

| | P1 | P2 | C | CEP | F | M | H | L | SH |
|------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| PCR Mix (Total volume 20 μ l) | | | | | | | | | |
| Buffer 10x ^(a) | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l |
| MgCl ₂ ^(b) | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM | 1.5 mM |
| dNTPs ^(c) | 200 μ M | 200 μ M | 200 μ M | 200 μ M | 200 μ M | 200 μ M | 200 μ M | 200 μ M | 100 μ M |
| Taq polymerase ^(d) | 1.25 U | 1.25 U | 1.25 U | 1.25 U | 1.25 U | 1.25 U | 1.25 U | 1.25 U | 1.25 U |
| BSA ^(e) | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l | 25 ng/ μ l |
| DNase free water ^(f) | FV | FV | FV | FV | FV | FV | FV | FV | FV |
| Primers concentration | 250 nM | 250 nM | 200 nM | 200 nM | 200 nM | 300 nM | 300 nM | 200 nM | 300 nM |
| Total DNA | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l | 100 ng/ μ l |
| PCR program | | | | | | | | | |
| Taq activation | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' | 94°C for 3' |
| N. of cycles | 40 | 40 | 35 | 35 | 35 | 45 | 45 | 16 | 45 |
| Denaturation | 94°C for 25'' | 94°C for 30'' | 94°C for 40'' | 94°C for 30'' | 95°C for 1' | 94°C for 30'' | 94°C for 30'' | 95°C for 10'' | 94°C for 25'' |
| Annealing | 57.5°C for 15'' | 53°C for 20'' | 60°C for 40'' | 53°C for 20'' | 40°C for 1' | 47°C for 30'' | 55°C for 20'' | 62°C for 30'' | 51°C for 20'' |
| Extension | 72°C for 20'' | 72°C for 30'' | 72°C for 40'' | 72°C for 30'' | 72°C for 30'' | 72°C for 30'' | 72°C for 40'' | 72°C for 60'' | 72°C for 10'' |
| Final elongation | 72°C for 10' | 72°C for 10' | 72°C for 7' | 72°C for 10' | 72°C for 7' | 72°C for 10' | 72°C for 10' | 72°C for 5' | 72°C for 5' |

Table 2SM. Amplification protocols and programs of each couple of primers selected in this study: ^{(a)(b)} 5Prime, Gaithersburg, USA; ^(c) dNTPmix, Euroclone S.p.A-Life Sciences Division; ^(d) PerfectTaq DNA Polymerase (5Prime, Gaithersburg, USA); ^(e) purified BSA 100 \times , New England Biolabs; ^(f) Water Mol. Bio. Grade, DNase–RNase and Protease free, 5Prime, Gaithersburg, USA; FV: up to the final volume achievement.