Kolesnick Protocol for Acid Sphingomyelinase PCR for Genotyping *Asm*-/- Mice

The genotyping of acid sphingomyelinase (*Asm*) has been problematic for some time. Much of the issue involved dimerizing of the primers to each other, thereby competing with genomic DNA, resulting in lower quality PCR. Primer dimerization occurs when there are homologous base pairs at the 3' end of the primers.

We have improved on the acid sphingomyelinase PCR by first removing two base pairs from one of the primers; removing the homologous base pair. In addition, the PCR was optimized for the new primers.

Below are the three primers that we are now using in addition to the optimized PCR conditions. Below is a picture of what our PCR now looks like. As you can see, we get a clear result and no primer dimer at the bottom of the lanes.

Primers:

GGCTACCCGTGATATTGC

AGCCGTGTCCTCTTCCTTAC

CGAGACTGTTGCCAGACATC

PCR Mix (polymerase from clonetech, Cat# 6050085, Advantage 2 PCR kit) :

9.1ul water
1ul 10X buffer
0.2ul dNTP
0.2ul of each primer
0.1ul 100X enzyme

Cycling conditions:

94 degrees 3'
94 degrees 15"
64 degrees 30"
68 degrees 90"

35X, then 72 degrees 7'

If you have any further questions, please do not hesitate to contact me at lermang@mskcc.org.

Gabriel Lerman
For Richard Kolesnick