## 16/06/2016

|                    | 16/06/2016         |      |                    | 16/06/2016 11:14        |       |  |
|--------------------|--------------------|------|--------------------|-------------------------|-------|--|
|                    | Additive: DMSO     | 0    | %                  |                         |       |  |
| 40 uL              | 1X_65ul 1.5ul Mg+  | 1ul  | Х                  | Master Mix (no primers) | 8 x   |  |
| Amount             | Compound           |      | Amount             | Compound                |       |  |
| 30.8 <sub>uL</sub> | $ddH_2O$           | 0.77 | 246 uL             | $ddH_2O$                | 246.4 |  |
| 0 <sub>uL</sub>    | DMSO 100%          | 0    | 0 <sub>uL</sub>    | DMSO 100%               | 0     |  |
| 4 <sub>uL</sub>    | 10x PCR buffer     | 0.1  | 32 <sub>uL</sub>   | 10x PCR buffer          | 32    |  |
| 3.2 <sub>uL</sub>  | dNTPs (2.5mM each) | 0.08 | 25.6 <sub>uL</sub> | dNTPs (2.5mM each)      | 25.6  |  |
| 0.4 <sub>uL</sub>  | Pf, 10μM           | 0.01 | 3.2 uL             | Pf, 10μM                | 304   |  |
| 0.4 <sub>uL</sub>  | Pr, 10 μM          | 0.01 | 3.2 <sub>uL</sub>  | Pr, 10 μM               |       |  |
| 0.8 <sub>uL</sub>  | DNA template       | 0.02 | 6.4 <sub>uL</sub>  | DNA template            |       |  |
| 0.4 <sub>uL</sub>  | Phusion Polymerase | 0.01 | 3.2 uL             | Polymerase              |       |  |
| 40 uL              | Total              | 1    | 320 uL             | Total   per tube ul:    | 38    |  |

Note: 250 and 500 bp Gell purified in 4 multiplies: 57, 58.5, 58, 58.5, 59 C (8 Total)

| 1x   | Initial Denaturation  | 95 | С | 5'   |
|------|-----------------------|----|---|------|
| 30 x | Denaturation          | 95 | С | 30'' |
|      | Annealing Temperature | 57 | С | 30'' |
|      | Elongation            | 72 | С | 30'' |
| 1x   | Elongation            | 72 | С | 7'   |
|      |                       | 4  | С | inf  |