10x GENOMICS®

TECHNICAL NOTE

Target Enrichment Using Custom Baits With The Chromium™ Genome Reagent Kit

INTRODUCTION

The Exome Demonstrated Protocol for "Application of the Chromium™ Genome Reagent Kits v2 for Exome Assays" (CG000059) provides detailed protocols for exome sequencing using the Agilent SureSelectXT Human All Exon V6 Reagent Kit. This Technical Note provides guidelines on how to prepare target enrichment libraries using probes from suppliers other than Agilent. Also highlighted are general protocol differences for the preparation of target enrichment libraries vs. whole genome libraries, both of which use the Genome Reagent Kits (CG00022 and CG00043).

METHOD

Modifications of the User Guide for Target Enrichment compared to the User Guide for Whole Genome Sequencing

The Exome Demonstrated Protocol has been modified compared to the Chromium™ Genome Reagent Kits v2 User Guide to provide optimal exome application performance using the Agilent SureSelectXT Human All Exon V6 Reagent Kit. Specific differences include:

- During GEM generation and barcoding, the input mass is increased from the standard 1.25ng for genome libraries to 1.5ng input for target enrichment libraries.
- The post GEM cleanup as well as SPRIselect cleanup throughout the library construction for target enrichment aim to maximize library recovery. As a consequence, the libraries contain adaptor dimers and are not suitable for sequencing without target enrichment.
- Shearing the sample prior to End Repair and A-tailing is required when taking samples through the target enrichment protocol. The target fragment size for Agilent SureSelectXT Human All Exon V6 Kit is between 150 to 200 bp. Therefore, the target shearing size of the post-GEM library is 225 bp which accounts for the Illumina Read 1 sequence and the 10x™ Barcode that are added to the molecules during GEM incubation.
- The Sample Index PCR cycles are increased from 10 cycles for genome libraries to 12 cycles for target enrichment libraries to ensure that library yields are sufficient for target capture.

Recommendation of library preparation modifications when using custom probes

When using target enrichment kits other than Agilent SureSelectXT kit the Exome Demonstrated Protocol is generally applicable, though it may require additional optimization tailored to the specific target enrichment kit. In addition, please follow the target enrichment kit manufacturer's protocol for target hybridization and capture, with the following recommendations:

- Follow the shearing recommendations provided by the target enrichment kit manufacturer, with a size

adjustment that is 40bp larger than a standard DNA sample. The increase of 40bp in target fragment size is to account for the Illumina Read 1 sequence and the 10x™ Barcode that are added to the molecules during the GEM incubation. For example, the target fragment size for Agilent SureSelectXT Human All Exon V6 Kit is between 150 to 200 bp. Therefore, the target shearing size for the post-GEM library is 225bp, 40bp larger than the target fragment size. Use recommended settings for the Covaris instrument to achieve the desired target peak size.

- The SPRIselect ratios specified in the Demonstrated Protocol aim to maximize library recovery, including fragments as small as 150bp. Adjust the SPRIselect ratio accordingly if the target fragment size for your target enrichment kit is larger than 200bp, along with an appropriate target shearing size.
- The SPRIselect ratios specified in the Demonstrated Protocol ensures maximal library recovery, which includes the presence of adaptor dimers. Therefore, if the SPRIselect ratio specified in the Demonstrated Protocol is maintained, it is not recommended to sequence these libraries prior to target enrichment.
- We recommend increasing the library input into target enrichment to 1 µg, as determined by Agilent Bioanalyzer smear analysis and described in section 4.7 c) of the Exome Demonstrated Protocol, to account for the presence of adaptor dimers and the differences in qPCR quantification between users.
- We strongly recommend that the library be lyophilized with IDT xGEN® Universal Blockers for blocking the Illumina sequencing handles (P5, P7, read 1, and read 2) to avoid off-target pulldown, even if blocking oligos for Illumina sequencing handles are supplied with the target enrichment kit.
- During target enrichment, all incubation and wash steps occurring at elevated temperatures are crucial to the success of target enrichment and should only be conducted in thermal cyclers with heated lid. Avoid using thermomixers except for room temperature incubations since thermomixers have inferior temperature control at elevated temperatures.
- Since the 10x libraries are indexed prior to target enrichment, use the standard Illumina P5 and P7 primers in the post-capture PCR.
- We recommend using Kapa Library Amplification Kit for post-capture library amplification.
- Always amplify the entirety of the captured library during post-capture library amplification, rather than amplifying a subset of the library (e.g. 50%). This ensures maximum library diversity for targeted sequencing.

DISCUSSION

The Chromium Genome Reagent Kit User Guide for Target Enrichment combined with this Technical Note provide guidance on preparing Target Enrichment libraries using probes from suppliers other than Agilent. Final libraries are ready for Illumina sequencing and can be analyzed with the Long Ranger™ analysis pipelines that leverage the Linked-Reads created by 10x™ Barcodes. This provides insight into haplotype phasing, structural variant detection, and greater accuracy in SNP and indel calling. Please see additional documentation on our support website for more detail.

REFERENCES

- Chromium™ Genome Reagent Kits v1/v2 User Guide (CG00022/CG00043)
- Exome Demonstrated Protocol – “Application of the Chromium™ Genome Reagent Kits v2 for Exome Assays” (CG000059)

Notices

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For technical information or advice, please contact our Customer Technical Support Division online at any time.

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