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Application Brief

Genome Sequencer FLX System

Unidirectional Sequencing of Amplicon Libraries Using GS FLX Titanium emPCR Kits (Lib-L)

Summary

This Application Brief describes an alternative approach for the design of Amplicon libraries that enables the use of GS FLX Titanium emPCR Kits (Lib-L) for clonal amplification, as opposed to the Lib-A kits typically used for Amplicon library preparation. This method provides additional workflow flexibility when unidirectional sequencing of Amplicon libraries is desired.

454 Life Sciences Corp., a Roche Company
Branford, CT, 06405 USA

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim, Germany

Roche Diagnostics Corp.
Roche Applied Science
Indianapolis, IN, 46250 USA

Background

GS FLX Titanium emPCR Kits (Lib-A) provide reagents for bidirectional sequencing of Amplicon Libraries within a single kit. This is enabled by supplying individual "A" and "B" aliquots of DNA Capture Beads, Amplification Primer, Enrichment Primer, and Sequencing Primer. Most amplicon-based applications benefit from bidirectional sequencing and sufficient reagents have been supplied in the GS FLX Titanium emPCR Kit (Lib-A) to support this need. However, some users may prefer to employ unidirectional sequencing of amplicons, using only "A" or only "B" reagents. By alternating the use of "A" and "B"-only designs with GS FLX Titanium emPCR Kits (Lib-A) one can enjoy maximum flexibility of use with GS FLX Titanium emPCR Kits (Lib-A). However, an alternate approach is enabled in this Application Brief by which users who primarily wish to employ unidirectional sequencing may have similar flexibility in their experiments via the use of GS FLX Titanium emPCR Kits (Lib-L). The primary benefits of this approach are two-fold: users need not maintain or dispose of unneeded components (i.e. "B" components if only unidirectional "A" sequencing is desired); and having 70 million DNA Capture Beads of one type, as in the GS FLX Titanium emPCR Kits (Lib-L), enables greater flexibility with respect to the range of acceptable emPCR enrichment results regardless of the sequencing Run format being used (i.e. sufficient enriched beads are generated to employ the maximum recommended PicoTiterPlate loading density).

Amplicon Design

Amplicon library design principles described in the "Guidelines for Amplicon Design" section of Technical Bulletin 013-2009: Amplicon Fusion Primer Design Guidelines for GS FLX Titanium Series Lib-A Chemistry should be followed when designing primers for this application. The only exception is with respect to the design of the fusion primers, which must be modified to accommodate amplification using the GS FLX Titanium emPCR Kits (Lib-L) as follows:

Forward primer (Primer A-Key):

5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID-template-specific-3′

Reverse primer (Primer B-Key):

5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-MID-template-specific-3′

As in the Technical Bulletin, the blue text identifies the GS FLX Titanium Primer A and Primer B sequences, herein modified for use with GS FLX Titanium emPCR Kits (Lib-L). The four-base library "key" is shown in red, optional Multiplex Identifier (MID) tag in orange or yellow, and the template-specific region in green.

Amplicon libraries may be generated using this primer design strategy according to the *Amplicon Library Preparation Method Manual, GS FLX Titanium Series (October 2009)* and prepared for sequencing as outlined below.

emPCR Amplification

Caution: Amplicon libraries prepared using this method must be clonally amplified using GS FLX Titanium emPCR Kits (Lib-L), not GS FLX Titanium emPCR Kits (Lib-A) .

Depending on experimental needs, follow the procedures outlined in the emPCR Method Manual - Lib-L LV, MV, or SV for Large Volume, Medium Volume, or Small Volume emulsion formats, respectively. No modifications are required for this application and denaturing of library DNA is not required prior to adding this material to the DNA Capture Beads.

Sequencing

Amplicon libraries prepared according to these methods should be sequenced according to the *Sequencing Method Manual, GS FLX Titanium Series (October 2009)*. No modifications to the protocol are required to sequence these libraries. Importantly, as outlined in Section 3.2.3.2, Step 4 (p. 7), Amplicon Control Beads must be added when sequencing these libraries.

As with Amplicon Libraries generated using GS FLX Titanium emPCR Kits (Lib-A), the appropriate data processing pipeline must be employed. As outlined in Section 3.3.4, Step 9 (p. 17) of the *Sequencing Method Manual, GS FLX Titanium Series (October 2009)*, you must select "Full Processing for Amplicons" if you are performing on-instrument data processing. Off-instrument processing may also be performed and should be carried out using Amplicon Signal Processing.