VWR We Enable Science

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GE Healthcare

FAST SIMPLE SEQUENCING PREP WITH GE HEALTHCARE PRODUCTS

DNA Sequencing Made Simple

Widely used around the world, Sanger sequencing is the Gold Standard in DNA sequencing due to its high accuracy on very long read-lengths using a small amount of target molecules. Next-generation Sequencing (NGS) came next which brought the power of sequencing an entire genome in a couple of days. The sequence can be less accurate due to errors introduced by lack of depth and coverage, which are often compromised due to the high price of NGS. The reads are shorter than in Sanger sequencing, so can be challenging to align to the known reference genome with confidence.

Sanger Sequencing

It was used to sequence the Human Genome but now it is used for validation of novel sequence from Next-Generation Sequencing (NGS) platforms, as well as for quality control in the pharmaceutical industry. The process of growing colonies, purifying out the DNA prior to Cycle Sequencing reaction, then purifying again before the Sanger sequencing run is still regarded as time-consuming and tedious.

The challenge of Sanger sequencing is getting high quality reads and long read-lengths. Using our workflow, a pass rate of 96%, read-lengths of >650bp is achieved in nearly half the steps of the traditional workflow.

Starting with a ligation reaction, which doesn't even need to be especially high-yielding, the addition of TempliPhi for isothermal rolling circle amplification (RCA), will generate micrograms of high quality DNA. The product of TempliPhi can be used directly in the Cycle Sequencing reaction. Once that reaction is finished a simple purification step to remove unincorporated dyes and dNTPs by gel filtration yields Sanger sequencing ready template. Specially formulated for Sanger sequencing, AutoSeq gel filtration columns or the high-throughput version, AutoScreen, in 96-well plate format are available.

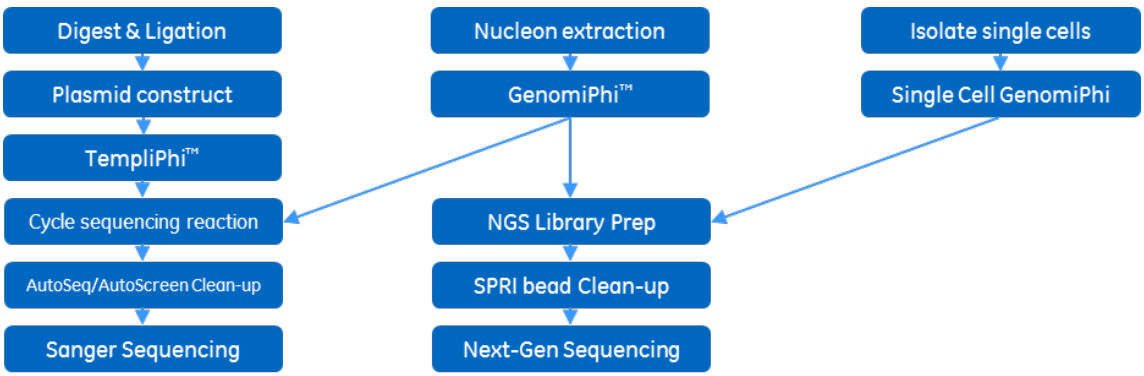

Fig 1. Workflows for different types of sample and sequencing

Difficult template

GC-rich template can compromise Sanger sequencing results. For this common problem, TempliPhi Sequence Resolver was invented. Used just like regular TempliPhi, this specially formulated kit resolves the issues posed by GC-rich template.

Next-generation Sequencing

The easiest part of NGS is hitting the "Sequence" button on your HiSeq. The challenging parts are all the steps that lead up to hitting that button. There are several platforms with their own unique challenges, but we will focus on the most widely used one – illumina platforms.

To obtain the greatest coverage across the whole genome, or even just the whole exome, a large amount of short pieces of the genome must be generated that can be attached to the flow-cell of the instrument. In order for that attachment to occur each adaptor must be ligated to each fragment of DNA – one at each end. In order to get full coverage, you must start with as much full length genomic DNA as possible. The next step is the library prep process which consists of first fragmenting the gDNA, end-repairing & A-tailing, ligating the adaptors, several rounds of PCR using the adaptor sequences as forward and reverse priming sites, then cleaning up with magnetic beads. Finally, the sequence ready fragments are eluted from the magnetic beads and bound to the flow-cell.

As you may guess, with each step of the library prep process, there is the possibility of loss of template DNA and ultimately gaps in the final sequencing coverage. Those missing pieces could include an important single or multi-nucleotide variant, insertion/deletion ("Indel") or transposed element that may hold the key to a disease pathway. The goal of the researcher is to have as few gaps as possible – complete genomic coverage. To do this, they will increase the depth – the amount of times that the material is sequenced, e.g. 30X, 100X, etc., but the results are limited to the material attached to the flow-cell and it's also very expensive to perform even 30X, let alone 100X. So, what can they do?

Phi29 Whole Genome Amplification

The discovery that the reaction of the Phi29 DNA polymerase of RCA works on linear DNA as well as circular DNA, opened up the world of whole genome amplification. This is called multiple-strand displacement amplification (MDA). Suddenly, the very small amount of full length human genomic DNA in a cell could be amplified, yielding micrograms of DNA from nanograms. This process enabled numerous downstream analyses from a single sample: PCR, qPCR, microarray, and, of course, sequencing. Making lots of DNA decreases the chance of having gaps in NGS coverage. The use of the MDA reaction of our GenomiPhi kits prior to NGS library prep is standard in any sequencing lab. GenomiPhi can, of course, be used prior to Sanger sequencing, too.

TempliPhi amplifies a circle of DNA very efficiently because the primers that bind and guide the reaction, just sweep around and around the circle, spinning out long stretches of DNA. The GenomiPhi amplification of linear DNA is less efficient and requires multiple binding sites across the genome, but one way to ensure that the DNA will be amplified is for the template to be greater than 1 Kb.

Better Extraction Methods

Sometime is the 1990s everyone started using spin column extraction kits, because they were easy and safer to use than the standard Phenol:Chloroform method. The principle of these kits is that the DNA or RNA is bound to a column, the unwanted material is washed away, then finally the DNA or RNA is eluted. The multiple spins, applications of buffers as well as the efficiency of the initial binding of the desired template and the final elution, limits the final amount of template as well as the genomic coverage. In addition, all those steps fragment and damage the template. So, even though Phenol is dangerous to use and even prohibited at many research sites, researchers are returning to Phenol:Chloroform, because the quality and length of template obtained is unmatched.

Other challenges are posed by the specific material being extracted from. For plants and seeds, the challenge is removing all the polysaccharides without damaging the template. For Cancer researchers, the challenge is extracting from Formalin-fixed Paraffin-embedded (FFPE) tumor samples, which is essential to trace a possible tumorigenic mutation to archived tumors.

What researchers need is a kit that can give them the quality and length of template of Phenol:Chloroform without Phenol, geared toward their target material. Nucleon kits offer the ease-of-use, quality and length of template of Phenol:Chloroform without Phenol. In addition, there is a kit for plant and fungal material called PhytoPure, another for Blood & Cultured Cells called BACC, and a third for Hard Tissue and FFPE, simply named Nucleon HT.

You may have already guessed that the DNA extracted with Nucleon is perfect for GenomiPhi whole genome amplification. This gives the added assurance that the researcher needs to reduce those sequencing coverage gaps as much as possible.

Single Cells or Trace Amounts of DNA Template

Up to now our knowledge of the genome has been limited to populations of cells. The variation in genome sequence between cells was impossible to characterize. You may ask, why would we need to know those differences? One example is the formation of a tumor. Somatic mutations randomly occur when cells undergo mitosis. Most somatic mutations occur in a part of the genome that will have no downstream effect on gene expression or the mutation will trigger the cell to die. A tumor generally starts from the accumulation of somatic mutations. To understand tumorigenesis, is to understand when and where a tumor will form. To characterize tumorigenesis, the heterogeneity of a tumor must be characterized. With our ability to isolate single cells, lyse them open to release their genome and sequence it by NGS, we come closer to the possibility of predicting where and when a tumor will form. The challenge here is the amount of DNA: a single human cell only has about 7 picograms of DNA. That's where Single Cell GenomiPhi is essential. This kit lyses the cell and amplifies its entire genome in a single tube in about two hours. The typical yield is from 4 to 7 micrograms, which is more than enough for sequencing its whole genome

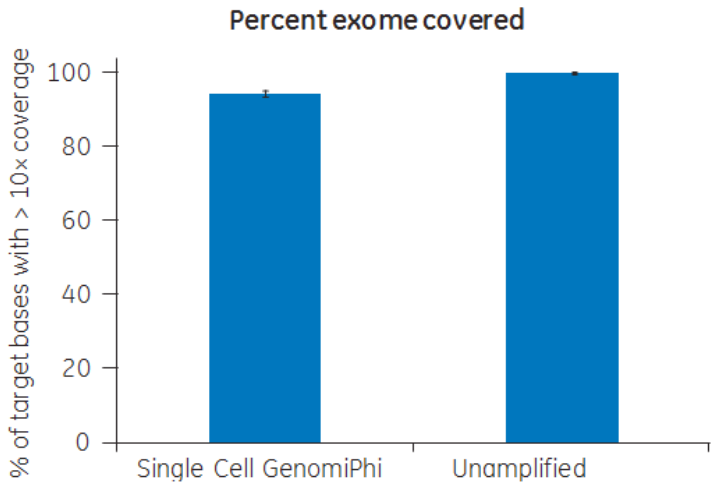

Fig 2. gDNA amplified with Single Cell GenomiPhi DNA Amplification Kit results in a high percentage of exome sequence coverage when run in whole exome sequencing (10x coverage).

Summary

However and whatever type of DNA you need to sequence, GE Healthcare Life Sciences offers innovative products to enable the high quality results, quickly and easily.

Description	Product code
TempliPhi 100 / 500	25-6400-10 / 25-6400-50
TempliPhi 2000	28-9642-86
TempliPhi Sequence Resolver	28-9035-31
GenomiPhi V2	25-6600-30, 25-6600-31, 25-6600-32
Single Cell GenomiPhi	29-1081-07, 29-1080-39
AutoSeq columns	27-5340-01, 27-5340-02, 27-5340-03
AutoScreen 96-well plates	259-005-98
Nucleon Blood & Cultured Cells	RPN8501, RPN8502, RPN8512
Nucleon PhytoPure for Plant & Fungi	RPN8510, RPN8511
Nucleon HT for Tissue & FFPE samples	RPN8509

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