RIESEBERG LAB SSR PCR PROTOCOL

Adapted by Kate Ostevik September 4, 2009

1. Prepare PCR Master Mix (MX)

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| PCR MX without M13 tails:  11.1 μL ddH2O  1.5 μL 10X PCR Buffer  0.3 μL dNTP (10mM)  0.5 μL Reverse Primer (10μM)  0.5 μL Forward Primer (10μM)  0.1 μL Taq Polymerase | PCR MX with M13 tails:  11.05 μL ddH2O  1.5 μL 10X PCR Buffer  0.3 μL dNTP (10mM)  0.5 μL Reverse Primer (10μM)  0.05 μL Forward Primer (10μM)  0.5 μL M13 Primer  0.1 μL Taq Polymerase |

* ALWAYS keep the Taq on ice
* Make enough MX for the number of reactions you will be setting up + 4 to account for pipetting error
* Vortex all reagents before adding EXCEPT FOR TAQ
* Add H20 first and Taq last
* You need to have designed your primers with M13 tails in order to tag your fragments with the M13 dyes

1. Aliquot 14 μL of PCR MX into tubes
2. Add 1 μL of DNA template to samples (DNA concentration should be between 20-100 ng/μL)