Supporting Text: Polymerase Error Rates and the Accuracy of ϕ29 Cloning

A problem in estimating the sequence accuracy of DNA cloned by ϕ29 polymerase rolling-circle amplification (RCA) is that there are not good measurements of the overall error rate for the enzyme. The work of Esteban *et al.* (1) on the fidelity of the enzyme is the most often quoted. However, that paper measures the misinsertion rate for incorrect nucleotides using an exonuclease-deficient mutant. Therefore the proofreading activity of the ϕ29 polymerase 3' exonuclease is deliberately not included in these measurements. This appears to be ignored in later work (2) that cites Esteban *et al.* as reporting an error rate of 5×10^{-6}. Also, this later work calculates the error rate (*ER*) as

$$ER = mf / (bp \times d),$$

where *mf* is the mutation frequency, *bp* is the size of the mutational target in base pairs, and *d* is the number of template doublings. Using this equation the error rate was estimated at 3×10^{-6}. But, because the ϕ29 RCA reaction is not a doubling process, this analysis is inappropriate.

We believe that the true error rate for ϕ29 polymerase must be ~1×10^{-6} or lower, as it is for other replicative DNA polymerases with proofreading (1, 3). We have made a number of simplified models of the RCA reaction. For example, consider a four-stage reaction in which 1,000 copies are made from the original input molecule. Three consecutive rounds that each gives 100-fold amplification, yielding a total amplification of 10^9-fold, follow this initial stage. Assuming an error rate of 1×10^{-6} and a 5-kb circular DNA we get:

Step	Copies	Total bp	New Errors	Propagated Errors	Total Errors	fraction mutant
0	1	$5x10^3$	0	0	0	0%
1	10^3	$5x10^6$	5	0	5	0.5%
2	10^5	$5x10^8$	500	500	1000	1%
3	10^7	$5x10^{10}$	$5x10^4$	$1x10^5$	$1.5x10^5$	1.5%
4	10^9	$5x10^{12}$	$5x10^6$	$1.5x10^7$	$2x10^7$	2%

By this type of reasoning, we predict that the majority of the molecules resulting from 10^9-fold amplification by the ϕ29 RCA reaction will be identical to the starting molecule even if the error rate is as high as 10^{-5} and the amplification in secondary rounds is as low as 10-fold.

1. Esteban, J. A., Salas, M. & Blanco, L. (1993) *J. Biol. Chem.* **268,** 2719–2726.
2. Nelson, J. R., Cai, Y. C., Giesler, T. L., Farchaus, J. W., Sundaram, S. T., Ortiz-Rivera, M., Hosta, L. P., Hewitt, P. L., Mamone, J. A., Palaniappan, C. & Fuller, C. W. (2002) *BioTechniques* **32,** Suppl., 44–47.
3. Kunkel, T. A. (2004) *J. Biol. Chem.* **279,** 16895–16898.