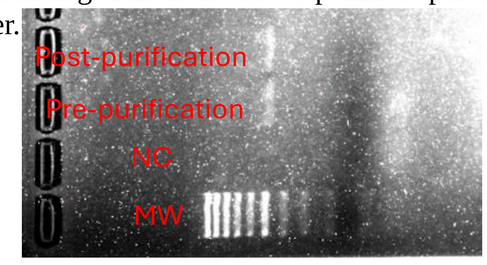
R primer chromatogram:
Allele can be seen at 446 in the sequence file and shows A:G as it is the reverse complement to what is being amplified with the forward primers



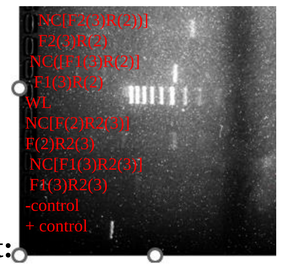
Bottom to top: MW, 138, 625, 863, 569, 409
good amp in 138, all other DNA types amplified with secondary banding. This is the last experiment performed this summer.



Bottom to top: MW, F1(3)R(2), F(2)R2(3), F2(3)R(2)
This is another experiment done to find the most ideal set of primers to use on meditation samples to send to Cornell



Bottom to top: MW, NC, S(not purified) , S(purified)
This gel shows the successful purification of a single band of amplicon. bands are of similar brightness and it seems that the purified sample has less dust at the 100 range than the unpurified.



Primer selection experiment:
This is the first experiment done to find the most ideal set of primers to use on meditation samples to send to Cornell

References

1. Koenig, Alexandra M., et al. "Intergenerational gene× environment interaction of FKBP5 and childhood maltreatment on hair steroids." *Psychoneuroendocrinology* 92 (2018): 103-112.
2. Ising M, Maccarrone G, Brückl T, Scheuer S, Hennings J, Holsboer F, Turck CW, Uhr M, Lucae S. *FKBP5* Gene Expression Predicts Antidepressant Treatment Outcome in Depression. *International Journal of Molecular Sciences*. 2019; 20(3):485. https://doi.org/10.3390/ijms20030485
3. Zannas, Anthony S., et al. "Gene–stress–epigenetic regulation of FKBP5: clinical and translational implications." *Neuropsychopharmacology* 41.1 (2016): 261-274.