**Recipe (20 μl reaction volume)**

100 ng DNA

1× reaction buffer

0.2 mM dNTP

1.5 mM MgCl2

0.5 uM each primer (sequences below)

1 unit Taq polymerase

**Cycling conditions:**

Initial denaturation at 95 °C

35 cycles of: 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 1 min

Final extension at 72 °C for 5 min

**Primers:**

Two primer pairs were used per reaction.

1. Positive control (amplifies region outside the duplication boundary and produces a product in both duplicated and non-duplicated strains)

Forward: 5**′-**CAGTCGTCGACAGTCATCGT-3**′**

Reverse: 5**′-**GAGCTGAAGGGATCTGCAAC-3**′**

2. Includes the duplication boundary and only yields a product in duplicated strains

Forward: 5**′-**CTCTGCCCCAGAGAACAGTC-3**′**

Reverse: 5**′-**TGATAGAGGCCACACAGCAG-3**′**

**Interpreting results:**

On a gel, a duplication-containing strain has two bands. A non-duplicated strain has only the larger amplicon.

Non-duplicated band: ~225 BP
Duplicated band: ~175 BP