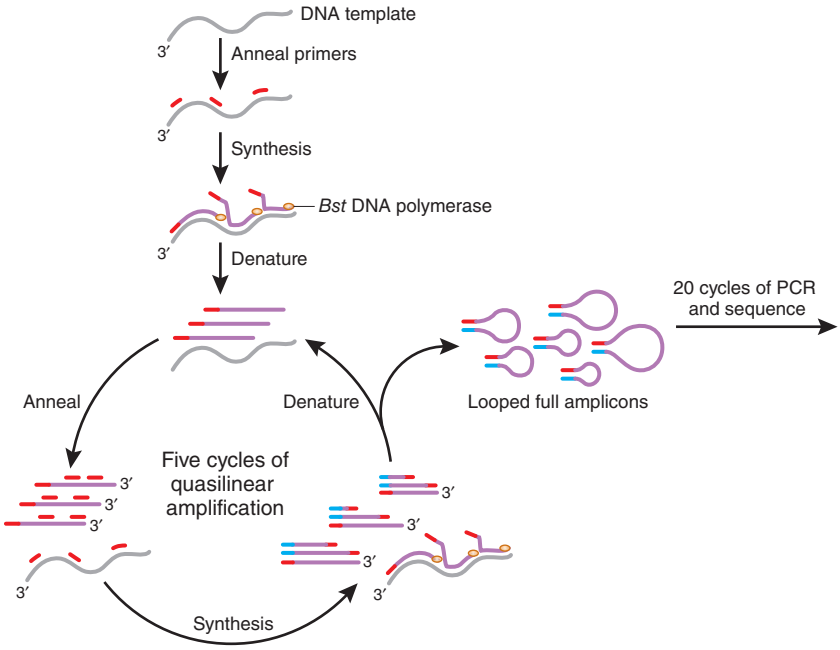

Katie Vicari

Figure 1 Simplified diagram of the MALBAC reaction. MALBAC primers (red) having a 27-nt common sequence followed by eight random nucleotides are annealed to the genomic DNA template. Strand-displacement synthesis generates partial amplicons, which are subsequently denatured from the template at 94 °C. Priming to new positions on the genomic DNA template generates more partial amplicons, which increases coverage of the genome with a resulting reduction in amplification bias. Priming and extension on the partial amplicons yield complete amplicons having the MALBAC primer sequence at the 5′ end (red) and its complementary sequence at the 3′ end (blue). Denaturation at 94 °C regenerates the original template and a now larger and more diverse pool of partial amplicons. Full amplicons form loops, which may be resistant to subsequent amplification and hybridization. Full amplicons are generated for five cycles and then exponentially amplified by 20 cycles of PCR using primers complementary to the common region of the MALBAC primers. *Bst*, *Bacillus stearothermophilus*.

isothermal strand displacement and Taq DNA polymerase for PCR.

To overcome the false-positive errors, Zong *et al.*[4] sequenced several single cells from a highly homogeneous cell culture. Briefly, a single cell was isolated from the culture and propagated through three cell divisions to obtain highly clonal kindred cells. DNA from three of these cells was amplified by MALBAC and analyzed by high-throughput sequencing. Sequences of single cells were compared with the predominant sequence of a bulk DNA extraction from the culture, allowing the identification of rare cell-specific alleles. It remains to be seen how well such an analysis can be carried out with more-complex cell populations such as tumor cells. Variants found in all three cells were deemed validated, whereas variants found in only one or two cells were false positives generated by MALBAC. Thus, it appears that statistical strategies using multiple single cells will still be required for many studies using MALBAC. There may be ways to reduce the false-positive error rate by adapting the protocol to use a high-fidelity enzyme, such as phi29 DNA polymerase, or a thermostable DNA polymerase with strong proofreading activity. The 20 cycles of PCR that are required might also be optimized to reduce error rates.

A second limitation of MALBAC involves regions of the genome that are reproducibly underrepresented. In the experiments of Zong *et al.*[4], about 20–30% of known single-nucleotide variants could not be accurately genotyped even when multiple cells were pooled in a single MALBAC reaction. Sequences in certain contexts can be difficult for DNA polymerases to copy. For instance, regions of secondary structure in the DNA template might be poorly replicated during the strand displacement or PCR steps. A difficult template sequence for either of the DNA polymerases used would result in poor recovery in the final MALBAC product. A published analysis of the sequences in the human genome that are consistently underrepresented would help users of MALBAC better design their research strategies.

As with PCR and MDA, there will be much to learn about MALBAC reaction mechanisms and the DNA products that are generated. For example, chimeric DNA rearrangements that occur in MDA[10] have been detected in MALBAC. However, their rate of formation has not yet been reported. Although uncertainties remain, MALBAC is a notable advance because it highlights the importance of the initial priming events in whole-genome amplification. The data demonstrate that bias can be reduced by controlling this crucial phase of the reaction and that genotyping of a diploid cell at the single-nucleotide level is feasible. A period of rapid improvement and testing of applications can be anticipated.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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