Vent PCR

Vent polymerase has exonuclease activity. Because of this, it has a tendency to backup and repair mistaken incorporation. Taq polymerase lacks this activity. For this reason, the fidelity of DNA replication by Vent is much higher than that of Taq. As a general rule, try to amplify up your DNA using Vent if you plan to express it as a protein or otherwise do not wish to tolerate any mutations. However, Vent requires some tooling to work properly, and can be problematic for some fragments sized greater than 1 kb. It all depends….

1. For a standard 100 ul reaction

    76.5 – X ul of QH2O
    10 ul of 10X Vent polymerase buffer (supplied with enzyme from NEB)
    **X ul of 100 mM $MgSO_4$
    5 ul of Primer A (20 uM stock)
    5 ul of Primer B (20 uM stock)
    2 ul of dNTP mixture (25 mM stock)
    1 ul of template DNA (~1-3 ng/ml stock)
    0.5 ul of Vent polymerase (2 U/ul stock)
    (** use between 0.5 to 3 ul, see note 4 below)

2. Overlay with a drop of mineral oil

3. PCR cycling

    95C for 30 seconds
    Primer melting temp minus 4 or 5C for 45 seconds
    72C (as a general guideline, use ~ 30 seconds per 500 bp of amplified fragment.

    Perform about 30 cycles

4. Note. Sometimes, you can get better results, and even results at all by varying the reaction MgSO4. NEB supplies 100 mM MgSO4 for this purpose. Use from 0.5 to 3 ul of this stock MgSO4 per reaction. It is entirely empirical whether and how much will enhance the product.