

MALBAC Single Cell WGA Kit

Multiple Annealing and Looping-based Amplification Cycles

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Rapid, Reliable and Reproducible Whole Genome Amplification for Single Cells

Nucleic acid analytical platforms (e.g. qPCR, microarray and high throughput sequencing) often require the sample to meet specific requirements for quantity and quality. Therefore, Whole Genome Amplification (WGA) is often required for analyzing samples with limited quantity, such as single circulating tumor cells, micro-biopsies, and single blastomere.

WGA methods are in general prone to amplification bias, PCR-based WGA introduces sequence-dependent bias mainly due to the exponential amplification. Multiple Displacement Amplification (MDA), which uses random priming and the strand-displacing phi29 polymerase under isothermal condition, has provided improvements over PCR-based methods but still exhibits considerable bias, again due to nonlinear amplification.

What is MALBAC technology?

Single cell whole genome amplification and sequencing is highly desirable for many applications such as IVF preimplantation screening and genotyping of rare circulating tumor cells. Existing amplification methods are hindered by non-uniformity across the genome and poor reproducibility between single cell WGA replicates.

Single Cell WGA Genome Kit is based on MALBAC (Multiple Annealing and Looping Based Amplification Cycles) technology, which carries out close-to-linear pre-amplification cycles of the entire genome using a mixture of highly-processive DNA polymerases with strand displacement activity, followed by an exponential amplification by PCR to a sufficient amount for various downstream analyses. The unprecedented uniformity provided by MALBAC allows accurate detection of both single point mutations and copy number variations of individual cells. Researchers have used MALBAC to study the generation of new-born mutations in a cancer cell line and were able to measure the mutation rate directly.

Applications of MALBAC

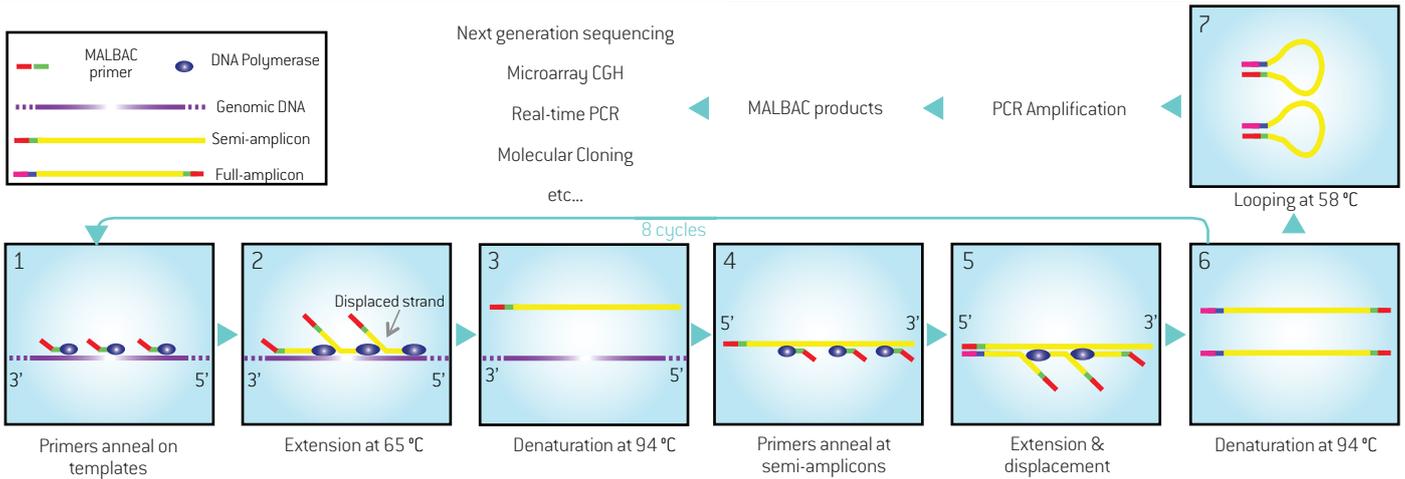


- Mammalian cells:**
- Pre-implantation genetic screening (PGS)
 - Pre-implantation genetic diagnosis (PGD)
 - Genotyping of transgenic animals
 - Phasing of individual human genomes
 - Genotyping of sperm cell
 - Embryo and stem cell
 - Forensic specimens analysis
 - Typing of transgenic animals

- Tumor:**
- Somatic genetic variation analysis
 - Tumor evolution and development
 - Tumor stem cell
 - Circulating tumor cells

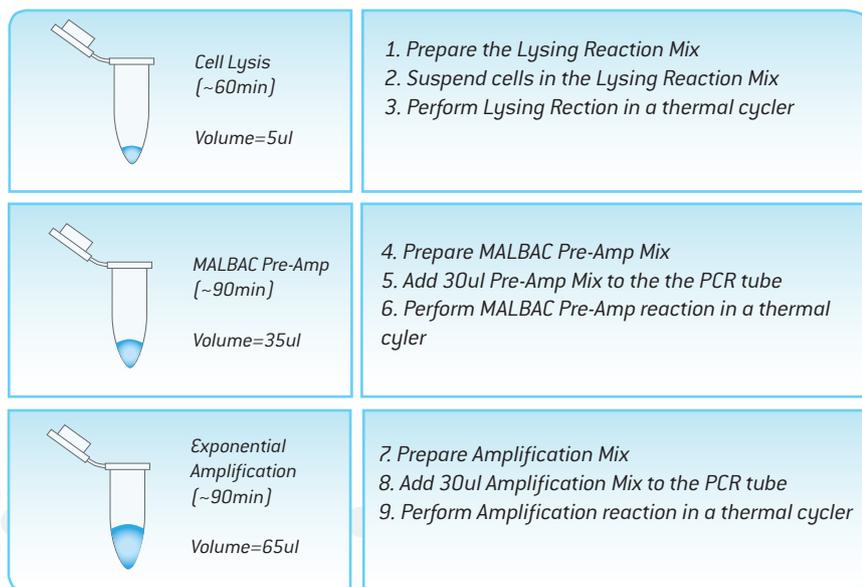
- Bacteria:**
- Genotyping of microorganism
 - Metagenomics study
 - Pathogen analysis

MALBAC Single Cell WGA Assay Principle



- 1.) MALBAC primers, each having a common 27-nucleotide sequence and 8 variable nucleotides, hybridise evenly to the templates at 0°C.
- 2-3.) At 65°C, DNA polymerases with strand-displacement activity generates semi-amplicons with variable lengths (0.5 to 1.5 kb), which are then melted off from the template at 94°C.
- 4.) 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.
- 5.) Priming and extension on semi-amplicons yield complete amplicons having the MALBAC primer sequence at 5' end and its complementary sequence at the 3' end.
- 6.) Denaturation at 94°C regenerates the original template and a now larger and more diverse pool of semi-amplicons.
- 7.) Full amplicons form loops, which may be resistant to subsequent amplification and hybridization.
- 8.) Full amplicons are generated for 8 cycles and the exponentially amplified by about 14-21 cycles using primers complementary to the common region of the MALBAC primers.

MALBAC Single Cell WGA Assay Workflow



2-4ug of DNA

Product Description	Size	Cat #
Single Cell Whole Genome Amplification Kit	10 Reactions	#YK001A
Single Cell Whole Genome Amplification Kit	50 Reactions	#YK001B