Fluent BioSciences

PIPseq™ UDI-96 Kit

User Guide

Doc ID: **FB0003694**
Revision: **1**
State: **Release**

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Introduction

The Fluent BioSciences PIPseq™ UDI-96 Kit enables multiplex sample preparation of PIPseq Single Cell 3' gene expression libraries for next-generation sequencing on the Illumina platform. Each kit provides 96 pre-mixed unique pairs of i5 and i7 index primers packaged in a single-use 96-well breakable plate with a pierceable foil seal.

Recommended Use

The recommended application for this kit is PIPseq library preparation of single cell 3' gene expression libraries to be sequenced on an Illumina platform. Users can break away a single column of 8 pre-mixed, unique indexes without the need to thaw the entire plate for each experiment. Further, the pierceable foil seal ensures single use of each pre-mixed index pair to avoid index contamination across wells. The volumes provided are sufficient for preparation of up to 96 reactions. The 96 well plate should be stored at -20°C.

The PIPseq UDI-96 Kit should only be used with Fluent BioSciences PIPseq 3' Single Cell RNA Kits. These indexes have not been tested for compatibility with other single cell library preparation kits or technologies.

Note: Users should not mix index primers provided by the PIPseq 3' Single Cell RNA Kits and the index primers in the UDI-96 Kit as the lengths of the sample indexes differ. Instead, it is recommended to utilize index primers from one kit or the other, as determined by the user's desired number of samples for multiplexing.

Users should refer to the kit specific User Guide prior to use of the PIPseq UDI-96 Kit primers for PIPseq library preparation.

Compatible PIPseq 3' Single Cell RNA Kits

Product Name	Catalog Number	User Guide
PIPseq T200 3' Single Cell RNA Kit v3.0	FBS-SCR-T200-2-V3	FB0003114
PIPseq T100 3' Single Cell RNA Kit v3.0	FBS-SCR-T100-2-V3	FB0003657
PIPseq T20 3' Single Cell RNA Kit v3.0	FBS-SCR-T20-4-V3	FB0002130
PIPseq T2 3' Single Cell RNA Kit v3.0	FBS-SCR-T2-8-V3	FB0001026

Index Plate Overview

The PIPseq UDI-96 plate provides 96 pre-mixed unique pairs of i5 and i7 index primers which are compatible with PIPseq 3' Single Cell RNA Kits. These pairs are sequentially labeled by column (Fig 1).

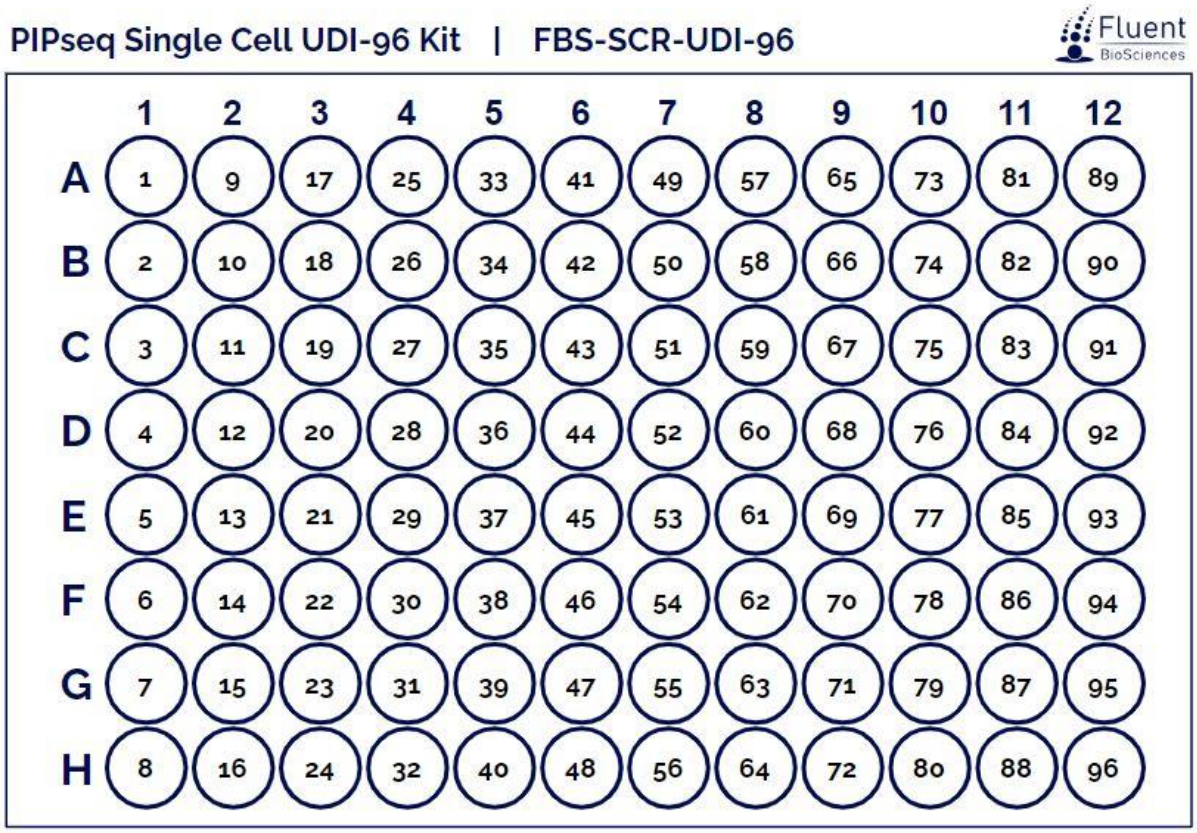

Figure 1. Plate map for the UDI-96 kit where each well contains a unique i5/i7 index pair and the wells are labeled sequentially by column. This configuration is intended to allow users to break apart the plate by column so that index pairs can be used most efficiently one row at a time.

PIPseq Single Cell Gene Expression Library Overview

The PIPseq 3' Single Cell gene expression library (Fig 2) comprises the standard Illumina paired-end sequences P5 and P7. Read 1 comprises the barcode information while Read 2 will contain the cDNA fragment information. PIPseq utilizes a unique mixture of Nextera and TruSeq sequences for Read 1 and Read 2, however these libraries are compatible for standard Illumina sequencing. The sample index lengths are 10 bases each for i5 and i7.

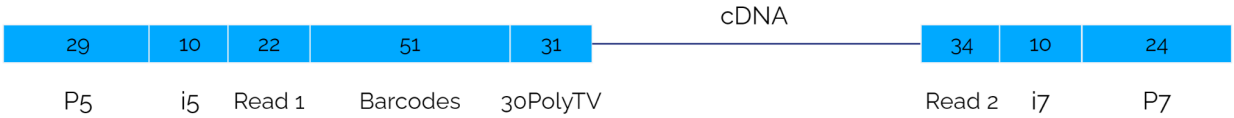

Figure 2. Structure of the PIPseq single cell gene expression library.

PIPseq UDI-96 Kit Protocol

Users should refer to the PIPseq kit-specific User Guide prior to use of the PIPseq UDI-96 Kit primers for PIPseq library preparation. The protocol presented below is an example sample indexing PCR that should replace the corresponding section within the PIPseq kit-specific User Guide.

Reagent Preparation

Upon receipt of the PIPseq UDI-96 Kit, store in a -20˚C freezer.

Prior to each experiment, determine the desired number of indexes necessary for sample multiplexing (see Index Pooling Guidelines). To break apart a column (strip), remove the plate from the -20˚C freezer, securely grip the desired strip and press along the perforation while pulling the strip away from the rest of the plate. Return the remainder of the plate to the -20˚C freezer. Thaw the index strip for 10 min at room temperature prior to use.

Sample Index PCR (< 96 samples)

⚠ **Before starting the Sample Index PCR, choose the appropriate sample index sets to ensure that no sample index combinations overlap in a multiplexed sequencing run (Index Pooling Guidelines for recommendations). There are 96 pre-mixed i5 and i7 indexes provided with this kit to allow for unique dual indexing of 96 samples. Note that each index well contains sufficient reagent volume for 1 individual reaction.**

1. Determine the number of libraries that will be amplified and pooled for sequencing while ensuring that a valid index combination is chosen according to color balance guidelines in the Index Pooling Guidelines section below.
2. Thaw the selected number of index strips at room temperature for 10 minutes.
3. Mix briefly by vortexing and then centrifuge the index strip on a benchtop minifuge to collect all of the primers at the bottom of the well.
4. Thaw Library Prep Mix B on ice. Once thawed, flick the tubes several times, pipette mix 10 times, and then briefly centrifuge to collect (do NOT vortex).
5. Orient the strip according to the provided plate map (Fig 1) to ensure the correct indexes will be removed. With a pipette tip, pierce the desired wells and transfer the volume of primer mix required for the PCR reaction (see step 6).

   ⚠ **CAUTION: Ensure pipette tips are not reused across wells to avoid cross-contamination of indexed primers. Do not reuse primer if the seal has been previously pierced to avoid cross-contamination of indexed primers.**
6. To the 20 µL of each PIPseq gene expression library, add the following in the order in which they appear in the table below.

Reagent	Volume Per Sample (µL)
Cleaned library DNA	20
UDI-XX (one well of UDI-96 plate)	5
Library Prep Mix B	25
Total	**50**

7. Mix the reactions by pipetting up and down 10 times at the 25 µL stroke, and briefly centrifuge to collect all liquid at the bottom of the tubes.
8. Proceed with the sample index PCR according to the specific PIPseq Kit specific User Guide.

Sample Index PCR (96 samples)

1. Thaw the UDI-96 plate at room temperature for 15 minutes.
2. Mix briefly by vortexing and then centrifuge the plate (280 x g for ~1 min) to collect all of the primers at the bottom of the well.
3. Thaw Library Prep Mix B on ice. Once thawed, flick the tubes several times, pipette mix 10 times, and then briefly centrifuge to collect (do NOT vortex).
4. Orient the plate according to the provided plate map (Fig 1) to ensure the correct indexes will be removed for each sample. With a pipette tip, pierce the desired wells and transfer the volume of primer mix required for the PCR reaction (see step 5).

⚠ **CAUTION: Ensure pipette tips are not reused across wells to avoid cross-contamination of indexed primers.**

5. To the 20 µL of each PIPseq gene expression library, add the following in the order in which they appear in the table below.

Reagent	Volume Per Sample (µL)
Cleaned library DNA	20
UDI-XX (one well of UDI-96 plate)	5
Library Prep Mix B	25
Total	**50**

6. Mix the reactions by pipetting up and down 10 times at the 25 µL stroke, and briefly centrifuge to collect all liquid at the bottom of the tubes.
7. Proceed with the sample index PCR according to the specific PIPseq Kit specific User Guide.

Index Pooling Guidelines

The PIPseq 3' Single Cell gene expression libraries may be pooled for sequencing, taking into account the differences in cell number and read depth requirements. Samples prepared with the PIPseq UDI-96 Kit will have distinct index combinations to avoid color balance failures in sample demultiplexing. Refer to Illumina documentation for discussion of appropriate color balance combinations for the selected sequencing platform. These libraries are dual-indexed with 10-base i5 and i7 indexes. Read 1 length must be ≥ 51 bases and the recommended read 2 length ≥ 66 bases. Sequencing depth will vary based on your application needs but it is recommended to start with a depth of 20,000 reads per cell.

Users may download a sample sheet with the index sequences for use with Illumina Experiment Manager from the PIPseq UDI-96 Kit product page at www.fluentbio.com.

Well position	Index ID	i5 bases in adapter sequence	i7 bases in adapter sequence
A1	UDI-1	TCGTGGAGCG	CGCTCAGTTC
A2	UDI-2	CTACAAGATA	GAATTGAGTG
A3	UDI-3	TATAGTAGCT	ATATGAGACG
A4	UDI-4	TGCCTGGTGG	CTTATGGAAT
A5	UDI-5	ACATTATCCT	TAATCTCGTC
A6	UDI-6	GTCCACTTGT	GCGCGATGTT
A7	UDI-7	TGGAACAGTA	AGAGCACTAG
A8	UDI-8	CCTTGTTAAT	TGCCTTGATC
A9	UDI-9	GTTGATAGTG	CTACTCAGTC
A10	UDI-10	ACCAGCGACA	TTCTACAGAA
A11	UDI-11	CATACACTGT	GAACATACGG
A12	UDI-12	GTGTGGCGCT	CCTATGACTC
B1	UDI-13	ATCACGAAGG	TAATGGCAAG
B2	UDI-14	CGGCTCTACT	GTGCCGCTTC
B3	UDI-15	GAATGCACGA	CGGCAATGGA
B4	UDI-16	AAGACTATAG	GCCGTAACCG
B5	UDI-17	TCGGCAGCAA	AACCATTCTC
B6	UDI-18	CTAATGATGG	TCCAATTCTA
B7	UDI-19	GGTTGCCTCT	CTAATGATGG
B8	UDI-20	CGCACATGGC	TCGGCCTATC
B9	UDI-21	GGCCTGTCCT	AGTCAACCAT
B10	UDI-22	CTGTGTTAGG	GAGCGCAATA
B11	UDI-23	TAAGGAACGT	AACAAGGCGT
B12	UDI-24	CTAACTGTAA	GTATGTAGAA
C1	UDI-25	GGCGAGATGG	TTCTATGGTT
C2	UDI-26	AATAGAGCAA	CCTCGCAACC
C3	UDI-27	TCAATCCATT	TGGATGCTTA
C4	UDI-28	TCGTATGCGG	ATGTCGTGGT
C5	UDI-29	TCCGACCTCG	AGAGTGCGGC
C6	UDI-30	CTTATGGAAT	TGCCTGGTGG

C7	UDI-31	GCTTACGGAC	TGCGTGTCAC
C8	UDI-32	GAACATACGG	CATACACTGT
C9	UDI-33	GTCGATTACA	CGTATAATCA
C10	UDI-34	ACTAGCCGTG	TACGCGGCTG
C11	UDI-35	AAGTTGGTGA	GCGAGTTACC
C12	UDI-36	TGGCAATATT	TACGGCCGGT
D1	UDI-37	GATCACCGCG	GTCGATTACA
D2	UDI-38	TACCATCCGT	CTGTCTGCAC
D3	UDI-39	GCTGTAGGAA	CAGCCGATTG
D4	UDI-40	CGCACTAATG	TGACTACATA
D5	UDI-41	GACAACTGAA	ATTGCCGAGT
D6	UDI-42	AGTGGTCAGG	GCCATTAGAC
D7	UDI-43	TTCTATGGTT	GGCGAGATGG
D8	UDI-44	AATCCGGCCA	TGGCTCGCAG
D9	UDI-45	CCATAAGGTT	TAGAATAACG
D10	UDI-46	ATCTCTACCA	CAGTAGTTGT
D11	UDI-47	CGGTGGCGAA	TATCCAGGAC
D12	UDI-48	TAACAATAGG	AGTGCCACTG
E1	UDI-49	CTGGTACACG	GGCCATCATA
E2	UDI-50	TCAACGTGTA	ACATGGTGTC
E3	UDI-51	ACTGTTGTGA	GACAGACAGG
E4	UDI-52	GTGCGTCCTT	TCTTACATCA
E5	UDI-53	AGCACATCCT	TTACAATTCC
E6	UDI-54	TTCCGTCGCA	AAGCTTATGC
E7	UDI-55	CTTAACCACT	TATTCCTCAG
E8	UDI-56	GCCTCGGATA	CTCGTGCGTT
E9	UDI-57	CGTCGACTGG	TTAGGATAGA
E10	UDI-58	TACTAGTCAA	CCGAAGCGAG
E11	UDI-59	ATAGACCGTT	GGACCAACAG
E12	UDI-60	ACAGTTCCAG	TTCCAGGTAA
F1	UDI-61	AGGCATGTAG	TGATTAGCCA
F2	UDI-62	GCAAGTCTCA	TAACAGTGTT
F3	UDI-63	TTGGCTCCGC	ACCGCGCAAT
F4	UDI-64	AACTGATACT	GTTCGCGCCA

F5	UDI-65	GTAAGGCATA	AGACACATTA
F6	UDI-66	AATTGCTGCG	GCGTTGGTAT
F7	UDI-67	TTACAATTCC	GATAACAAGT
F8	UDI-68	AACCTAGCAC	TTGTTCCGTG
F9	UDI-69	TCTGTGTGGA	AAGTACTCCA
F10	UDI-70	GGAATTCCAA	ACGTCAATAC
F11	UDI-71	AAGCGCGCTT	GGTGTACAAG
F12	UDI-72	TGAGCGTTGT	CCACCTGTGT
G1	UDI-73	ATCATAGGCT	GTTCCGCAGG
G2	UDI-74	TGTTAGAAGG	ACCTTATGAA
G3	UDI-75	GATGGATGTA	CGCTGCAGAG
G4	UDI-76	ACGGCCGTCA	GTAGAGTCAG
G5	UDI-77	CGTTGCTTAC	GGATACCAGA
G6	UDI-78	TGACTACATA	CGCACTAATG
G7	UDI-79	CGGCCTCGTT	TCCTGACCGT
G8	UDI-80	CAAGCATCCG	CTGGCTTGCC
G9	UDI-81	TCGTCTGACT	ACCAGCGACA
G10	UDI-82	CTCATAGCGA	TTGTAACGGT
G11	UDI-83	AGACACATTA	GTAAGGCATA
G12	UDI-84	GCGCGATGTT	GTCCACTTGT
H1	UDI-85	CATGAGTACT	TTAGGTACCA
H2	UDI-86	ACGTCAATAC	GGAATTCCAA
H3	UDI-87	GATACCTCCT	CATGTAGAGG
H4	UDI-88	ATCCGTAAGT	TACACGCTCC
H5	UDI-89	CGTGTATCTT	GCTTACGGAC
H6	UDI-90	GAACCATGAA	CGCTTGAAGT
H7	UDI-91	GGCCATCATA	CGCCTTCTGA
H8	UDI-92	ACATACTTCC	ATACCAACGC
H9	UDI-93	TATGTGCAAT	CTGGATATGT
H10	UDI-94	GATTAAGGTG	CAATCTATGA
H11	UDI-95	ATGTAGACAA	GGTGGAATAC
H12	UDI-96	CACATCGGTG	TGGACGGAGG

Oligonucleotide Sequences

Part Number	Name	Sequence (5' - 3')
FB0003671	Library P7 Index	CAAGCAGAAGACGGCATACGAGATXXXXXXXXXXGTCTC GTGGGCTCGGAGATGTGTATAAGAGACAG
FB0003672	Library P5 Index	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXXXA CACTCTTTCCCTACACGACGC

Document Revision Summary

Doc ID: **FB0003694**
Revision: **1**
Revision date: September 2022

General Changes:

- First revision

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