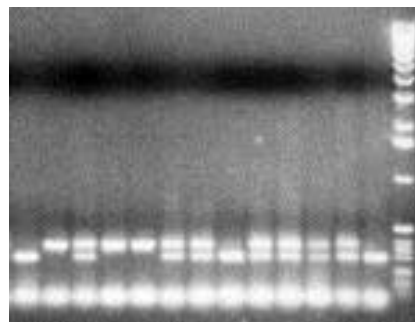
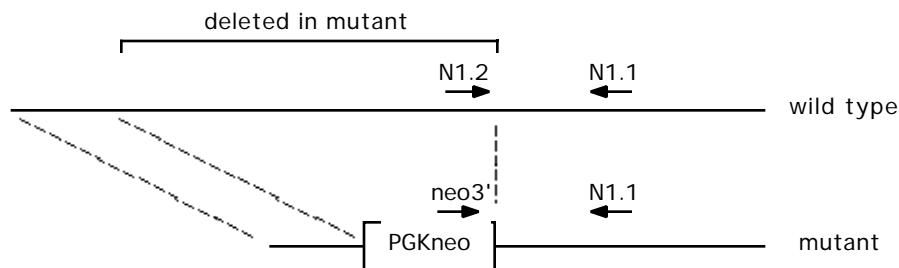


## Typing the Notch1 Mutation



N1. 1: 5' GAT ATC GTG GTG CAT ACC CTC CTG 3'  
 N1. 2: 5' GTG GTC TAG GAT GCT TGG GTC TAG 3' gives a 300 bp wild type product

Neo 3' : 5' GTC AGT TTC ATA GCC TGA AGA ACG 3'  
 N1. 1: gives a 380 bp mutant product

If all three primers are used together, the mutant band is preferentially amplified in the heterozygote, but one can usually distinguish heterozygotes from homozygotes.

### PCR Typing Yolk Sacs

Yolk sac is digested O/N at 50-55°C in 100 µl of PCR Lysis Buffer containing 100 µg/ml proteinase K (either with continuous gentle agitation, or with a brief vortex in the morning followed by 1-2 hours more digestion). Digested samples can be stored at room temperature.

### Lysis Buffer For Yolk Sac (aliquots containing ProK can be stored at -20°C)

50 mM KCl  
 10 mM Tris pH 8.3  
 2 mM MgCl<sub>2</sub>  
 0.1 mg/ml gelatin  
 0.45% NP-40  
 0.45% Tween-20

### PCR Typing Ear Punches

Heat ear punch in 300 µl 10 mM NaOH/0.1 mM EDTA at 95°C for 10 min. Store at RT.

Use 4 µl of each DNA sample with 8 µl of water in each PCR reaction. Denature at 95°C for 10 minutes in the PCR machine. At 85°C add 8 µl of the cocktail (freshly made and premixed; can be added through the oil.)

| Cocktail                | per reaction |
|-------------------------|--------------|
| 100 ng/µl primer 1      | 1.2 µl       |
| 100 ng/µl primer 2      | 1.2 µl       |
| 100 ng/µl primer 3      | 1.2 µl       |
| 10X PCR buffer          | 2.0 µl       |
| 20 mM MgCl <sub>2</sub> | 2.0 µl       |
| 10 mM dNTPs             | 0.4 µl       |
| 5 U/µl Taq              | 0.2 µl       |

### Cycle conditions

94°C 0.5 min  
 62°C 0.5 min  
 72°C 1.5 min  
 39 cycles

Run the whole sample on an agarose gel with markers, a no DNA negative control, and positive controls. The primers are in massive excess and will make a low molecular weight smear on the gel. The most typical problem with the procedure is a failure of the reaction due to too much DNA, especially if tissue is taken from a big tail cut etc. This results in a DNA smear from 100 bp to several kb.