

Division of Science Sequencing Core Facility Overview



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Sample Preparation

Flow Cytometry

- Sort subpopulations of cells
- Sort single cells



Fluidigm C1

- Isolate single cells on microfluidic chip
- Lysis, RT, and amplification on chip



Nucleic acid isolation

- Manual – Phenol, Column, Bead
- Automated (AutoGenprep 965, ABI 6100)

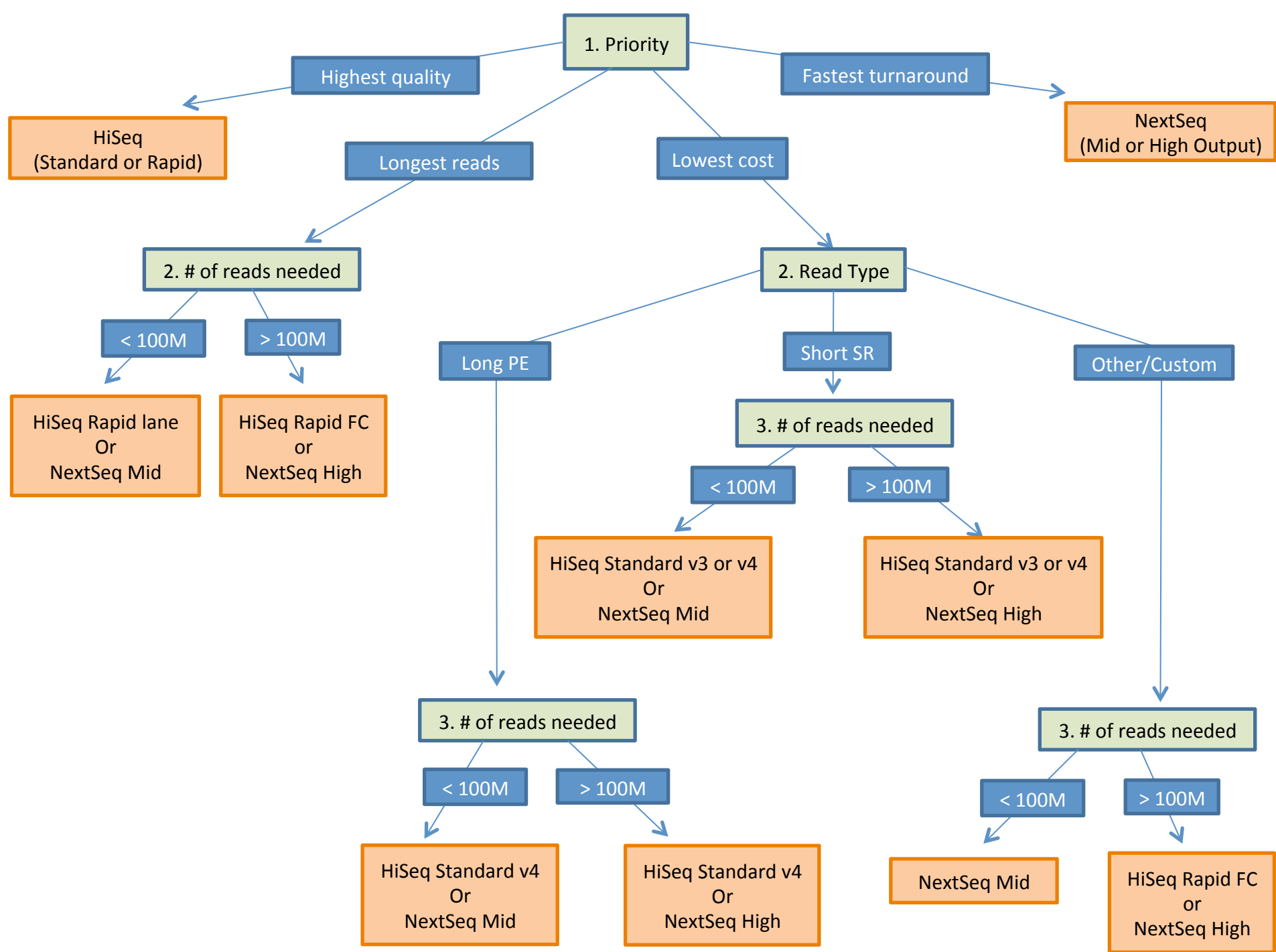


RNAseq Guidelines

Application	Description	Read Length	Number of Reads	PE
Gene expression profiling (GEX)	<ul style="list-style-type: none">• Gene level abundance	1x50 bp	≥10M	no
mRNA-Seq	<ul style="list-style-type: none">• Transcript level abundance• Discover novel features• Variant detection	2x75 bp	≥25M	yes
Enriched RNA-Seq	<ul style="list-style-type: none">• Coding Region Interrogation• High Splice Junction Sensitivity• Discover rare mRNA features• Variant detection	2x75 bp	≥25M	yes
Total RNA-Seq	<ul style="list-style-type: none">• Coding + ncRNA Interrogation• Transcript level abundance• Discover novel features• Variant detection	2x75 bp	≥50M	yes

Special Considerations:

- Low diversity libraries
- Lack of reference sequence



Choosing a Library Preparation Method

- Experimental Goal
- Species-related restrictions
- Starting RNA quantity
- Multiplexing strategy
- Automated vs. manual prep
 - Automation adds ~\$50/sample but saves time & improves consistency
- Self-run vs. core service
 - Cost: Service adds ~\$120/sample (including ~\$70 for QC/Quant)
 - Quality: Service guarantees sequencing output
 - Turnaround: Service = 2 weeks, self-run = 2-3 days



Ancillary Equipment



Shearing

- Covaris
- g tube

Size selection

- Bead-based
- Pippin Prep
- E-gel



Library QC and Quantification

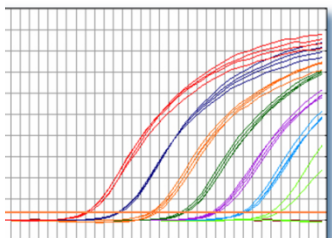
Nanodrop: Nucleic acid purity (salts, organics)



Qubit: Accurate nucleic acid concentration

Bioanalyzer / TapeStation:

Nucleic acid concentration,
Library size, Adapter dimers



QPCR: Concentration of adapter-ligated inserts

Sequencing Instruments


	HiSeq		NextSeq	
	High Output	Rapid Run	High Output	Mid Output
Max Clusters/ Lane	250M	150M	400M	130M
Cost/Base	\$	\$\$	\$	\$\$
Wait times	Long	Medium	Short	Short
Run Time (days)	3-6	1 - 2	1 – 2	1 – 2

Sequencing Cost Examples

	Clusters/ Lane	Cost
High Output SR 50	200M	\$912
Next Seq High SR 75	400M	\$1675
Next Seq Mid PE 75	130M	\$1396
Rapid Lane PE 150	150M	\$2302
Next Seq High PE 150	400M	\$4188

Sequencing Submission

← https://bauer-minilims.rc.fas.harvard.edu/minilims/plugins/Core/logout.php ★ Google 🔍 ⚙️ ⬇️ 🏠 🔑




Bauer Center Sequencing Core
FAS Center for Systems Biology

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Username

Password

Login



[\[New users register here\]](#)

✕ 1P

Sequencing Submission

Group_Member
PI
Sample
Alert
Line_Item
Invoice
User
Samples
Submission
Reagent
Reagent_Request
Sequencing_Price

Billing

Line_Item
Invoice
Invoice Generator

Sequencing

Make Sequencing
Request
Submissions
QC Lanes
Billable Items

Reagents

Make Reagent
Request
Reagents
Reagent Requests
Add New Reagent

Adding Submission

Cancel Submit

* Name	<input type="text" value="SUB00826"/>	
Illumina Run	<input type="text"/>	
Run Type	<input type="text"/>	
* Status	<input type="text" value="NEW"/>	⬆ ⬇ ⬆
* Group	<input type="text" value="Bauer_Core"/>	⬆ ⬇ ⬆ +
* Contact Name	<input type="text" value="Claire_Reardon"/>	⬆ ⬇ ⬆ +
* Email	<input type="text" value="claire@cgr.harvard.edu"/>	
* Phone	<input type="text" value="617-384-7913"/>	
* PI	<input type="text" value="Claire_Reardon"/>	
* PO/Billing Code	<input type="text"/>	?
* Billing Type	<input type="text" value="Billing_Code"/>	⬆ ⬇ ⬆
PO File	<input type="button" value="Browse..."/> No file selected.	?
Index List	<input type="button" value="Browse..."/> No file selected.	?
Destination Directory	<input type="text"/>	?
Comments	<input type="text"/>	?

* = Required field

Sequencing Submission

Create Flowcell
Illumina Flowcells

Run Data

Illumina Runs
Illumina Lanes
Lane Libraries

Enter details about your Sample(s) below.

Sample details [Add Sample](#)

No.	Project	Group	External_ID *	Run_Type *
1		Bauer_Core		

continued....

No.	Sample_Type *	Volume_(ul) *	Concentration *	Concentration_Units *
1				

continued....

No.	Fragment_Size_(bp) *	Sample_Prep_Kit_Used *	Read_Length *	Read_Type *
1				

continued....

No.	Low_Diversity_Library *	Index_Type *	Is_Dual_Indexed	Index_Sequence ?
1				

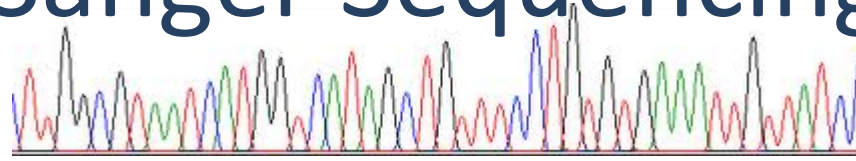
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No.	Reference_Genome *	Qubit_Performed *	Bioanalyzer_Performed *	Bioanalyzer_File
1				Browse... No file selected.

Sequencing Timeline

Library preparation:	self-run: 1 – 3 days service: 1 week
Library QC and quantification:	self-run: 1 – 2 days service: 1 week
Online submission + drop-off:	self run: minutes
Wait for flow cell to fill:	Full flow cell: None Single lane: days - weeks
Sequencing run time:	Rapid Run or NextSeq: 1-2 days High Output: 3 – 6 days
Analysis:	2 days

Sanger Sequencing



Applications:

- Plasmid Sequencing
- Genotyping, Microsatellite

Run Types:

Staff-Run:

- \$2.50/reaction
- User performs BigDye reaction and cleanup
- Drop off by 5pm, data delivered 9 am next day

Self-Run:

- \$38 per plate, \$6.33 per 16 samples
- User performs all steps
- Training to use ABI 3730xl and ABI 3130xl sequencers



Questions?

Sequencing Staff :

illumina_submission-list@lists.fas.harvard.edu

Sequencing User List:

https://lists.fas.harvard.edu/mailman/listinfo/high_throughput_sequencing

Sanger Sequencing:

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Claire Reardon:

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