K-ras genotyping (LSL, G12D)

Note: Carl has done seperate pcr using U1-wt; and U1-Mut as sepearte PCR. 3 primer PCR does not work well with Carl and Marcelo. Although it has worked well with Jacks lab and Lowe lab. One should always try the three primer system before using the two primer system.

Notes:

- This reaction looks for the presence of the stop cassette NOT for the mutation. Samples must be sequenced to look for mutation.
- Run a large gel at 100V for at least 40-50 min to allow the two bands to separate well!
- Run 2 % gel

Primers:

K-ras-U1 5' – CGCAGACTGTAGAGCAGCG – 3'
K-ras-wt 5' – GTCGACAAGCTCATGCGGG – 3'
K-ras-mut 5' – CCATGGCTTGAGTAAGTCTGC – 3'

Size of bands:

Wt 500 bp
Mut 550 bp

PCR conditions:

94 °C for 3min
94 °C for 30 sec
60 °C for 1:30min
72 °C for 1min
30 cycles
72 °C for 5min
10C for ever

50µl reaction

2µl DNA
1µl K-ras-U1
1µl K-ras-wt
1µl K-ras-mut
1µl dNTPs (10mM)
1µl Taq
5µl 10x buffer
add H2O

20ul reaction (Bin Xue using GoTaq to give more reliable results)

1 µl DNA
0.5 µl K-ras-U1
0.5 µl K-ras-mut
10 µl GoTaq

8 µl H2O