HAVEY'S LAB MICROSATELLITE PROTOCOL

SOME PRIMERS "TAILING" BACKGROUND:
Attach ABI forward sequence to either forward or reverse primer of microsatellite pair (to the shortest sequence of primer pair to save few cents). The last three bases of ABI forward primer are ...*GCC*, if microsatellite primer starts with let's say *CC*, then erase these two *CC*, because they are already present at the end of the ABI forward sequence (we also save few cents here). ABI forward sequence is (20 mer) 5-3' (this sequence brought Mike from San Diego's 2004 meeting, P227: A low cost high efficient method for genetic mapping using indel markers, page 128, 2004):

5′ GACGTTGTAAAACGACGGCC 3′

Label 5' end of this primer with FAM. In PCR use 0.2 uM concentration of ABI forward (FAM labelled), 0.2 uM concentration of normal length microsatellite primer and 0.05 uM concentration of microsatellite-tailled primer (with ABIfor sequence). Use 30 cycles, volume of reaction is 15 ul. Original protocol was published in Nature Biotechnology 5 years ago with M13 tails (Markus Schuelke. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18, 233-234). Group in our building tailed few primers with M13 sequence but they didn't work for them. Stick to ABI forward tailing!

Procedure (15 ul volume, 40 ng DNA):
-Dilute DNA to 8ng/ul, take 5 ul to yield 40 ng of DNA er reaction.
-Prepare MIX containing:
1) 1x PCR buffer (1.5 ul, it has to contain $MgCl_2$)
2) ABIforward FAM labeled primer to 0.2 uM (e.g. if you have 10 uM stock take out 0.3 ul)
3) Microsatellite specific tailed primer 0.05 uM (0.075 ul)
4) TAQ DNA polymerase 0.8 ul
5) dH_2O up to 10 ul, mix well add to each sample 10 ul of PCR mix.

Cycling conditions:
Recommended starting point:
95°C 5 min, then 30 cycles of 94°C 45 sec annealing temperature 30 sec, 72°C 1 min 30sec, extension step at the end for 8 min at 72°C, 12°C until recovery.

This are recommendation which work well for majority of samples, sometimes you have to cycle for few cycles less or more (depending on peak heights), sometimes you have to use special blend of better TAQ DNA polymerase, higher $MgCl_2$ concentration.

Preparation of samples for analysis:

After PCR add 80 ul of dH_20, take out 2 ul, put in 96 well plates, mix with 10 ul of formamide (use ABI Hi-Dye formamide) and fluorescent marker Chimerx's GeneFlo 625 ROX labelled (prepare marker+formamide mix: 1 ml of formamide and 40 ul of marker), tape with foil, spin down, take over Biotech.

Results will be posted on FTP in GeneScan format, use program Genotyper to analyze the data. In the case that a lot of samples have to high peaks a lot of samples didn't inject or have shifted injection it is probably necessary to more dilute the PCR samples. Try several serial dilutions above 80 ul, run and chose the best dilution.