

An Introduction to NGS at UT: The UT GSAF

Scott Hunicke-Smith

Updated: 9/23/14

Where to find more information

gsaf.cssb.utexas.edu

THE UNIVERSITY OF TEXAS AT AUSTIN Spaces People Browse Create Search

Genomic Sequencing and Analysis Facility User Support Wiki

Home Page Edit Share Tools

Ready to submit samples? Use these links

7/25/13 NOTE: UT's new version of Confluence requires you to "Log In" in the upper right corner BEFORE selecting an online submission form link.

To submit a new project or get detailed pricing information, use these links. You'll be given a 6-digit request ID to refer to your request later. Your requests stay here until you're ready to submit samples. You return to this link when you're ready to submit your samples.

- 1. Log in in the upper right corner with your EID (don't have an EID, or haven't submitted a sample to us before? [Please go read this page](#)).**
- 2. Choose your project type:**
 - 1. Bacterial metagenomics project** - Simplified submission page for bacterial 16S metagenomics (**you must log in first - upper right corner**)
 - Metagenomics assay: This service starts with normalized (i.e. equal concentration) DNA samples and includes amplification of the V4/V5 regions of the bacterial 16S gene [as shown on this web page](#) and at least 10,000 2x250 bp paired-end sequences from the Illumina MiSeq platform for at least 95% of the samples submitted. PCR is performed in triplicate to minimize jackpot effects. Gel-based QC is performed on a sampling of 10% of the samples post amplification. DNA is expected as input; the GSAF does not provide DNA extraction services at this time. [See this web page for sample input guidelines and note that DNA concentrations should be normalized before submission.](#) (The GSAF can normalize your sample concentrations for an additional charge.)
 - 2. All other projects (you must log in first - upper right corner)**
 - Download sample submission template (required for jobs with more than 30 samples; optional otherwise)** for the general, non-bacterial metagenomics submission page ONLY: Projects must be submitted via the online project submission form above to specify **project** details like platform, contact info, etc., but if you'd rather fill out an Excel template for your **sample** info instead of the web form, use this template. You'll upload it when you request a new sequencing job. This form is required for more than 30 samples; suggested for more than 10.

Latest news

11 Sep 14
Scott Hunicke-Smith presented GSAF updates at the Byte Club meeting. The presentation covers new user-accessible equipment, the NextSeq 500 and HiSeq V4 upgrade, pricing changes, new library prep methods, and new data storage options. [Here is a PDF copy of the presentation.](#)

Search

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- Companion Animal Interest Group: Information
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What the GSAF does

- Sample QC
 - By Fluorimetry/Qubit and BioAnalyzer
 - Self-service and full-service
- Library Preparation
 - Construction of NGS sequencing libraries from DNA and RNA of all sorts
- Sequencing
 - Final sequencing library quantification
 - Operation of the Illumina HiSeq 2500, NextSeq, and MiSeq systems
- Advise and consult
 - On experimental designs, lab methods, and cost

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850 BioAnalyzer runs/year
273 384-well qPCR plates/year

7,500 samples/year,
190 sequencer runs/year

800 jobs (projects)/year

What the GSAF does not do

- Data analysis
 - See the [CSSB/CCBB Bioinformatics Consulting Group](#) or
 - Investigate [data analysis training available at UT](#) (both via CCBB)
- Design your experiment
 - You, the customer, are responsible for your experimental design including the detailed choices of library preparation, sequencer run type, etc.
 - Illumina has some excellent tools and training videos that can assist when planning out projects, please visit <http://support.illumina.com/> there is also an excellent resource for calculating coverage at http://support.illumina.com/downloads/sequencing_coverage_calculator.html
 - The coverage calculator will be extremely helpful when submitting your samples for sequencing and you need to determine the number of reads to request for each sample

How do I start a project with the GSAF?

The screenshot displays the GSAF (Genomic Sequencing and Analysis Facility) website interface. At the top, there is a navigation bar with the University of Texas at Austin logo and links for Spaces, People, Browse, and Create. A search bar is also present. The main content area is titled 'Submit Any Other Project Request' and includes a brief description: 'Enter info about your project and samples and see what our prices are. You'll be given a 6-digit request ID to refer to your request later. Your requests stay here until you're ready to submit samples.'

The form is divided into two main sections: 'Provide Sequencing Job Information' and 'Payment, PI and Contact Information'. The 'Provide Sequencing Job Information' section includes three text input fields for 'Brief Job Description (truncated at 255 characters)', 'Pro- or Eukaryote/Organism', and 'Type of Bioinformatics Analysis Desired, if any'. The 'Payment, PI and Contact Information' section includes a dropdown menu for 'P.I. *', a text input field for 'UT Account/Grant or PO Number *', and a text input field for 'Contact Name *'. A left sidebar contains a search bar and a list of navigation links, with 'Submit Any Other Project Request' highlighted.

How NGS sequencers work

- Please see this YouTube video for a great illustration:
 - <http://www.youtube.com/watch?v=77r5p8IBwJk>
- Important practical conclusions:
 - Sequencing library DNA must be properly formed and suited to your question
 - The quality and quantity of sequencing library is essential

Sequencer run types & price

	Run Type	2014 rates	2013 rates	\$/Mbp	\$/ (M reads)
HiSeq 2500 H.O. v3	1x50	N/A	\$955	\$0.12	\$5.85
	1x100	N/A	\$1,318	\$0.08	\$8.08
	2x50	N/A	\$1,656	\$0.10	\$10.15
	2x100	N/A	\$2,317	\$0.07	\$14.20
HiSeq 2500 H.O. v4	1x50	\$1,013	N/A	\$0.09	\$4.66
	2x75	\$2,091	N/A	\$0.06	\$9.61
	2x100	\$2,428	N/A	\$0.06	\$11.16
	2x125	\$2,766	N/A	\$0.05	\$12.72
NextSeq 500 H.O.	1x75	\$1,999	N/A	\$0.08	\$6.06
	2x75	\$3,329	N/A	\$0.07	\$10.09
	2x150	\$5,025	N/A	\$0.05	\$15.23
MiSeq V3	2x300	\$1,813	\$1,720	\$0.14	\$82.40
MiSeq V2	2x250	\$1,396	\$1,321	\$0.21	\$107.35

- All run types indicate (# of reads) x (read length)
 - e.g. “1x50” means single-end read, 50 bp long
- Any run type can be single- or dual-indexed

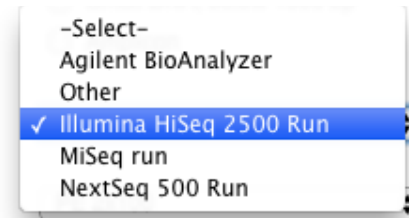
Sequencer run types & price

Requested Platform (will be set automatically for Metagenomics or ddRAD) *

Requested RunType (will be set automatically for Metagenomics or ddRAD) *

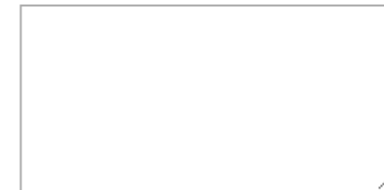
Buy sequencing by: (will be set automatically for Metagenomics or ddRAD) *

Special Sequencing Notes: ONLY for comments pertaining to running the Illumina Sequencer, lane layout, etc. DO NOT put LIBRARY PREPARATION instructions here.



A dropdown menu with the following options: -Select-, Agilent BioAnalyzer, Other, Illumina HiSeq 2500 Run (selected), MiSeq run, and NextSeq 500 Run.

- Fraction of Plate
 Number of Reads



An empty rectangular text input field with a small cursor icon in the bottom right corner.

Buying per lane? Select "Fraction of Plate", then for HiSeq 2500 High Output lanes, enter 12.5% for each lane, for HiSeq 2500 Rapid lanes, enter 50% for each lane, and for MiSeq lanes - one run is one lane (i.e. 100%).

- All run types indicate (# of reads) x (read length)
 - e.g. “1x50” means single-end read, 50 bp long
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Sequencer run types & price

Requested Platform (will be set automatically for Metagenomics or ddRAD) *

Requested RunType (will be set automatically for Metagenomics or ddRAD) *

Buy sequencing by: (will be set automatically for Metagenomics or ddRAD) *

Special Sequencing Notes: ONLY for comments pertaining to running the Illumina Sequencer, lane layout, etc. DO NOT put LIBRARY PREPARATION instructions here.

Illumina HiSeq 2500 Run

- ✓ -Select-
- PE 2x100
- PE 2x125
- PE 2x50
- SR 100
- SR 50

- All run types indicate (# of reads) x (read length)
 - e.g. “1x50” means single-end read, 50 bp long
- Any run type can be single- or dual-indexed

Sequencer layouts & capacity

- Data capacity per run:
 - HiSeq: 8 lanes/run, 220 million reads per lane
 - NextSeq: 1 lane/run, 330 million reads per lane
 - MiSeq: 1 lane/run, 13 or 22 million reads per lane
- Barcode capacity per run:
 - Single-indexes: typically MAX of 12 per lane, unless prepared by the GSAF who have >48 barcodes available
 - Dual-indexes: typically >12, up to 400 per lane
 - Single- and dual-indexes can be run in the same lane IF they can be separated after the run

Purchasing “by read”

- The UT GSAF is known for selling “by the read” and not just “by the lane”
- What this means:
 - Target vs. Minimum:
 - We aim to sequence “Target”
 - If we fall below Minimum, we will automatically re-queue your sample for more sequencing
 - You will be billed for the LESSER of Target or what you actually receive
 - Minimum order per instrument:
 - HiSeq: 20 million read-pairs
 - MiSeq: 1 million read-pairs
 - NextSeq: > of 20 million reads per sample or 40 million reads per job

Purchasing “by read”

“Fraction of Plate” allows you to enter a % below

Buy sequencing by: (will be set automatically for Metagenomics or ddRAD) *

- Fraction of Plate
- Number of Reads

Special Sequencing Notes: ONLY for comments pertaining to running the Illumina Sequencer, lane layout, etc. DO NOT put LIBRARY PREPARATION instructions here.



Buying per lane? Select "Fraction of Plate", then for:
HiSeq 2500 High Output lanes, enter 12.5% for each lane
MiSeq lanes - one run is one lane (i.e. 100%).
NextSeq 500 lanes - one run is one lane (i.e. 100%).

Fraction of Plate for Job

%

Enter Sample Information *

- Manually
- Attachment

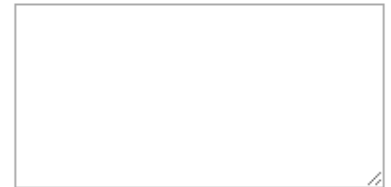
Purchasing “by read”

“Number of Reads” allows you to enter a number PER-SAMPLE on a later screen or via Excel file upload

Buy sequencing by: (will be set automatically for Metagenomics or ddRAD) *

- Fraction of Plate
 Number of Reads

Special Sequencing Notes: ONLY for comments pertaining to running the Illumina Sequencer, lane layout, etc. DO NOT put LIBRARY PREPARATION instructions here.



Buying per lane? Select "Fraction of Plate", then for:
HiSeq 2500 High Output lanes, enter 12.5% for each lane
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NextSeq 500 lanes - one run is one lane (i.e. 100%).

Enter Sample Information *

- Manually
 Attachment

Library Types

- DNA
 - Whole genome
 - Exome
 - ChIP
 - Amplicon
 - RAD
- RNA
 - Whole transcriptome – poly-A(+) or Ribosomal(-)
 - Small RNA
 - RIP

Library Types

- DNA – listing on LIMS

Library Prep Required *

Library Types: NOTE - if you require special library prep handling (at extra cost), you must select "Special Fragmentation" and enter details under the Library Type Specifications field

Yes
 No

Standard DNA Library
 Mate-Pair Library
 Exome or Custom Capture
 Special Fragmentation
 DNA low cost high throughput
 ddRAD genotyping
 ddRAD development phase 1
 ddRAD development phase 2
 16S Metagenomics

- RNA – listing on LIMS

Library Prep Required *

Library Types: NOTE - if you require special library prep handling (at extra cost), you must select "Special Fragmentation" and enter details under the Library Type Specifications field

Yes
 No

RNA low cost high throughput
 Standard RNA Seq Library
 DSN-normalized Library
 Ribosomal Removal
 Poly-A mRNA capture
 Special Fragmentation
 No RNA Enrichment

Library Types

- DNA

-
-
-
-
-

- Standard DNA Library
- Mate-Pair Library
- Exome or Custom Capture
- Special Fragmentation
- DNA low cost high throughput
- ddRAD genotyping
- ddRAD development phase 1
- ddRAD development phase 2
- 16S Metagenomics

- RNA

-
-
-

- RNA low cost high throughput
- Standard RNA Seq Library
- DSN-normalized Library
- Ribosomal Removal
- Poly-A mRNA capture
- Special Fragmentation
- No RNA Enrichment

What Reagents do I use for Library Prep if I want to do it myself?

- There are **many options** to choose from when selecting library prep reagents
- The GSAF mainly uses New England BioLabs (NEB) for all their library prep reagents
 - Manuals are easy to follow
 - Reagents are well priced
 - You can purchase the reagents as a “kit” or as individual modules of the kit, which can be very helpful!
- We’ve also published our protocols in Wiley’s Current Protocols:
 - PMID: 24984855 – DNA
 - PMID: 24733242 - RNA

Library Types & Sequencing

- Important concepts:
 - Single-end vs paired-end sequencing
 - Single-indexing, dual-indexing, and inline indexing

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Genomic Sequencing and Analysis Facility User Support Wiki / Home Page / Oligo Sequences

Illumina - all flavors

1 Added by Scott Patrick Hunicke-Smith, last edited by Scott Patrick Hunicke-Smith on Sep 24, 2014 (view change)

If this page isn't formatted well on your screen, try shrinking the left side bar.

Canonical ILLUMINA library design as of June 2012 (all 5'-3'), "TruSeq V3": NOTE all sequences shown are TOP STRAND 5' to 3'

<P5 primer/capture site><IndexRead2><Read1 primer site><template - gDNA, RNA, amplicon, whatever><Read2 primer site><IndexRead1><P7 primer/capture site>

If you'd like a different description, [this one from the Tufts core facility is quite good](#).

NOTE THAT THE SHADED PORTIONS SHOULD NOT BE CHANGED if you are designing your own primers!! The only flexibility one has is in the "template" section and in the two "index read" sections. Every other nucleotide shown matters as-is.

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<P5 primer/capture site><IndexRead2><Read1 primer site><template - gDNA, RNA, amplicon, whatever><Read2 primer site><IndexRead1><P7 primer/capture site>

primer or adaptor sequences, note also the reverse-complement of this is the Read 2 sequencing primer, but the Read 2 sequencing primer includes the 1 corresponding to the dA insert tail so sequencing starts with the insert)

6. **IndexRead1:**

The index sequence (usually 6 bp) - see many examples below in the **Barcodes** section. Within a lane, image analysis works best with as much base diversity as possible.

7. **P7 PCR primer/flowcell capture site:**

ATCTCGTATGCCGTCTTCTGCTTG

How do I start a project with the GSAF?

- Job Submission example
 - Saving vs. Submitting your job

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Genomic Sequencing and Analysis Facility User Support Wiki / ... / Create a Job Request

Submit Any Other Project Request

Added by Natalie B Hunt, last edited by Natalie B Hunt on Apr 09, 2014 (view change)

Enter info about your project and samples and see what our prices are. You'll be given a 6-digit request ID to refer to your request later. Your requests stay here until you're ready to submit samples.

Job Price Estimate for RequestID: 8c2aea

Base Pairs: 600 Reads: 2400000.0 Fraction: -1.0 List of LibTypes: DNA low cost high throughput Size of list of lib types is: 1 LibType: DNA low cost high throughput Batches: 2 PI: int

Description	Quantity	Rate	Total
Labor Hours	9.5	\$34.88	\$331.36
QC/Oligo Kits	1.00	\$16.10	\$16.10
Sample Prep Kits	1.13220	\$53.00	\$60.01
Sequencer Prep	0.0	\$52.00	\$0.00
Sequencer Kits	73.85	\$3.66	\$270.29
Sequencer Time	0.0	\$8.85	\$0.00
Total			\$677.76

Note that charges for special services such as ribosomal depletion or GSAF-supplied reagents are not included but will be added before billing if applicable.

This is a PRICE ESTIMATE suitable for budget planning, grant applications etc. Formal quotations can be requested by forwarding this estimate to gsaf@utlists.utexas.edu with the subject line "Formal quote request."

Modify Job Information Modify Sample Information Save & Email for Reference Submit Request Delete Request

Save and Email – just for you!

Submit Request – goes to GSAF for review/approval

How do I start a project with the GSAF?

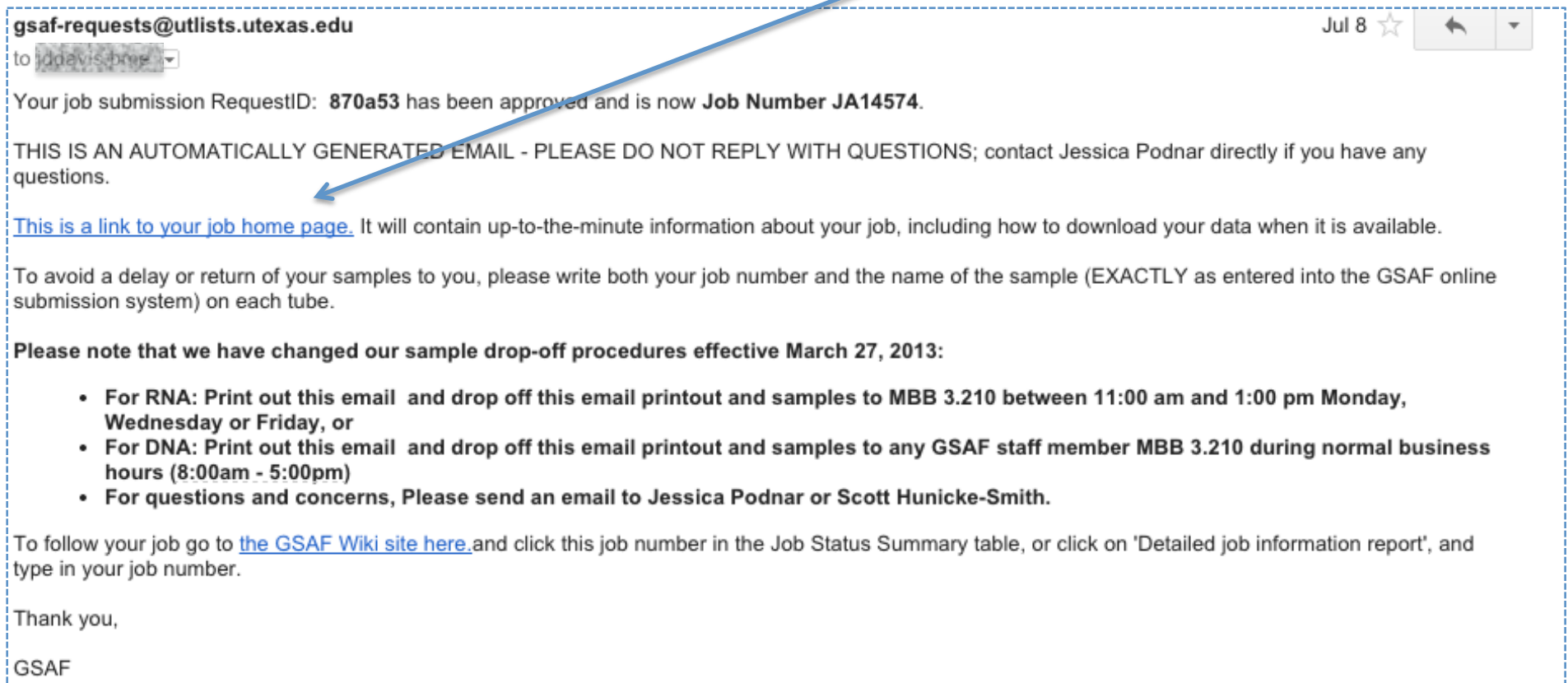
- Job Submission example
 - Editing your job BEFORE submitting it
 - You cannot edit your job once you submit it



The screenshot displays the GSAF user support wiki interface. On the left is a navigation menu with a search bar at the top. The menu items include: Pricing, How to submit samples to the GSAF, Submit Samples to the GSAF (expanded), Create Job Request, Return to Job Request (highlighted with a blue arrow), Sequencer Queues, Current Job Status, Job Info Report, and Historical Billing Info. The main content area shows the page title 'Return to Job Request' under the GSAF logo. It includes a breadcrumb trail: 'Genomic Sequencing and Analysis Facility User Support Wiki / Home Page / Submit Samples to the GSAF'. Below the title is a lock icon and text: 'Added by Joe A Cruz, last edited by Joe A Cruz on Jan 20, 2012 (view change) show comment'. A note states: 'Use this with your 6 character request ID to modify job info in a previous request. You'll get an email confirming any changes.' Below this is a form titled 'Lookup Job Request by Request ID' with a 'Request ID #' label and an input field. At the bottom of the form are 'Submit' and 'Reset' buttons.

How can I tell where my project is?

- Your job submission email – link to your job home page



gsaf-requests@utlists.utexas.edu Jul 8 ☆

to jddavis@brwe

Your job submission RequestID: **870a53** has been approved and is now **Job Number JA14574**.

THIS IS AN AUTOMATICALLY GENERATED EMAIL - PLEASE DO NOT REPLY WITH QUESTIONS; contact Jessica Podnar directly if you have any questions.

[This is a link to your job home page.](#) It will contain up-to-the-minute information about your job, including how to download your data when it is available.

To avoid a delay or return of your samples to you, please write both your job number and the name of the sample (EXACTLY as entered into the GSAF online submission system) on each tube.

Please note that we have changed our sample drop-off procedures effective March 27, 2013:

- **For RNA: Print out this email and drop off this email printout and samples to MBB 3.210 between 11:00 am and 1:00 pm Monday, Wednesday or Friday, or**
- **For DNA: Print out this email and drop off this email printout and samples to any GSAF staff member MBB 3.210 during normal business hours (8:00am - 5:00pm)**
- **For questions and concerns, Please send an email to Jessica Podnar or Scott Hunicke-Smith.**

To follow your job go to [the GSAF Wiki site here](#), and click this job number in the Job Status Summary table, or click on 'Detailed job information report', and type in your job number.

Thank you,

GSAF

How can I tell where my project is?

- Your job home page

GSAF Data System: Job Home Page for Job JA14227

This page contains up-to-the-minute information about your job. If a section is blank (e.g. QC Information, or Billing information), it means that we have not yet done that task for your job. This information is pulled direction from our production databases, so as soon as the information is available it will appear on this page.

Job ID: JA14227

Description: ddRAD protocol development

Sample receipt date: 17-Mar-14

Job completion date: 20-May-14

This project has 3 samples and there are 4 QC runs associated with this job.

More information is available below - simply expand the sections to see more.

- ▶ QC Information
- ▶ Job Summary
- ▶ Billing Information
- ▶ Sequencing Data (expand to request new data access keys)
- ▶ Total sequencing data generated (expand for detail)

How can I tell where my project is?

- The sequencing queues

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Genomic Sequencing and Analysis Facility User Support Wiki / Home Page / Submit Samples to the GSAF

Sequencer Queues

Added by Scott Patrick Hunicke-Smith, last edited by Scott Patrick Hunicke-Smith on Aug 21, 2014 (view change)

Sequencer Queues

- View the Illumina MiSeq sequencing queue
- View the Illumina NextSeq 500 sequencing queue
- View the Illumina HiSeq PAIRED-END run sequencing queue

Sample ID	Quantity	Library	Barcode	Barcode	Operator	Project ID	Quantity
Con_p17				Illumina Barcode p17	Jake Grohman	JA14743	1
FHC				Illumina Barcode #29	Jake Grohman	JA14743	
FHC_DDX6				Illumina Barcode #31	Jake Grohman	JA14743	1
HeLa_p19				Illumina Barcode #19	Jake Grohman	JA14743	
HT29_p32				Illumina barcode #32	Jake Grohman	JA14743	10
LoVo_p18				Illumina Barcode #18	Jake Grohman	JA14743	10
SW480_p28				Illumina Barcode #28	Jake Grohman	JA14743	10
SA14140	1	3_5minFrag		TS24	Douglas Wu	JA14730	10
2x125	1	Pool11	TCTTTCCC	GTGAAACG	Matthew I	JA14486	10
Start Date:	1	Pool 8	multiple		Matthew I	JA14486	10
CA14045	1	22_66_031	TAATCTTA	TCCGCGAA	Matthew I	JA14486	
slide ID:	1	22_66_015	TAATCTTA	TAATGCGC	Matthew I	JA14486	
Sequencer:	1	22_66_019	ATAGAGGC	CGGCTATG	Matthew I	JA14486	
Barcodes:	1	22_66_021	GGCTCTGA	CGGCTATG	Matthew I	JA14486	
DUAL INDEX	1	22_66_043	TATAGCCT	AGCGATAG	Matthew I	JA14486	
	1	r3_1		ATCACG	LIN QIU	JA14637	21
	1	r2_11		TGACCA	LIN QIU	JA14637	21
	1	r3_11		TTAGGC	LIN QIU	JA14637	21

Expand section to see queue

How do I get my data?

- Your data delivery email comes to you automatically
-

GSAF Data Key Available

New access keys have been generated for each of your data files. Anyone with access to your data keys will be able to download your data. We have limited the time window for download to 72 hours.

[Access your data for JA14699 from sequencing run SA14130 here.](#)

This link will be accessible for ten days from the date of this email.

You may request *new* access keys and find many details about your job on your [job home page](#).

Please see [this page for more information about downloading your data](#).

Sincerely,
GSAF

How do I get my data?

- Your job home page – you can access ANYTIME

GSAF Data System: Job Home Page for Job JA14699

This page contains up-to-the-minute information about your job. If a section is blank (e.g. QC Information, or Billing information), it means that we have not yet done that task for your job. This information is pulled direction from our production database.

Job ID: JA14699

Description: heavy chain amplicons (~400 bp) to be sequenced by 2 x 300 bp MiSeq

Sample receipt date: 02-Sep-14
The job has not been completed

This project has 23 samples and there are 4 QC runs associated with this job.

More information is available below - simply expand the sections to see more.

- ▶ QC Information
- ▶ Job Summary
- ▶ Billing Information
- ▼ Sequencing Data (expand to request new data access keys)

Clicking this link will re-generate an email with new data keys to any email addresses listed on the project

Sequencing run SA14130: [\(select this link to generate access keys for SA14130\)](#)

File	# of read-pairs	md5sum
3173poMIM_S1_L001_I1_001.fastq.gz	818911	ee328eaff02e5c9a1ec4dcd81a8d41d7
3173poMIM_S1_L001_R1_001.fastq.gz	818911	1d5b7ad4823722d486ecb3f27ccba411
3173poMIM_S1_L001_R2_001.fastq.gz	818911	3353e10330d5ecd45bc5b7fa39993911
3173poMock_S2_L001_I1_001.fastq.gz	694653	08b9c1da413b7d0523a0aaaf3f5c9734
3173poMock_S2_L001_R1_001.fastq.gz	694653	5d28f88d02b3942c2344a61b2e1982e3
3173poMock_S2_L001_R2_001.fastq.gz	694653	027d6f4b9b3ccac56c8d81a6d6070d32

Frequently Asked Questions

Example Projects

- RNA-seq, poly-A mRNA enrichment for differential expression
- Bacterial whole-genome assembly
- ChIP-seq
- ddRAD

Thank you from the GSAF!



The GSAF: Jessica Podnar, Matt Barnette, Heather Diedrick, Terry Heckman, Gabby Huerta, Mani Singh