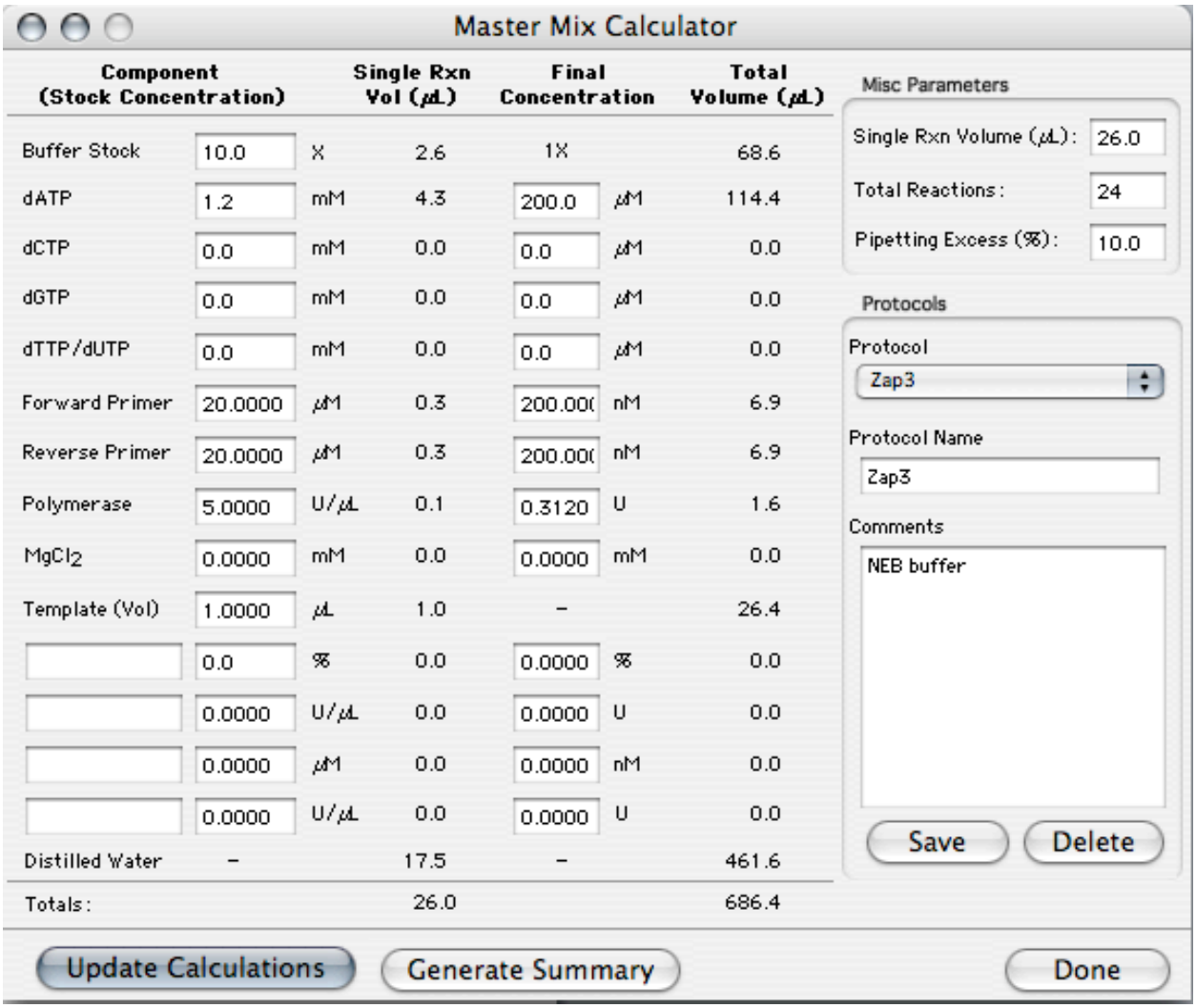
ZAP GENOTYPING PROTOCOL

GENE NAME: Z/AP (LacZ/hPLAP Reporter)
PCR PROTOCOL NAME: ZAP3 (NOT Zp3)

PCR REAGENTS: 10X NEB THERMOPOL PCR BUFFER
NEB THERMOPOL TAQ POLYMERASE
dNTPs (1.25 uM Standard Working Concentration)

PRIMERS: ZAP3 Forward (20uM Standard Working Concentration)
ZAP3 Reverse (20uM Standard Working Concentration)

REACTION VOLUME: 26 ul (25 ul + 1ul DNA)
Add Master Mix to DNA



THERMOCYCLER CONDITIONS:

95 C for 3 min

95 C for 30 sec
60 C for 30 sec  40 cycles
72 C for 2 min

72 C for 2 min
4 C forever

ELECTROPHORESIS CONDITIONS:

1% Agarose Gel (e.g. 1.5 g of Agarose per 150 ml of 0.5X TBE), 5ul EtBr
Use 20-well combs (not 30-well combs): 1 comb/gel
Add 4ul of Xylene dye per PCR reaction
Use 100 bp Ladder (5ul)
Load maximum volume per well
Run 80 V for 0.5 hour
Score absence (-) or presence (+) of a ~300bp product

COMMENTS: