With the support of a grant from the Office of the Vice President for Research, the Genomics Center has adopted a novel suite of self-serve instruments to give UMN researchers direct access to genomics tools. Traditionally, due to the large-size, complexity, and expense of instruments, a researcher’s ability to use a new technology has been limited to dropping off samples at the Genomics Center and then wait for processing. Now, by selecting a new breed of smaller, user-friendly, and specialized instruments for purchase, researchers can access workstations within our labs for direct access, giving users ultimate flexibility with experimental design and project turnaround.

### Illumina iSeq 100

Big advantages come in small packages. Measuring just one cubic foot in size, the iSeq desktop sequencer was selected as a DIY instrument due to its portability and easy-to-use system. It utilizes Illumina’s one-channel sequencing by synthesis technology to give extraordinarily fast sequencing turnaround while maintaining high data quality. Even as the smallest Illumina sequencer to-date, it is still able to produce over 1.2 billion base pairs in as little as 9 hours for a 50-bp single-read run.

#### Spotlights on DIY Sequencing

**Benefits.**
Researchers can now have walk-up access with overnight results plus user control from library prep to data demultiplexing.

**Output.**
A 300-cycle cartridge is available that accommodates 36 single-read to 150 paired-end read lengths, each producing 4 million reads per run.

**Location.**
The iSeq is centrally installed at our CCRB location for easy access for St. Paul and Minneapolis researchers. Cartridges are available for purchase at our St. Paul and CCRB sites.

**Load and go.**
After thawing the reagent cartridge, libraries are loaded into a dedicated reservoir in the cartridge, the flow cell is inserted, and the run is set-up with the guidance of a step-by-step user interface – all within 10 minutes.

### Applications

Even as a low-throughput instrument, the iSeq is a flexible system with a wide range of applications that is ideal for the study of bacteria and viruses.

- Small-genome sequencing
- Targeted resequencing
- Amplicon sequencing
- v4 amplicon sequencing
- Library evaluation before a NovaSeq run
- Viral and microbial sequencing

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**CONTENTS**

- RATES 2
- TRAINING 3
- RESERVING 4
- CARTRIDGES 4
- LIBRARY QC 4
- CONSUMABLES 5

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**DIY INSTRUMENTS**

- **Short-read Sequencing**
  - iSeq 100 | CCRB
  - next-gen@umn.edu

- **Long-read Sequencing**
  - GridION | Snyder
  - next-gen@umn.edu

- **Single-cell Sequencing**
  - ddSeq isolator | Mobile
danie786@umn.edu

- **Gene Expression**
  - ddPCR QX200 | NHH
exprn@umn.edu

- **Quantitative PCR**
  - QuantStudio 5 | All sites
exprn@umn.edu

- **Quality Control**
  - TapeStation 2200 | CCRB
umgc-qc@umn.edu

- **Quantitate DNA/RNA**
  - QuBit 3.0 | All sites
umgc-qc@umn.edu

- **Shearing DNA/RNA**
  - Covaris S220 | Snyder
umgc-qc@umn.edu
Pricing for the iSeq

The 300-cycle cartridge supports a variety of read lengths with the sequencing run time increasing with longer read lengths. Illumina charges the same cost for the cartridge, no matter the read length selected.

A full list of read length options for the 300-cycle cartridge can be found on page four.

Illumina will release the 500-cycle cartridge in early 2019 that will provide a longer 2 x 250 bp read length.

Table is a low-throughput sequencing comparison with internal rates. The iSeq DIY rate is the UMGC cartridge costs ($621.85) + instrument usage charge for two days ($207.40). Queue time does not include sample QC or library prep time. Please contact us for a quote with our most up-to-date pricing.

*Note on Data Release.

For clients using our standard sequencing service, data released by the UMGC includes trouble-shooting runs, demultiplexing, generation of QC and FastQC files, informatics, and releasing data to MSI. These services are not available with the client-operated DIY iSeq option.
Required Training with the Next-Gen Staff

Whether you are new to NGS protocols or have experience operating Illumina instruments, training on the iSeq instrument is required before users can schedule their own independent run.

The UMGC provides required group training at our CCRB site under the direction of Aaron Becker – lead scientist for NGS instrumentation. Providing space is available, up to two scientists from one lab can attend the training in CCRB at a cost of $162.58/lab.

Training will be a full demonstration using an actual library to give users the ability to independently sequence their own future run. Topics will cover library QC requirements through sample sheet set-up for demultiplexing, as well as run monitoring in BaseSpace.

<table>
<thead>
<tr>
<th>Location</th>
<th>Time</th>
<th>Cost</th>
<th>Dates</th>
<th>Reservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group training will be at our main sequencing lab in 1-210 CCRB.</td>
<td>Scheduled from 9:30 - 1:30 and will take ~3.5 hours. Extra time is for questions.</td>
<td>If space permits, group training is up to 2 people from 1 lab at $162.58/lab.</td>
<td>Flexible training dates are available. Contact <a href="mailto:next-gen@umn.edu">next-gen@umn.edu</a>.</td>
<td>Once training is complete, users will be able to access the Instrument Reservation portal.</td>
</tr>
</tbody>
</table>

Training Topics

1. Workstation and lab overview.  
This portion of the training will briefly go over access to the CCRB lab space, location of relevant equipment - ice machine, reagent freezers, etc. - and workstation etiquette and available consumables at the dedicated client bench.

2. Library QC.  
Accurately assessing the library fragment distribution and its quantity by a fluorometric method is an essential step to the NGS workflow. Inaccurate estimation of the library size or the amount of library can lead to over or under-clustering your sequence run producing lower Q30 scores and reduced data output.

3. Cartridge features and iSeq operation.  
We will walk trainees through the main features of the iSeq instrument, the components of the pre-filled cartridge, and the flow cell to give a solid understanding of Illumina sequencing instruments. This will give new users the ability to perform basic trouble-shooting and maintenance of their sequencing runs.

4. Loading a run.  
The bulk of the training session will be a demonstration on loading an actual sequencing run. Attendees will learn how to pool libraries based on QC; how much diversity to add to certain libraries by adding PhiX; using the nM calculator for diluting the final library; populating and uploading a sample sheet; preparing and loading the cartridge; and configuring a run using the Local Run Manager.

5. Run Monitoring in BaseSpace.  
Each lab will need their own Illumina BaseSpace account for tracking the run, demultiplexing, and analyzing the quality of the run by generating FASTQ files. Users can easily sign-up for a secure Illumina genomics computing hub account that allows for easy sharing, collaboration, and data management. BaseSpace provides 1 Tb of storage for free.

Local storage is not available on the iSeq and data will be deleted after each run due to limited storage space.

2019 iSeq DIY User Guide | 3
Reserving the iSeq

The iSeq is reserved in 2-day increments to accommodate the varying run times due to read length options, and also accounts for the workstation time to dilute your final sequencing library, plus time to also prepare and load the cartridge.

After training, and with the guidance of the Illumina iSeq System Guide and the interface on the iSeq, clients should be able to independently use the iSeq without further support from the UMGC staff.

Availability of the iSeq can be found on our Instrument Reservation portal. The CCRB lab can be accessed between 9:00 AM - 5:30 PM Monday-Friday.

Purchasing a Cartridge

The Illumina 300-cycle iSeq reagent cartridge and flow cell kit are available for purchase ($621.85) with an EFS number at our CCRB location, or if arranged ahead of time, the frozen cartridges can be picked up at our other sites. Clients are responsible for thawing the cartridges prior to sequencing and will manage the thawing in their lab. Please note below the amount of time needed to completely thaw the cartridge; any alteration in the thawing protocol will compromise the sequencing run.

It is highly recommended to have your library QC complete and that you are satisfied with the quality of your library before you begin to thaw your cartridge. A quality library will lack adaptor and primer dimers at 80-85 bp and 120 bp, respectively, and will ideally have a size between 200-800 bp.

The Genomics Center does not receive a discount from Illumina on the iSeq cartridges. We do charge users our time to order, unload, track, and store iSeq cartridges, and to avoid this additional cost, clients can purchase cartridges directly from Illumina.

<table>
<thead>
<tr>
<th>Method</th>
<th>Temperature</th>
<th>Min. Thaw Time</th>
<th>Stability</th>
<th>Packaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temp</td>
<td>20 - 25°C</td>
<td>9 hrs</td>
<td>18 hrs in water bath</td>
<td>Do not remove</td>
</tr>
<tr>
<td>Water bath</td>
<td>20 - 25°C</td>
<td>6 hrs</td>
<td>18 hrs in water bath</td>
<td>Do not remove</td>
</tr>
<tr>
<td>Fridge</td>
<td>2 - 8°C</td>
<td>36 hrs</td>
<td>72 hrs in the fridge</td>
<td>Do not remove</td>
</tr>
</tbody>
</table>

Table of thawing options for the cartridge.

Library QC

There is no formal training for the QC instruments; a staff member will guide new users on the QuBit and TapeStation during their first QC sign-up session. In the iSeq training session, the NGS team will go over how to use your library quantification and sizing results in the nM calculator for the final library dilution before sequencing.

QC DIY access is available in our CCRB site for both the QuBit 3.0 fluorometer plus reagents for fast library quantification, as well as the Agilent Tapestation 2200 for assessing library quality. Please contact our QC team at umgc-qc@umn.edu for signing up for the DIY QC instruments and for details on QC instruments at our other sites.
CONSUMABLES

Supply List

The UMGC provides a dedicated workbench for DIY users that is stocked with standard lab consumables, and we also provide a 4°C fridge with aliquoted QC supplies. Other than the iSeq cartridge, no additional sequencing reagents are needed - inside the cartridge are all the reagents needed for a sequencing run, including the denaturation reagent, primers, buffers, and cluster generation reagents.

1. QC your library to ensure you have a quality library before sequencing. It is recommended to not thaw your cartridge until you are satisfied with your library.

2. Purchase a cartridge and flow cell and thaw before arriving in CCRB for sequencing.

3. Use the quantatification and sizing from your QC to dilute your final library pool to 1 nM in at least 10 uL before coming to CCRB.

4. Sign-up for a free BaseSpace account (1 Tb of space) for storing your sequencing run.

5. A flash drive will be needed to transfer your samplesheet to the iSeq.

<table>
<thead>
<tr>
<th>Client Responsibility</th>
<th>Workstation Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. QC your library to ensure you have a quality library before sequencing. It is recommended to not thaw your cartridge until you are satisfied with your library.</td>
<td>1. PhiX at 10 nM</td>
</tr>
<tr>
<td>2. Purchase a cartridge and flow cell and thaw before arriving in CCRB for sequencing.</td>
<td>2. Resuspension buffer (RSB)</td>
</tr>
<tr>
<td>3. Use the quantatification and sizing from your QC to dilute your final library pool to 1 nM in at least 10 uL before coming to CCRB.</td>
<td>3. Illumina iSeq Sequencing Guide</td>
</tr>
<tr>
<td>4. Sign-up for a free BaseSpace account (1 Tb of space) for storing your sequencing run.</td>
<td>4. Rainin pipettes and tips</td>
</tr>
<tr>
<td>5. A flash drive will be needed to transfer your samplesheet to the iSeq.</td>
<td>5. 1.5 mL tubes</td>
</tr>
<tr>
<td>7. Ice Bucket and ice</td>
<td></td>
</tr>
</tbody>
</table>

QUESTIONS

DIY iSeq instrument
Aaron Becker  |  CCRB
next-gen@umn.edu

DIY QC instruments
Archana Deshpande  |  All sites
deshp008@umn.edu

DIY General Assistance
Shea Anderson  |  All sites
ande3650@umn.edu

Director
Dr. Kenny Beckman  |  CCRB
kbeckman@umn.edu

Illumina provides excellent support for the iSeq instrument. Please use the links below for more information.

The iSeq Sequencing System Guide provides instructions for starting a run on pages 18 - 30.

Illumina iSeq Training Video Links:
1. How to Start a Run
2. Does My Run Look Good?
3. Local Run Manager

RESOURCES

CONNECT

Subscribe to the Genomics Center’s newsletter to receive news, event info, technology updates, and more.

Each fall we host a technology seminar and vendor fair, which include breakout sessions, promotions and raffles. Visit our website for upcoming dates.

Stay up to date on the latest - follow us on social media.