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REVIEW

The History and Advances of Reversible Terminators Used in New Generations of Sequencing Technology

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Abstract DNA sequencing using reversible terminators, as one sequencing by synthesis strategy, has garnered a great deal of interest due to its popular application in the second-generation high-throughput DNA sequencing technology. In this review, we provided its history of development, classification, and working mechanism of this technology. We also outlined the screening strategies for DNA polymerases to accommodate the reversible terminators as substrates during polymerization; particularly, we introduced the "REAP" method developed by us. At the end of this review, we discussed current limitations of this approach and provided potential solutions to extend its application.

Introduction

Dideoxy sequencing method, developed by Frederick Sanger et al. in 1977, contributed substantially to development of biological sciences in the past several decades [1]. As the core technology, it brought the great successful completion of the Human Genome Project (HGP) [2]. However, high cost and low throughput inherited within the method had limited its application to meet the ever-expanding appetite for large scale genome sequencing projects, including *de novo* sequencing of more species, whole-genome resequencing, and deep sequencing. Therefore, the second-generation sequencing technology, characterized by its high speed, high throughput, and lower cost, appeared on the scene [3–5].

Since 2005, the second-generation sequencing technologies have experienced a rapid development: different sequencing platforms came to market and data produced by these new technologies mushroomed exponentially. These achievements not only provided large amount of raw data for scientific research, but also uncovered new scientific ideas, and revolutionarily changed our ways of working in life sciences [3–7]. Now, the third-generation sequencers, represented by single molecule real-time sequencer (SMAT) from PacBio [8], are also on the horizon. But due to the relatively high single read error rate (~15%) [9], the second-generation sequencing technologies are still the mainstream in whole-genome sequencing markets.

So far, technologies employed in the second-generation sequencing platforms are of two main types based on sequencing chemistry: "sequencing by synthesis" and "sequencing by ligation" [10,11]. Reversible termination sequencing is one of the sequencing-by-synthesis strategies popularized by Illumina/Solexa due to its wide adoption in the worldwide sec-

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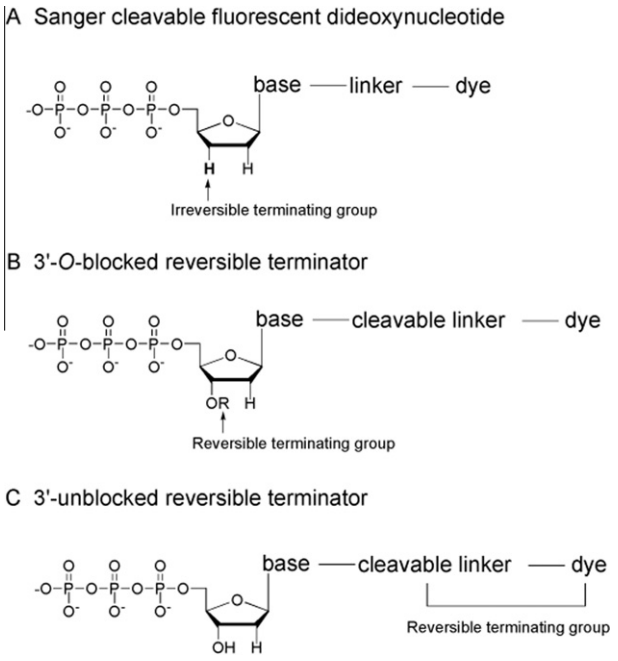

Figure 1 Structure schematic of irreversible and reversible terminators used in sequencing technologies

ond-generation sequencing market (with 80–90% market share) [12]. In the following sections, we give a brief introduction on the developmental history and current status of this technology, discuss the limitations, and suggest potential solutions of its uses in detail.

The history and classification of reversible termination sequencing technology

Reversible termination sequencing technology was first reported by Dr. Jingyue Ju from Colombia University [13]. The prime difference between this approach and the traditional Sanger sequencing method is that the former uses modified nucleotide analogous to terminate primer extension reversibly, while the latter employs dideoxynucleotide to irreversibly terminate primer extension (**Figure 1**) [5].

With the development over the last decade, several reversible terminators were produced. They can be classified into two types based on the difference of the reversible blocking groups [11–23]. One type is 3′-*O*-blocked reversible terminators. As shown in Figure 1B, the blocking group –OR [reversible terminating (capping) group] is linked to the oxygen atom of the 3′-OH of the pentose, while the florescence label is linked to the base, which acts as a reporter and can be cleaved [12–18]. The other type is 3′-unblocked reversible terminators [19–23] as shown in Figure 1C. In this case, the reversible termination group is linked to the base as well as the florescence group which not only is a reporter but also functions as part of the reversible terminating group for termination of the primer extension. These two types of reversible terminators have their advantages and disadvantages: the 3′-*O*-blocked reversible terminator contains a 3′ reversible blocking group, thus should render better termination effect; the 3′-unblocked reversible terminator, on the other hand, is easier to be accepted by the DNA polymerases due to the lack of a modified moiety at the 3′-OH. Because polymerases have evolved for billions of years to discriminate between ribonucleoside triphosphates and 2′-deoxyribonucleoside triphosphates, they have evolved to inspect the 2′- and 3′-positions of their substrates closely. For example, although the only difference between dideoxyribonucleoside and deoxyribonucleoside is the presence or absence of oxygen atom at the 3′ position, accepting a dideoxyribonucleoside for all known DNA polymerases will prevent further catalytic elongation of additional nucleoside afterward [17,24].

For the first type, there are three commercially-available reversible terminators with a blocking group at 3′-OH (structures shown in **Figure 2A–C**): the 3′-ONH_2 reversible terminator developed by Dr. Steven A. Benner and his colleagues from Foundation for Applied Molecular Evolution (FfAME) [17,18]; the 3′-*O*-allyl reversible terminator created by Jingyue Ju and his colleagues from Colombia University [5]; the 3′-*O*-azidomethy reversible terminator developed and used by Illumina Solexa [12]. All three commercial reversible terminators were reported to show good performance in reversible termination function: achieving nearly 100% of 3′-O blocking efficiency and florescent label group cleavage after primer extension termination [12–18].

For the second type, hitherto, there is only one commercial 3′-OH unblocked reversible terminator named ‘‘virtual terminator’’, which is developed by Helicos BioSciences Corporation (**Figure 2D**) and employed by the first ‘‘single molecule’’ sequencer to hit the market in the emerging third generation of DNA sequencing platform [19]. In addition, the ‘‘Lightning terminator’’ recently developed by Michael L. Metzker’s group also belongs to 3′-OH unblocked reversible terminator (the lower panel in **Figure 2E**), and is unique in using UV light to cleave the fluorescent group [20–23].

Table 1 Comparison of four main second-generation sequencing platforms

Feature	Roche/454	Ion-torrent (318)	SOLiD5500	Hiseq2000
Read size (bp)	500–1000	~200	60	100–150
Throughput	700 Mb	1 Gb	180 Gb	600 Gb
Time required/run	23 h	2 h	14 days	8 days
Coverage/dpl h-genome	10×	20×	100×	60×
Run/dpl h-genome	43	60	0.5	0.33
Days/h-genome	43	5	7	8
Cost ($USD)/run	7000	4000	27,000	22,000
Cost ($USD)/h-genome	300,000	240,000	13,500	2400

Note: dpl stands for diploid.