RTSF acquires NextSeq 500 and HiSeq 4000 Sequencers

(HiSeq 2500 will be retired February 15, 2017)

Both instruments have significantly Increased Output
Decreased Cost/nucleotide.

See Pages 2-3 for critical changes in library construction requirements

NextSeq System

Exome, transcriptome, and targeted resequencing.

HiSeq System

Production-scale genome, exome, transcriptome sequencing, and more.

	NextSeq System	HiSeq system	Current System
	NextSeq 500	HiSeq 4000	HiSeq2500
Output Range	20-120 Gb	125-1500 Gb	10-1000 Gb
Run Time	11-29 hr	<1-3.5 days	<1-6 days
Reads per Run	130-400 million	25-5 billion	up to 2 billion
Max Read Length	2 x 150 bp	2 x 150 bp	2 x 250 bp Rapid , 2 x 150 bp High Output

Price List for NextSeq 500 and HiSeq4000 - Effective February 1, 2017

https://rtsf.natsci.msu.edu/genomics/pricing/

Important changes for labs who construct their own libraries for sequencing on the HiSeq4000.

Library Preparation:

- Libraries must now have an insert size of 450 bp. or smaller. The shorter fragments will cluster more efficiently than larger fragments. Therefore, if your library size distribution is wide, you will have an imbalance of reads resulting in the majority of your sequences coming from the smaller library molecules.
- If using the Illumina TruSeq DNA Nano kit, follow the protocol using the 350bp insert size. This is achieved by doing a magnetic bead size selection. The beads are included within the kit.
- If using the Illumina TruSeq stranded mRNA library preparation kit, the standard fragmentation time is 8 minutes. This will result in libraries with inserts ranging from 120 to 200bp with a median size of 150 bp. For other insert sizes see table below:

  To modify the fragmentation of the RNA to allow for longer RNA fragments, the time of fragmentation can be shortened. This can be accomplished during the Purify and Fragment mRNA procedures by modifying the thermal cycler Elution 2 - Frag - Prime program: 94°C for X minutes followed by a 4°C hold for the thermal cycler. Determine X based on the length of the desired RNA. A range of suggested times and sizes is described in Table 21.

Table 21

Library Fragmentation Time

Time at 94◦C (Minutes)	Range of Insert Length (bp)	Median Insert Length (bp)	Average Final Library Size (Bioanalyzer bp)
0	130-150	200	467
1	130-310	190	439
2	130-290	185	410
3	125-250	165	366
4	120-225	160	326
8	120-210	155	309
12	115-180	140	272

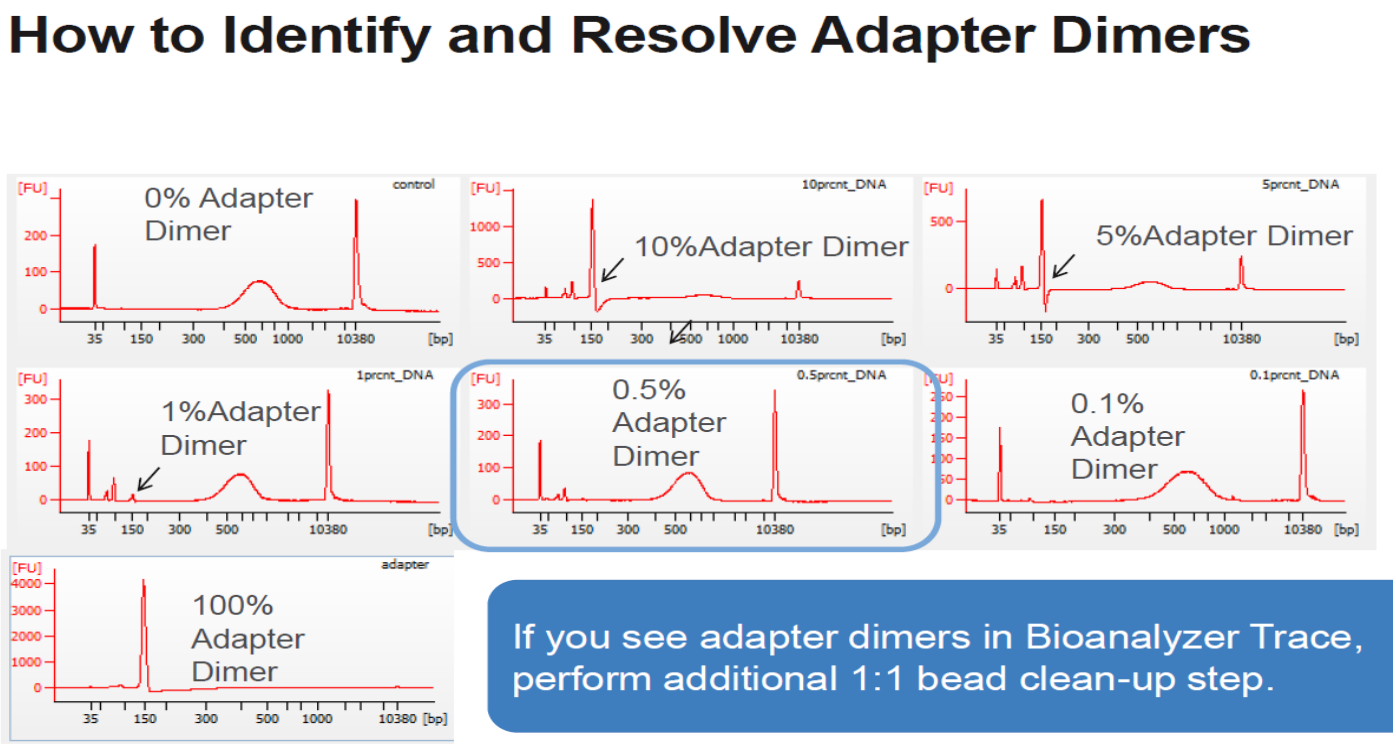
Insert length determined after clustering and sequencing with a paired-end sequencing run.

Skip the incubate RFP procedures (fragmentation) for samples requiring 0 minutes fragmentation time. Instead, place the sealed plate on the pre-heated thermocycler. Cole lid and incubate plate at 80 degrees for 2 minutes to elute the primed mRNA form the RNA Purification Beads. Then, immediately place the plate on the magnetic stand and proceed to the Synthesize First Strand cDNA process.

- For other library preparation kits and methods, please review their instructions on size selection as each library preparation protocols will have different methods.

- **Library preparation clean-up:**

With the preference for amplification of smaller fragments with the HiSeq 4000, it is important to clean-up your libraries to ensure that you do not have a large amount of adaptor contamination. If adaptor contamination is greater than 1%, you will significantly reduce the number of true reads. The optimal target is less than 0.5%. For example, if your adaptor contamination is 5%, you could have up to 60% of your reads being from adaptor molecules.



Adaptor Dimers are formed when the P5 and P7 adaptors ligate to one another rather than to an inserted library.

Sources of Primer and Adaptor Dimer formation:

- Poor Quality of Input material
- Insufficient input amount
- Clean-up method. If using beads, improper bead handling
- Enzymatic failures

Please see us if you have any questions regarding library preparation and the new instruments.

Additional Information:

https://www.illumina.com/systems/hiseq-3000-4000/applications.html

https://rtsf.natsci.msu.edu/_rtsf/assets/File/HiSeq4k_NextseqMSU.pdf