How to sequence single- and dual-indexed libraries together

Summary

- Single-index sequencing is ideal for fast processing of workflows that require a low level of multiplexing.
- Many researchers have turned to dual indexing for applications that require high levels of multiplexing, and there is a misconception that co-sequencing of single- and dual-indexed libraries together is challenging. In reality, combining these methods helps save time and costs.
- The addition of one simple step to the workflow of the Invitrogen™ Collibri™ Stranded RNA Library Prep Kit for Illumina™ Systems enables robust construction of single-indexed cDNA libraries for strand-specific RNA sequencing that can be co-sequenced with dual-index Collibri Stranded RNA or other dual-indexed libraries.

Introduction

Indexed sequencing allows multiple libraries to be pooled and sequenced together, and requires the addition of a unique identifier (index) to DNA samples during library preparation. Libraries are then pooled and sequenced, and index reads are used during downstream analysis to identify and separate the sample libraries.

The choice between single indexing (where one index read is performed) or dual indexing (where both an index 1 read and index 2 read are required) depends on various factors. The main benefit of single indexing is that it has a shorter run time, as only the i7 index needs to be read, and is ideal for workflows that don't involve high levels of library multiplexing or don't require ultrahigh resolution. However, the increased throughput of current DNA sequencing instruments means that sample multiplexing is attractive, making it more economically viable to combine single- and dual-indexed samples in a single workflow.

The ability to sequence single- and dual-indexed libraries together offers the potential to combine dual-indexed multiplexing of numerous libraries with single indexing of a few libraries in a single workflow, saving precious time. The Collibri Stranded RNA Library Prep Kit for Illumina Systems enables construction of cDNA libraries for strand-specific RNA sequencing using either single- or dual-indexed primers on Illumina next-generation sequencing (NGS) systems, from RNA samples of various quality, including FFPE samples. With a rapid and simple library preparation workflow, the kit provides high-quality, sequencing-ready libraries.

Method

Illumina NGS systems use different i5 index read strategies, but it is simple to co-sequence single-indexed and dual-indexed libraries together. After library preparation, all that is necessary is to specify the i5 index sequence in the sample sheet.

To pool single-indexed libraries prepared with the Collibri Stranded RNA Library Prep Kit for Illumina Systems with dual-indexed libraries, you will need to indicate an i5 index in the sample sheet, using the following sequences:

- TCTTTCCC for MiSeq™, HiSeq™ 2000, HiSeq™ 2500, and NovaSeq™ 6000 systems (Figure 1)
- AGATCTCG for iSeq™ 100, MiniSeq™, NextSeq™, HiSeq™ 3000, and HiSeq™ 4000 systems

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The reads can then be automatically identified, ready for further downstream analysis, reducing the need for two separate workflows.

[Header]												
IEMFileVersion	5											
Experiment Name												
Date												
Workflow												
Application												
Instrument Type												
Assay												
Index Adapters												
Description												
Chemistry	Default											
[Reads]												
[Settings]												
Adapter	AGATCGGAAGAGCACACGTCTGAACTCCAGTCA											
AdapterRead2	AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT											
[Data]												
Lane	Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Index_Plate_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description	
	1					IX001	ATGCCTAA	Universal	TCTTTCCC			
	2					IX002	GAATCTGA	Universal	TCTTTCCC			
	3					IX003	AACGTGAT	Universal	TCTTTCCC			
	4					IX004	CACTTCGA	Universal	TCTTTCCC			
	5					IX005	GCCAAGAC	Universal	TCTTTCCC			
	6					IX006	GACTAGTA	Universal	TCTTTCCC			
	7					IX007	ATTGGCTC	Universal	TCTTTCCC			
	8					IX008	GATGAATC	Universal	TCTTTCCC			
	9					IX009	AGCAGGAA	Universal	TCTTTCCC			
	10					IX010	GAGCTGAA	Universal	TCTTTCCC			
	11					IX011	AAACATCG	Universal	TCTTTCCC			
	12					IX012	GAGTTAGC	Universal	TCTTTCCC			
	13					IX013	CGAACTTA	Universal	TCTTTCCC			
	14					IX014	GATAGACA	Universal	TCTTTCCC			
	15					IX015	AAGGACAC	Universal	TCTTTCCC			
	16					IX016	GACAGTGC	Universal	TCTTTCCC			
	17					IX017	ATCATTCC	Universal	TCTTTCCC			
	18					IX018	GCCACATA	Universal	TCTTTCCC			
	19					IX019	ACCACTGT	Universal	TCTTTCCC			
	20					IX020	CTGGCATA	Universal	TCTTTCCC			
	21					IX021	ACCTCCAA	Universal	TCTTTCCC			
	22					IX022	GCGAGTAA	Universal	TCTTTCCC			
	23					IX023	ACTATGCA	Universal	TCTTTCCC			
	24					IX024	CGGATTGC	Universal	TCTTTCCC			

Figure 1. Example sample sheet for co-sequencing of libraries.

Conclusions

- Co-sequencing of single- and dual-indexed libraries saves times and increases efficiency.
- Only one additional step is required to perform co-sequencing of single- and dual-indexed libraries—specifying the i5 index sequence in the sample sheet.
- The Collibri Stranded RNA Library Prep Kit for Illumina Systems offers a rapid workflow for robust cDNA library preparation that includes single or dual indexes.

Find out more at **thermofisher.com/collibriRNA**

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