

## NIH-0617 Genotyping Strategies

| Reaction Components    | Vol (ul) |
|------------------------|----------|
| 2X Premix G            | 25       |
| 25mM MgCl <sub>2</sub> | 3.5      |
| 10mM dNTPs             | N/A      |
| Primer 20 uM           | 2.5      |
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| 5 U/ul Taq polymerase  | 0.5      |
| Water                  | 11       |
| Total mix volume       | 45       |
| Tail lysate extracted  | 2.5      |
| Total reaction volume  | 47.5     |

| Step | Temp | Time | Note               |
|------|------|------|--------------------|
| 1    | 95C  | 1'   |                    |
| 2    | 95C  | 17"  |                    |
| 3    | 58C  | 30"  |                    |
| 4    | 72C  | 40"  | Go to 2, 40 cycles |

### Primer Sequences (5' to 3'):

Mutant PCR: Primer 0617-5' and Primer LTR-rev, 385 bp

Recommended Wt PCR: Primer 0617-5' and Primer 0617-3', 446 bp

|                |                         |
|----------------|-------------------------|
| Primer LTR-rev | ATAAACCCCTCTGCAGTTGCATC |
| Primer 0617-5' | TGCTCTGAAGGACTCGTGACTGG |
| Primer 0617-3' | TAAGCGAAAGGATTCACTAATTC |

Samples were phenol-chloroform extracted

| Well | Sample    | Genotype |
|------|-----------|----------|
| 1    | 135       | het      |
| 2    | 137       | het      |
| 3    | 138       | het      |
| 4    | 145       | het      |
| 5    | 149       | het      |
| 6    | 163       | het      |
| 7    | 164       | het      |
| 8    | 167       | het      |
| 9    | ES DNA    | het      |
| 10   | wt lysate | wt       |
| 11   | water     | no amp   |

### Mutant PCR

MK 1 2 3 4 5 6 7 8 9 10 MK

