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How to Install Bioo Scientific's NEXTflex™ Barcode Indices for Illumina Experiment Manager

Introduction

Illumina Experiment Manager (IEM) is a user interface for assembling input CSV (comma-separated variables) data that the sequencer software can read directly. The information included has reagent barcode reference, run name, investigator name, sample names, read adapter sequences, indices used and other tracking information for the pooled samples. Since Bioo Scientific's indices are not included by default with IEM, specific index information needs to be added to the IEM data source.

Two Approaches

In order to incorporate NEXTflex indices, two methods are available:

Directly modify the resulting CSV file with corrected indices. All files generated by IEM can be opened in any text editor or Microsoft Excel after generation. At that point the user can directly modify indices (or any other element of the file) prior to loading into the Illumina sequencing software. While this may be the quickest option for a one-off sequencing run, we highly recommend setting up IEM with NEXTflex indices (method 2) so human input errors are minimized.

Set up custom SamplePrepKit files so indices are read directly by IEM. The remainder of this guide concerns setting up NEXTflex data files for repeated usability.

How to Set Up a SamplePrepKit File

IEM stores barcode sets as text files with a specific format. These barcode sets are then plugged into the various Applications listed in IEM. These barcode sets are labeled as SamplePrepKits.

Bioo Scientific has already generated the appropriate data files for the following NEXTflex Kits, which can be dowloaded below:

SamplePrepKit File Name	NEXTflex™ Barcode Kits Covered
NEXTflex™ 6nt Barcodes.txt	NEXTflex ™DNA Barcodes NEXTflex™ ChIP-Seq Barcodes NEXTflex™ PCR-Free Barcodes NEXTflex™ RNA-Seq Barcodes NEXTflex™ Bisulfite-Seq Barcodes* *Note: selected indices from full set
NEXTflex™ 8nt Barcodes.txt	NEXTflex-96™ DNA Barcodes NEXTflex-96™ ChIP-Seq Barcodes NEXTflex-96™ RNA-Seq Barcodes
NEXTflex™ 16S V4 12nt Barcodes.txt	NEXTflex™ 16S V4 Amplicon-Seq kits
NEXTflex™ 16S V1 – V3 12nt Barcodes.txt	NEXTflex™ 16S V1 – V3 Amplicon-Seq kits
NEXTflex™ qRNA 8nt Barcodes.txt	NEXTflex™ qRNA-Seq™ Kit

To install the above SamplePrepKit files, first locate the installation path for IEM. The default installation at time of writing on Windows 7 is:

C:\Program Files (x86)\Illumina\Illumina Experiment Manager

Within the **Illumina Experiment Manager** folder, navigate to the **SamplePrepKits** folder. Inside this folder are a number of pre-installed Illumina barcode sets.

Copy the appropriate downloaded **NEXTflex Barcodes.txt** file into the **SamplePrepKits** directory. The barcode set is now placed so IEM can read its contents.

# Plug-in the NEXTflex™ Barcode sets into IEM Applications

IEM requires that barcode sets are registered with each applicable application. Navigate back to the main directory and open the **Applications** folder. This folder should have a similar path to the below:

C:\Program Files (x86)\Illumina\Illumina Experiment Manager\Applications

The applications folder features separate sequencing build text files that provide specific settings. Amplicon pools may be handled differently from Resequencing, for example.

To incorporate the Bioo Scientific NEXTflex Barcode sets into a specific application, open the chosen application text file using a text editor such as Notepad on Windows. **You may need to open Notepad or other editors as Administrator in Windows 7 as IEM places write protections on its files.**

For example, I want to update **ChIP-Seq.txt** with Bioo Scientific's 6nt Barcodes. The file appears in Notepad below:
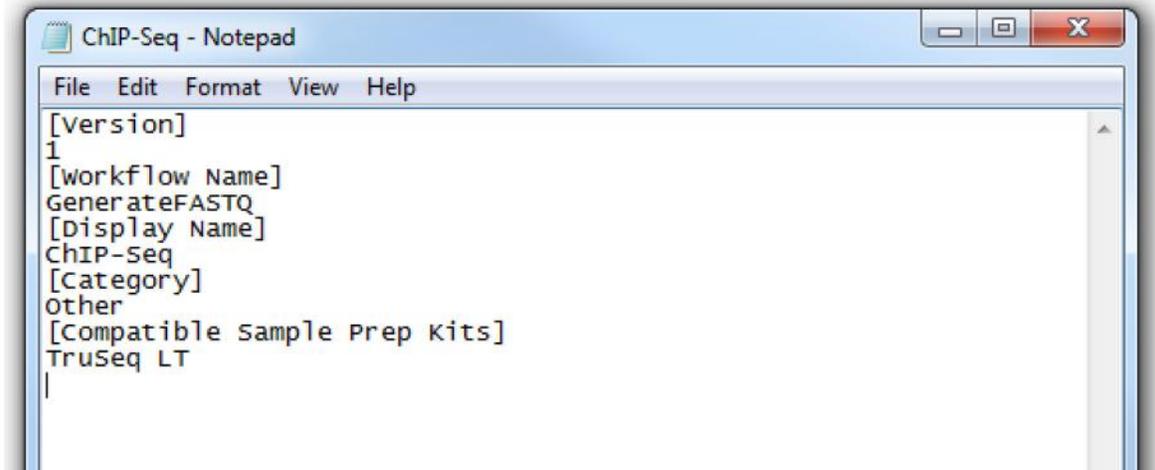
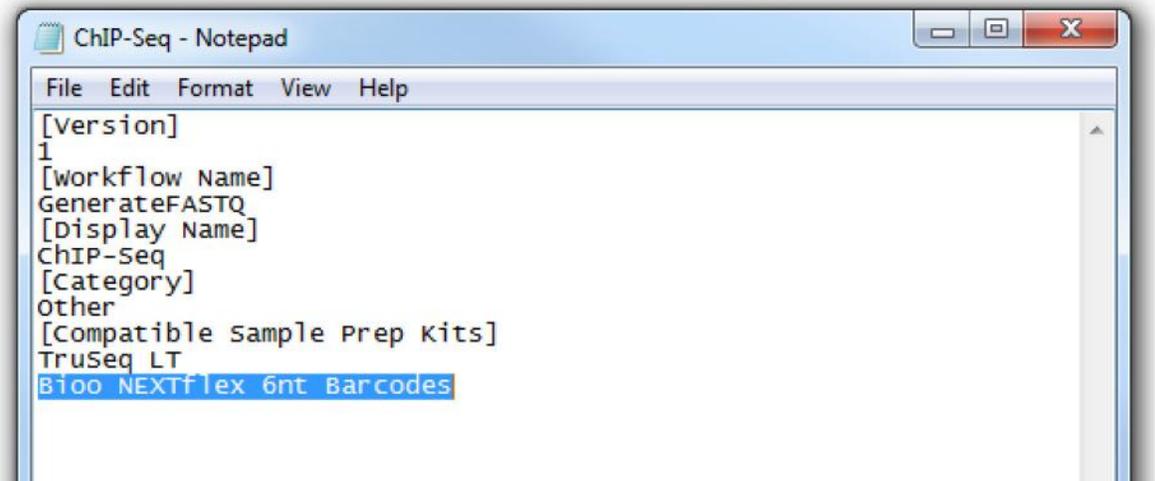To add the Bioo Scientific 6nt barcode set to the ChIP-Seq application, find the

[Compatible Sample Prep Kits]

line and paste the following underneath (in addition to other Sample Prep Kits mentioned):
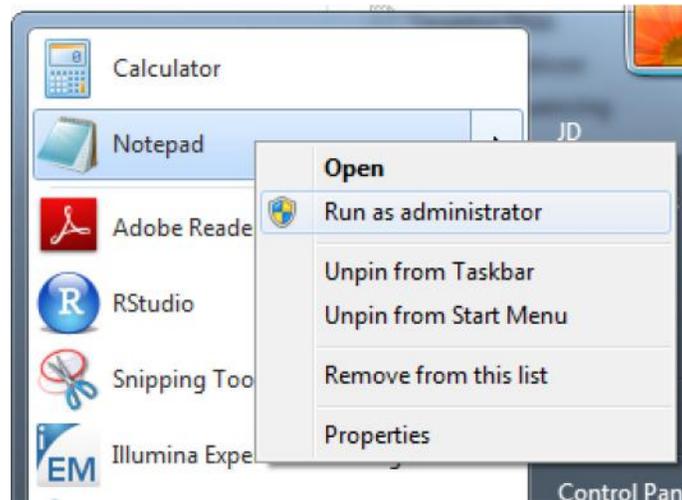
Bioo NEXTflex 6nt Barcodes

(For the 8nt Barcodes, paste Bioo NEXTflex 8nt Barcodes)

Below is the file after editing:

Save the file. If Notepad displays an Access Denied warning, reopen Notepad under Administrator mode and repeat the preceding steps.

Open IEM and Create a Sample Sheet

Now that the NEXTflex Barcode indices are properly set up, proceed with generating a new sample sheet for the next sequencing run as usual. When the Workflow Parameter screen appears, the Bioo NEXTflex adapters will be selectable in the barcode set dropdown menu:
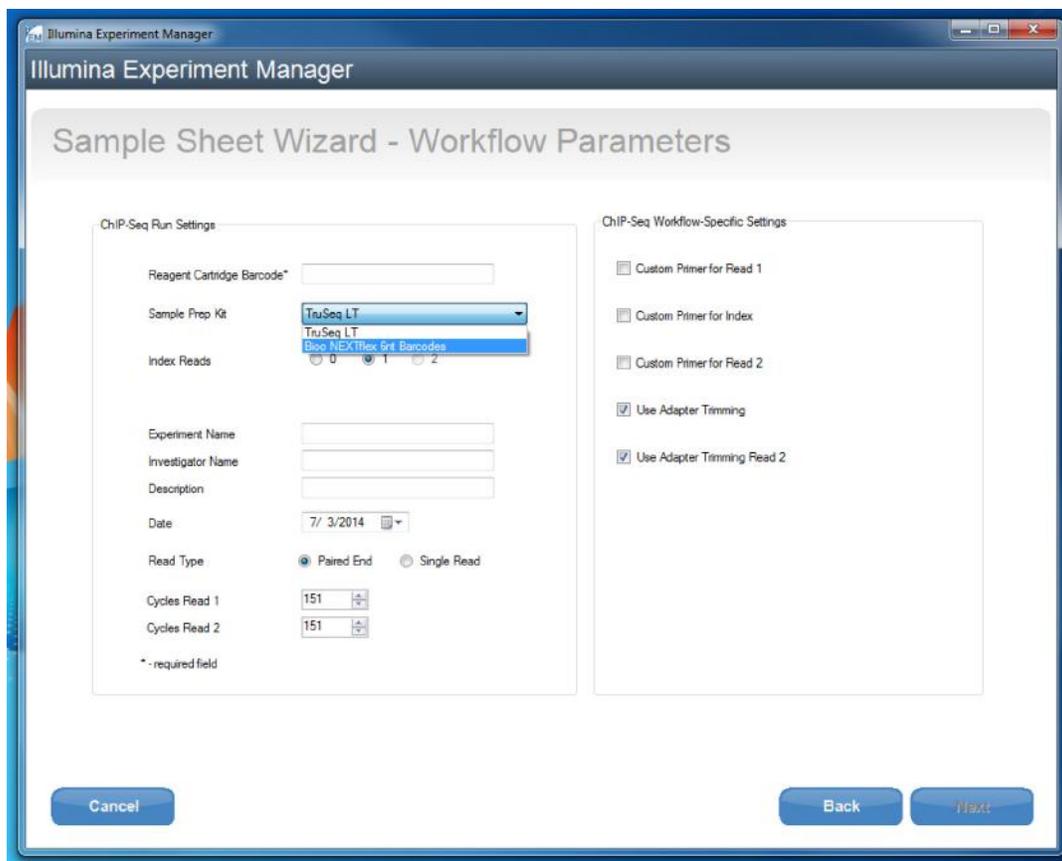
Moving to the Sample Selection spreadsheet, the Index1 (I7) column displays B001 through B048 (C001 to C096 for the 8nt Barcode set) which correspond to Barcodes 1 to 48 of the NEXTflex 6nt Barcode kits. At this point, the proper barcode indices have been implemented and are ready for repeated use through IEM.

If you have any questions, please contact Bioo Scientific at **1-888-208-2246, 1-512-707-8993** or by email at **BiooNGS@biooscientific**.com.