Quantitative Microsatellite Analysis (QuMA)

(Reference: Ginzinger DG, Godfrey TE, Nigro J, Moore DH 2nd, Suzuki S, Pallavicini MG, Gray JW, Jensen RH. Measurement of DNA copy number at microsatellite loci using quantitative PCR analysis. Cancer Res. 2000 Oct 1;60(19):5405-9.

I. Test PCR Efficiency of Reference Primers

Reference primers: D2S385
D3S1286
D3S1283
D3S1293
D3S1554
D11S1315
D12S1699
D21S1904

1. Thaw all reagents on ice, 10X buffer needs to be protected from light.

Master Mix – 96-well plate

	1X	110 X
10X Buffer	2 µL	220 µL
25mM MgCl2	2	220
25mM dNTPs	0.16	17.6
dH2O	7.54	829.4
20 µM probe (CA12.5 or 10.5)	0.2	22
5 U/µL Ampli Taq	0.1	11
	12	1320

Master Mix (MM)	12
DNA	4
Primers	4
	20

Set up:
27 x12µL MM = 324µL MM
8 x 4µL DNA = 108 µL MM

Pipet 16 up of above/well

2. After combining the master mix, pipet 324 µL MM into four microfuge tubes. Add 108 µL of the respective DNA dilutions, i.e. 1 (4ng), 1/10 (0.8 ng), 1/100 (0.16 ng) and 1/1000 (0.032 ng).
3. Make 4-fold dilutions of the std DNA. Starting with 150 µL @ 1ng/µL

DNA (µL)	dWater (µL)	Total Vol (µL)	Amt DNA (ng)
150	0	150	4
30	120	150	0.8
30	120	150	0.16

30	120	150	0.032

4. Dilute lyophilized primers in 1X TE to make 100 µM stock. Further dilute stock to 2 µM working stock (forward + reverse primer) in dwater.

i.e. 100 µM Forward primer = 2 µL
100 µM Reverse primer = 2 µL
distil water = 96 µL
100 µL of 2 µM Fwd+Rev working stock

5. Into each well using a repeator, pipet 16 µL MM + DNA mix and then 4 µL respective primer. Apply sealing film. Mix well and centrifuge at 1000 rpm for 5 mins.
6. Run appropriate PCR program.

PCR program for 10.5 CA Repeat (Shorter probe)

Stage 1: 95°C for 12 mins
Stage 1: 95°C for 20 sec
55°C for 30 sec
72°C for 30 sec
40X

PCR program for 12.5 CA Repeat

Stage 1: 95°C for 12 mins
Stage 1: 95°C for 20 sec
60°C for 30 sec
72°C for 30 sec
40X

Calculations
Example:

PCR Effficiency

Average Cts

Quantity	log	D11S1315	D3S1554	D12S1699	D3S1283	D3S1293	D3S1286	D21S1904	D2S385
1000	3.00	26.38	27.71614	25.82763	26.56695	**25.40224**	25.7119	26.65323	27.08798
200	2.30	29.01	31.12753	28.38162	29.02644	**28.02388**	28.22422	29.49981	30.16901
40	1.60	32.09	33.32945	31.50989	31.57286	**30.30248**	31.05193	32.94054	32.67458
8	0.90	34.02	35.53099	33.58813	34.20833	**30.97208**	33.42512	34.50719	34.76165
	Slope	3.7202	3.6692	3.7784	3.644	**2.7166**	3.7151	3.8632	3.652
	Efficiency	0.86	0.87	0.84	0.88	**1.33**	0.86	0.81	0.88

PCR Efficiency = 10^(1/slope)-1
Primers with PCR efficiency = 88%-105% are acceptable

Once primers to be used are determined, make a pool and repeat the efficiency test with the pooled primers and primer of gene of interest. The PCR efficiency of the pooled primers should also = 88%-105%

Proceed with experimental samples. Make sure to include normal DNA samples on your plate.

Calculations for DNA Copy Number

1. Calculate average Ct for all samples.
2. Subtract average Ct for the pooled reference from the average Ct of test sample to get Δ Ct.
3. Average the Δ Ct of all normal samples.
4. Calculate $\Delta\Delta$ Ct = Δ Ct(test)-Avg Δ Ct(Reference pool).
5. Relative DNA Copy # = $(2^{-\Delta\Delta Ct}) \times 2$