Using Vent® DNA Polymerases to Extend a Primer

Basic reaction conditions are 1X Vent$_R$® buffer (supplemented with additional $MgSO_4$ if necessary), 200–400 µM each dNTP, 0.2–1.0 µM primer, DNA template and enzyme (Vent$_R$®, Vent$_R$® (exo$^-$), or Deep Vent$_R$®, Deep Vent$_R$® (exo$^-$) DNA polymerase). Conditions for primer extension reactions are detailed below.

Buffer
It is important to use the buffer enclosed with the enzyme. All Vent® DNA polymerases prefer this low ionic strength, sulfate-containing buffer.

Enzyme Quantity
For Vent$_R$® or Deep Vent$_R$® DNA polymerase, (the Vent® DNA polymerases with proofreading exonucleases), use 1 to 2 units of enzyme (1/2 to 1 µl of the 2,000 units/ml stock) per 100 µl reaction volume. For different reaction volumes, scale the amount of enzyme added accordingly. For Vent$_R$® (exo$^-$) or Deep Vent$_R$® (exo$^-$) DNA polymerase, use at least 4 units of enzyme per 100 µl reaction volume. It is possible to use up to 16 units of Vent$_R$® (exo$^-$) or Deep Vent$_R$® (exo$^-$) DNA polymerase in 100 µl reaction mixtures since it has no exonuclease activity; these higher levels can often result in concomitantly higher yields. To obtain a storage stock of Vent$_R$® DNA polymerase at lower than 2,000 units/ml, Vent$_R$® storage buffer is available for making dilutions. This is preferable over dilution in 1X reaction buffer; enzyme stocks should never be diluted in water.

Mg^{+2} Concentration
2 mM $MgSO_4$ is often sufficient for primer extensions. Use the 10X buffer supplied to achieve a 1X level of $MgSO_4$ equivalent to 2 mM. For primer extensions requiring a higher level, supplement this 2 mM level to achieve a range of concentrations up to 6 mM. Please note that primer extensions beyond 2 Kb often require 2 mM to 6 mM $MgSO_4$. For convenience, a vial of 100 mM $MgSO_4$ is included in every Vent® enzyme package.

BSA
The presence of non-acetylated BSA at a final concentration of 100 µg/ml may increase activity with some reactions. This may be due to the neutralization of enzyme inhibitors in some DNA samples or the prevention of nonspecific adsorption of reagents to the reaction vessel. BSA is not necessary for enzyme stability. BSA is available separately free of charge if requested when placing an order. *Acetylated* BSA should not be used in reactions incubated at temperatures above 85°C.

dNTP Concentration
The 200-400 µM level of each dNTP is especially important for Vent$_R$® or Deep Vent$_R$® DNA polymerase (the Vent® DNA polymerases with proofreading exonucleases). This level of dNTPs will help ensure that the 3′→ 5′ proofreading exonuclease function of the Vent® DNA polymerases will not start to degrade DNA products. Degradation can occur upon complete depletion of the dNTP pool due to incorporation into your DNA product. To determine if there is a problem, compare an aliquot of the reaction mixture taken at an intermediate time point to the final product.

Polymerase Addition
The recommended order of addition of reaction components is: 10X buffer and H_2O, additional $MgSO_4$ if necessary, dNTPs, DNA template and primers, and lastly the polymerase. Keep the mixture at 0°–4°C while adding all reaction components. Alternatively, add the DNA polymerase following an initial high temperature incubation of all reaction components except the DNA polymerase. Please note that the 3′→ 5′ exonuclease activity of Vent$_R$® or Deep Vent$_R$® DNA polymerase will begin to degrade nonannealed primers at their 3′ end if incubated for prolonged periods. When reaction components are added in the recommended order, there is minimal interference from the 3′→ 5′ exonuclease activity with primer extension or other applications.

Primer Annealing
To maximize specificity, anneal your primer to the template for 1 minute or less at the highest temperature which will permit primer :template annealing. Please note that the reaction buffer composition for Vent® DNA polymerases is different from that of other DNA polymerases. This difference in buffers may require an adjustment in the annealing temperature for a primer. In general, if no *apparent* primer extension is seen, the annealing temperature should be attempted at both lower and higher temperatures; if false priming extensions are seen, the annealing temperature should be increased in 2°C increments.

Primer Extension
Calculate extension times by allowing 1 minute at 72°–75°C per kilobase of expected extension product. This ratio of extension time :product length can be used for product lengths from 50 up to 15,000 bp. Using higher than calculated extension times is not recommended for Vent$_R$® enzymes possessing proofreading exonucleases.

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