Protocol for geneotyping Itk-deficient mice using PCR

ASneo+: 5’ ATTGAACAAGATGGATTGCAC 3’
Asneo-: 5’ CGTCCAGATCATCCTGATC 3’
itk420: 5’ TGGGTGCTGACCCTTAAGAAG 3’
itkintron3a: 5’ GCCGTAATGAACAGGTGGTA 3’

Neo PCR

2 ul 10 x PCR (Perkin-Elmer, shipped with the enzyme)
2 ul RediLoad (Research Genetics; optional)
2 ul DMSO
3.2 ul 1.25 mM dNTP (1.25 mM each dA, dG, dC, dTTP)
0.79 ul 10uM ASneo+
0.79ul 10uM Asneo-
0.4ul Taq Polymerase (Perkin-Elmer)
7.82 ul dH2O
1 ul DNA (about 0.5-1 ug/ul)

Itk-WT PCR

2 ul 10 x PCR (Perkin-Elmer, shipped with the enzyme)
2 ul RediLoad (Research Genetics; optional)
2 ul DMSO
3.2 ul 1.25 mM dNTP (1.25 mM each dA, dG, dC, dTTP)
0.79 ul 10uM itk420
0.79ul 10uM itkintron3a
0.4ul Taq Polymerase (Perkin-Elmer)
7.82 ul dH2O
1 ul DNA (about 0.5-1 ug/ul)

PCR conditions:
If using PTC 100 from MJ Research: 93oC 30’’, 55oC 45’’, and 74oC 45’’ for 35 cycles followed with 74oC 3’ to finish up.
If using DNA Thermal Cycler 4800 from Perkin-Elmer: 93oC 45’’, 55oC 1’’, and 74oC 1’ for 35 cycles followed with 74oC 3’ to finish up.
The RediLoad is a DNA loading dye that does not interfere with the PCR reactions.
Including it in the reaction cocktail avoids addition of loading dyes to many samples later.

Do PCR in 96-well plate or in individual tubes with a total volume of 20 ul. Do the neo and itk-WT PCR separately and then take 10 ul from each reaction using the same DNA template, mix and load to a 1% agarose + 1% low-melting agarose gel (i.e. a 2% gel in fact).

Expected size:
475bp neo fragment (for most neo-containing knockouts, such as PMC1neo and PGKneo)
218 bp itk fragment for the wild-type itk locus.
In general, a single band at 475bp indicates itk-/-, a single band at 218bp indicates itk+/+, and the presence of both the 475bp and 218bp bands indicates itk+-.

The exception may arise where another knockout mice also contribute to the neo band. In that case, an itk-deficient specific product is necessary. PCR amplification of a 1.9 kb product encompassing the junction of the neo construct and the itk gene can be found in Liao and Littman, 1995, Immunity 3, 757-769. The combined results from itk-WT PCR and itk-KO PCR should answer the genotype of itk unambiguously.