RHS
Unique Genomic Solutions

AT THE FOREFRONT OF
SINGLE CELL GENETIC ANALYSIS

Reproductive Health Science Ltd is a developer of advanced single cell genomic technologies focused on improving health and research outcomes.

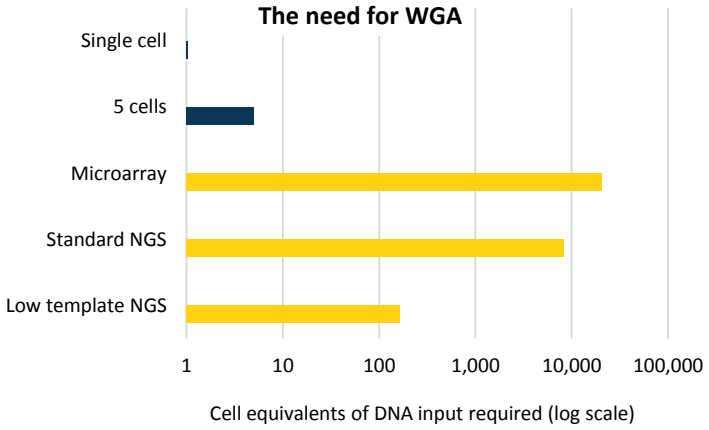
Fundamentals of Whole Genome Amplification

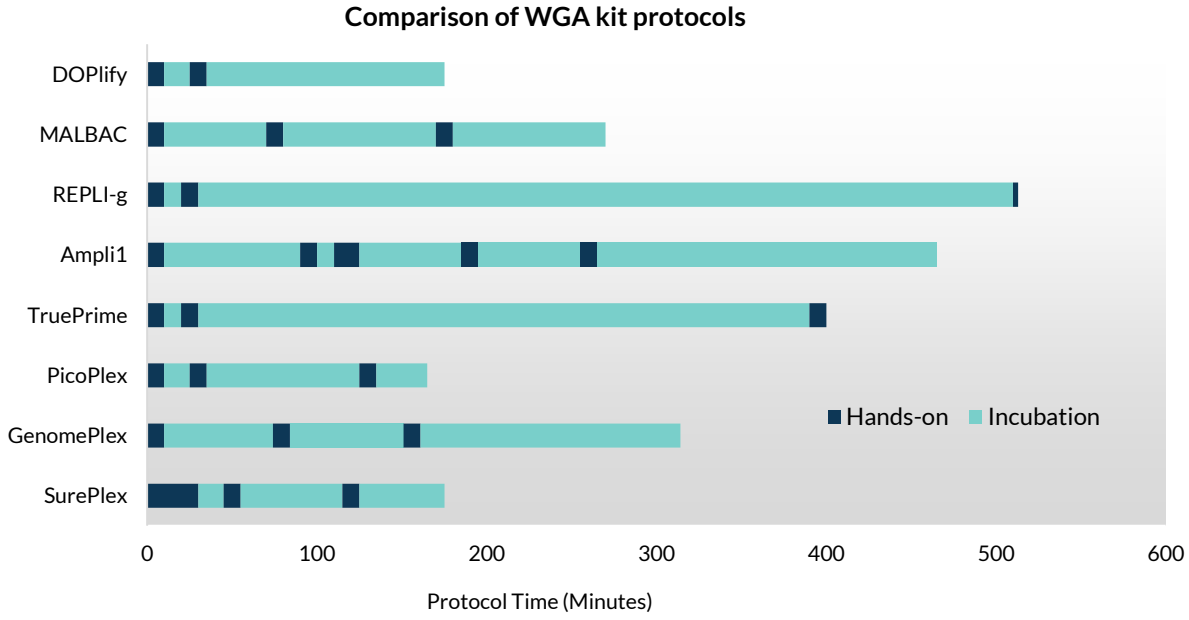
The genetic interrogation of single or small numbers of cells has application in a range of human, agricultural and environmental settings. The main technical challenge is the limited amount of DNA available for analysis. In order to increase the amount of DNA available, there is usually an amplification step needed.

Single cells contain approximately 6 picograms of DNA. Microarrays, Next Generation Sequencing and other genetic analysis platforms require significantly larger quantities of input DNA. The starting DNA therefore often requires whole genome amplification (WGA) to create 10^6 to 10^9 higher yields.

There are a small number of single cell whole genome amplification kits available on the market. These kits are sold as separate products and some are also included in single cell analysis kits such as those for Pre-implantation Genetic Screening for aneuploidy or karyomapping. It is seldom known or appreciated that the method of whole genome amplification used in these kits is not common and that each method has its own mechanism by which it copies the DNA template with different amplification biases.

The purpose of this Application Note is to summarise the theory behind each of the WGA methods and to compare and contrast the 4 main whole genome amplification technologies currently available in kits, including their hands on and incubation times.





Advanced Degenerate Oligonucleotide-Primed PCR (DOP-PCR)

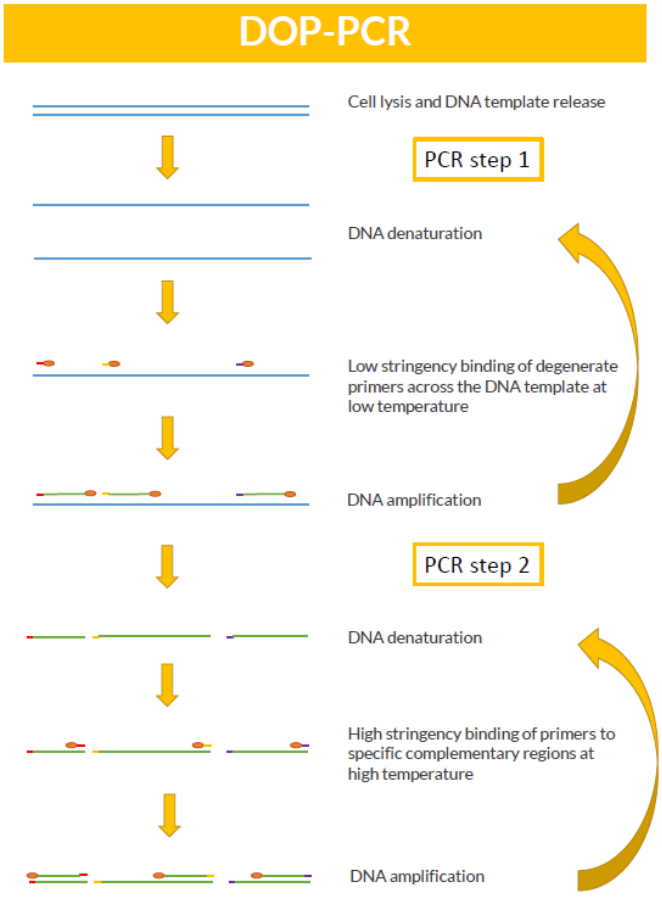
Example; DOPlify™ (Reproductive Health Science Ltd)

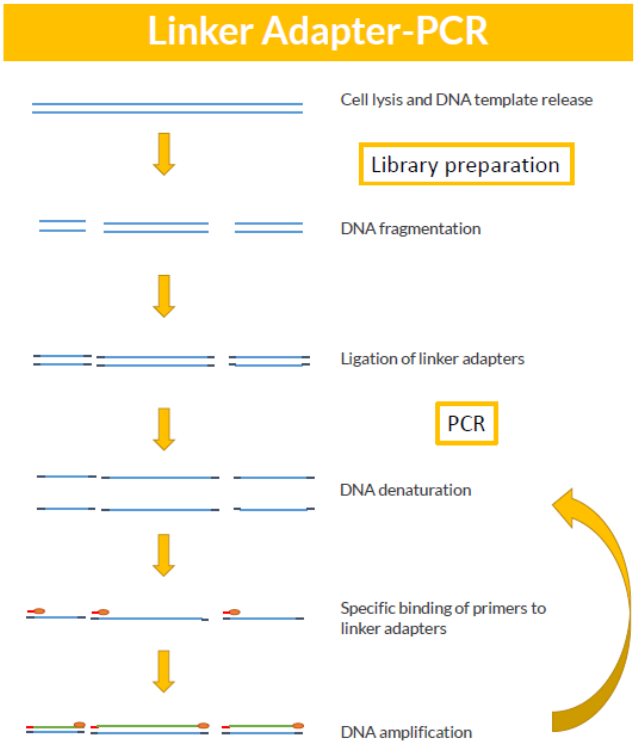
Used in: EmbryoCellect™ (Reproductive Health Science Ltd)

DOP-PCR uses a unique primer design with a 6bp anchor at the 3' end, a 6bp degenerate sequencing in the middle (ie a mixture of As, Cs, Ts and Gs in random order) and another anchor at the 5' end. This provides a mixture of 10^6 primer sequences.

The PCR program starts with a small number of low stringency cycles, where the primer binding temperature is low, allowing the 3' anchor to bind indiscriminately across the entire genome while the rest of the DOP primer does not need to bind (PCR step 1). This creates template that has incorporated the DOP primer at the 5' and 3' end of the amplicon.

The low stringency cycles are then followed with a larger number of high stringency cycles where the entire primer sequence is used (PCR step 2). This ensures that the primer binding and subsequent amplification in the later PCR cycles is more specific.





Linker-Adapter PCR (LA-PCR)

Examples; SurePlex (Illumina Inc), PicoPlex (Rubicon Genomics), GenomePlex WGA4 (Sigma), Ampli1 (Silicon Biosystems)

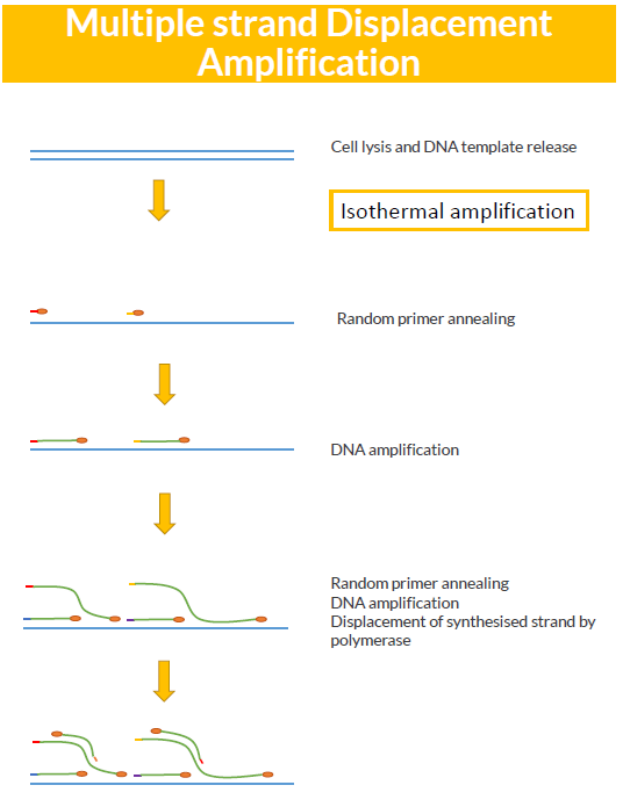
Used in: 24Sure, VeriSeq (Illumina), ReproSeq, Ion SingleSeq (Thermo Fisher)

In linker adapter PCR, the cell is lysed to release the template DNA and the DNA is fragmented.

Linkers containing a primer binding site are then enzymatically attached to the ends of the fragmented DNA template to create a template library.

With the addition of PCR primers complementary to the linker primer binding site, PCR is used to amplify the fragmented DNA that has linkers attached to the 3' and 5' ends of the fragment.

Multiple Displacement Amplification (MDA)



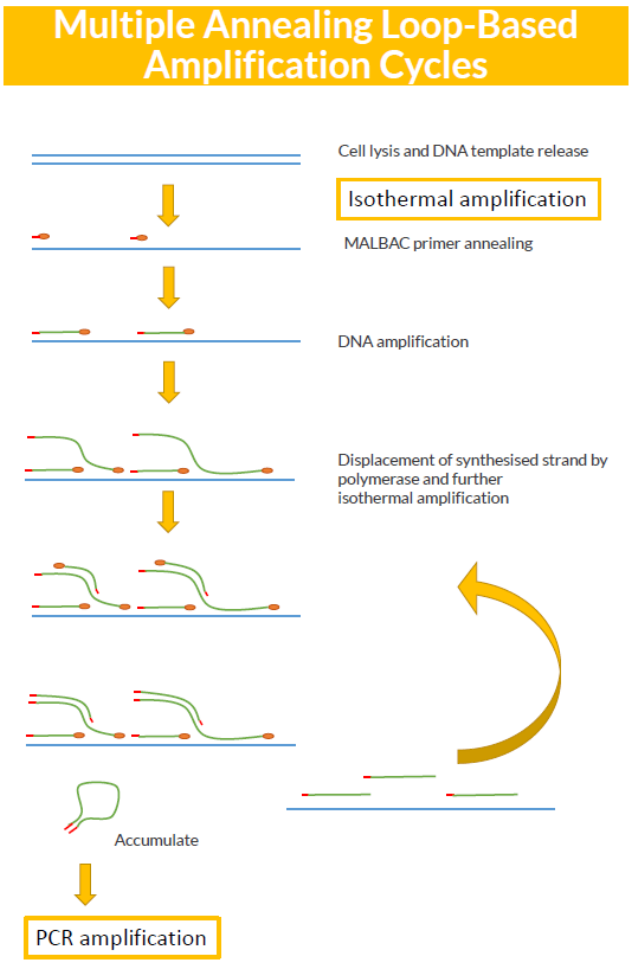
Example; RepliG (Qiagen)

Used in; GenetiSure (Agilent), Karyomapping (Illumina)

Multiple Displacement Amplification (MDA) does not employ PCR-based amplification. It instead uses a bacteriophage DNA polymerase that separates double stranded DNA into single stranded (strand displacement) and amplifies the single stranded template at a constant temperature (isothermal). Amplification of a fragment will continue unrestricted as long as there is single stranded DNA template available.

The very long fragments (approx. 2-100kb) amplified using MDA are well suited to PGD and SNP genotyping (e.g. karyomapping) due to the likelihood that a single amplicon (piece of replicated DNA) will contain a large region of a gene of clinical interest and will span a significant number of SNP markers.

Karyomapping data may reveal trisomy where the aneuploidy is of meiotic origin due to the presence of both haplotypes from one parent. Monosomy is detectable by the loss of heterozygosity for a chromosome. However, mitotic errors are not easily detectable.



Multiple Annealing and Looping-Based Amplification Cycles (MALBAC)

Example; MALBAC (Yikon Genomics)

MALBAC is a hybrid method combining MDA and Linker-Adapter PCR.

Cells are lysed and heated to release and denature the DNA template, making it single stranded.

Initial MDA-like isothermal amplification of the template is performed using specific MALBAC primers, which are comprised of 8 variable nucleotides and 27 common nucleotides. This incorporates the MALBAC primer into the template.

The subsequent PCR cycles are initiated from the MALBAC primer. Because the 27 nucleotides of the MALBAC primer are complementary to itself, synthesised DNA fragments that have the MALBAC primer at both ends (ie they have been forward and reverse amplified) will form stem loop structures that are no longer amplifiable. This pushes the PCR towards amplifying template rather than amplicons and increases the genome coverage.

WGA kit comparison						
Kit name	GenomePlex	PicoPlex/ SurePlex	Repli-G	MALBAC	DOPlify	Telenius DOP
WGA technology	Linker Adapter-PCR	Linker Adapter-PCR	Multiple Displacement Amplification	Hybrid	Advanced DOP-PCR	DOP-PCR
Launched	Feb-06	Jun-09	Oct-12	Oct-14	Jun-16	Jul-92
Lysis	Proteinase K digestion	Enzyme-based	Alkaline	Enzyme-based	Enzyme - based	Nil
Lysis volume (ul)	10	10	7	6	5	na
Lysis incubation time (mins) (a)	64	14	10	60	15	0
Template preparation	Yes	Yes	No	Yes	No	No
Template preparation incubation time (mins)	2	45	0	62	0	0
Cumulative volume (ul)	13	15	10	37	5	0
Amplification						
Cumulative volume (ul)	74	75	47	67.8	27	50
Amplification incubation time (mins) (b)	137.5	33.5	483	66	103	138
Total incubation time (mins) (a+b)	203.5	92.5	493	134	118	138
Yield from single cells	65-130ng/ul	25-65ng/ul	Up to 800ng/ul	30-55ng/ul	60-80ng/ul	Up to 140ng/ul
Fragment size range	0.1-1kb	0.2-1kb	2-100kb	0.3-2kb	0.2-2kb	0.1-1kb

Information sourced from Manufacturer's protocols and information sheets available on their websites.
Protocol times exclude PCR machine ramping duration.

RHS
Unique Genomic Solutions