Steps for Locus Development

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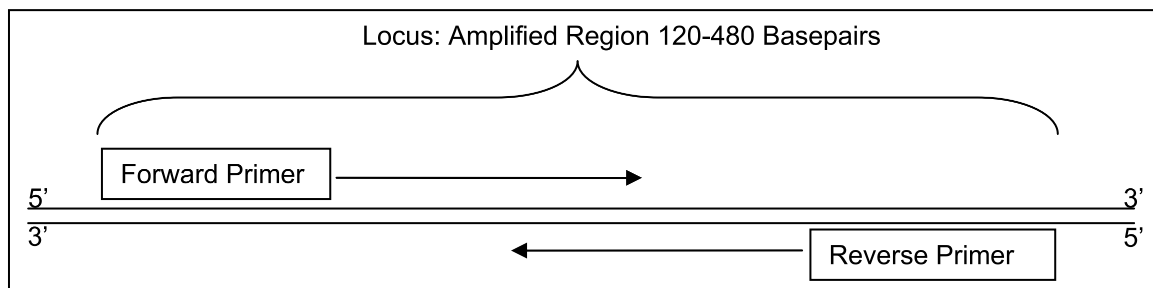
1. PCR clone
2. Sequence clone with forward and reverse primers (2 sequences)
3. Check sequence for microsatellites
    a. Dinucleotide: 5 or more perfect repeats
    b. Trinucleotide: 4 or more perfect repeats
    c. Tetranucleotide: 4 or more perfect repeats
4. Check sequence for accuracy
5. Check for vector sequence and delete any vector
6. BLAST sequence (this will help find any leftover vector, too)
7. Do a local BLAST from BioEdit, using your own database, to make sure you don't already have this particular clone
8. Design primers (can use online 'Primer3' site if you want)
    a. Region must have low repetitiveness and good sequence
    b. Usually at least 50% GC
    c. 3' end has at least one G/C
    d. At least 20bp long
    e. Tm 60°C-65°C (melting temperature, can check with IDT)
    f. Amplified region greater than 120bp
    g. No guanidine on 5' end of reverse primer (quenches fluorescence)
    h. No single nucleotide repeats more than 3 (AAAA etc)
    i. Run primer through IDT's Oligo Analyzer (http://scitools.idtdna.com/Analyzer/)
        i. Check for hairpins, self-annealing, hetero-dimer



9. Test primers for amplification on 6-12 samples (or genotype mix)
    a. Use the gradient block, 5°C on either side of Tm (less necessary if using HotStarTaq)
    b. Run on 1-2% agarose gel
10. Test positive primers for polymorphism and null alleles
    a. Amplify on 6 samples from each set (BRSY=OR/USDA, *Piri*=C,V,H)
    b. Run on 4% metaphor gel (read instructions!)
11. For loci that amplify consistently, order fluorescent primers
    a. 6-FAM, HEX or TAMRA on 5' end of Reverse primer, 100nmol scale