# IMSERC User Manual for the Organic GPC

## CONTENTS

## INTRODUCTION

This SOP provides quick instructions how to operate the instrument for gel permeation chromatography, also known as size exclusion chromatography (GPC/SEC). GPC/SEC is a type of high-performance liquid chromatography (LC) that separates molecules based on their size, hence 'size exclusion'. GPC/SEC is used to determine the molecular weight distributions of polymers.

Use of this instrument is allowed only by qualified users after receiving training by a staff member. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges.

This short set of instructions is meant to serve as a guide for 'routine' data collection on the instrument. Please read this standard operating procedure and acquaint yourself with the instrument. If during using the system, something happens that you do not understand, please ***stop*** and ***get help***. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable.

The Organic GPC is a general use instrument capable of analyzing synthetic polymers soluble in Tetrahydrofuran (THF) in 1kDa to 2000 kDa range. To keep the system simple and useful for a group of diverse users, the default methods are designed to run samples free from buffer and salts to obtain a quick analysis of sample composition. A default double Varian PolyPore column 7.6x250 mm is installed and may be changed in order for a user request of their own column. Please check NUcore for the most up-to-date notification regarding the instrument column.

All samples must be soluble in THF and prior to injection your sample must:

1. Must be filtered through a 0.2 µm PTFE filter.
2. Must have a blank THF sample to run at the end of a sequence.
3. Must have known concentration of the target analyte. If you do not know the concentration of your sample, you will not be able to perform MW calculations
4. **NOT** exceed a concentration of ≤ 1 mg/mL.
   a. Concentrations ≥ 1 mg/mL have the potential to contaminate the instrument.
   b. Concentrations ≥ 1m µg/mL may produce carryover which will affect the data quality of subsequent users.

You are responsible for maintaining instrument quality prior to and post sample analysis. Always check the system backpressure is less than 95 bar before starting your sample run. If the backpressure remains high after your sample run, you are required to file a bug report. Do NOT leave the instrument and end your reservation while the instrument is running as it affects other users' reservations.

## SAFETY

All users of IMSERC must review the general safety policies at http://imserc.northwestern.edu/about-policies.html.

Familiarize yourself with the location of standard safety stations like eye wash and shower stations found inside BG170. Protective eyewear is required in this room, and gloves should be removed when using the computer.

| Hazard | Location | PPE Required/Hazard Mitigation |
|---|---|---|
| Samples | BG170 – Organic GPC | Eye Protection, Gloves |

To become an independent user of this instrument, you must have the following safety training and certificates which are offered at https://learn.northwestern.edu:

- Laboratory Safety
- Personal Protective Equipment

Upon completion of the certificate, it will take an overnight to filter through the different systems and get into the files that NUCore uses.

## DATA MANAGEMENT

Your personal data folder is created during training which must be located under your supervisor's group folder. See a staff member if you do not have a personal folder on this instrument yet. Your personal file folder must contain your Lastname_Firstname. Inside your personal folder you will create new folder which references the year the data was collected. Inside this folder you can access your individual acquisitions and processed data files from that calendar year.

Every new year, you are responsible to create a new data folder, so that the data is collected in the new annual folder.

Over time, files from previous years will be archived to a larger file server to save space on the local drive. If you do not use the designated file structure as outlined in this manual, it may result in a failure to back up your data. See IMSERC staff for clarification if you have any questions.

Once inside the 'annual' folder, you may label/identify your sample data according to your needs.

> **Example:** PI name > Your Lastname_Firstname > YYYY> sampleinfo

> **Specific Example:** Einstein > Currie_Marie > 2020 > PMMA_standard

Data on this instrument are copied on 'imsercdata.northwestern.edu' under 'MS/Others/LS_org every 1 hour. Please follow instructions at http://imserc.northwestern.edu/about-general-faq.html#data for details about data access. Please remember to use the northwestern VPN when accessing data from a personal computer.

## SOFTWARE

Data acquisition and analysis may be performed with Astra. The icon to access this software is located on the instrument in the upper right corner of the computer screen.

For offline analysis after your instrument reservation is complete, please see Data Acquisition on page xx.

## SAMPLE PREPARATION

The table below outlines recommendations to ensure proper instrument care and sample analysis.

| Detail | Recommendation | Consideration |
|---|---|---|
| Vials | Agilent Vial 5182-0714, Agilent cap 5185-5865 | The Autosampler picks up the vial. With the wrong vial dimensions, the sample will drop during transport. |
| Sample Solvent | Tetrahydrofuran | The mobile phase utilized THF and it is performed under isocratic conditions. |
| Concentration / Purity | ≤1.0 mg /ML | Please avoid any additives, DMF, DMSO or similar solvents, strong acids (i.e. HCl, $H_2SO_4$, $HNO_3$) and bases. They are not column friendly. |
| Volume | 400 µL | If volume available is too low, use Agilent vial spring loaded insert 5182-8872 |
| Labeling | Name and solvent | Sample identification & safety / proper disposal |

## QUICK START ACQUISITION CHECKLIST

1. Begin reservation in NUCore.

2. Check if the LC is running in recycle mode.

3. Open Astra.

4. Open 'New Sequence From Template.

5. Add sample(s)

    a. Enter sample name, dn/dc value, injection volume, concentration.

6. Click run.

7. Save sequence in appropriate folder.

8. Open LC(online).

9. Open Sequence table.

10. Add samples

    a. Enter sample name, vial location, method, injection volume.

11. Confirm sequence information matches Astra.

    a. Samples, method duration, injection volume.

12. Click Apply.

13. Click Run.

14. Analyze data.

15. Save processed data and close file.

16. End Reservation in NUCore.

## DATA ACQUISTION

Before you start, please know the LC is left running so that the column and all the detectors are in 'ready-to-inject' mode. If the flow is turned off, please notify IMSERC-MS staff.

The Orbit recycle system should be in recycle mode. The waste indicator light should be off. In this mode, the solvent is going from the column, back into the solvent bottle. Dawn Heleos-II will indicate recycle as yellow. Once you start running samples through the Astra software, the Orbit Recycle System will automatically be switched to waste mode. The waste indicator light will come on, and the flow will then be going to the waste container. At the end of each sequence, you must run a blank to clean out the column before the flow of solvent can go back into the solvent bottle.

### WRITE AND RUN A SEQUENCE IN 'ASTRA'

1. The computer screen is by default, deactivated. You must start your reservation through NUCore to be able to turn on the computer screen. If screen is already on, start your reservation through NUCore.

2. Open Astra. You can select the Astra icon located on the top right screen or on the lower tool bar.

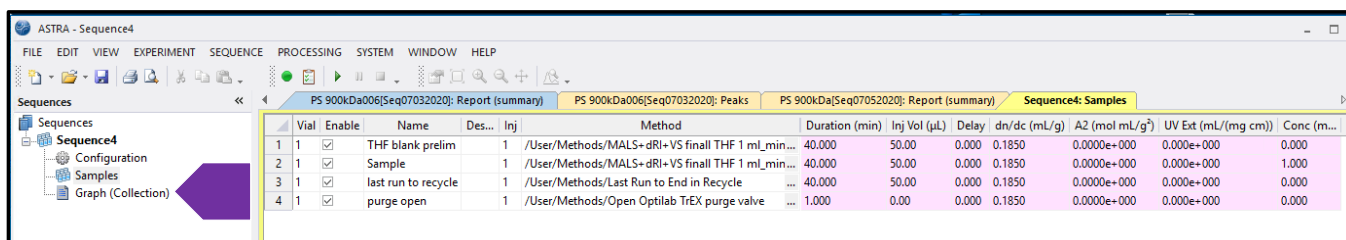3 . Click on 'File' located in the top left window. Go to "New," then click "Sequence From Template."
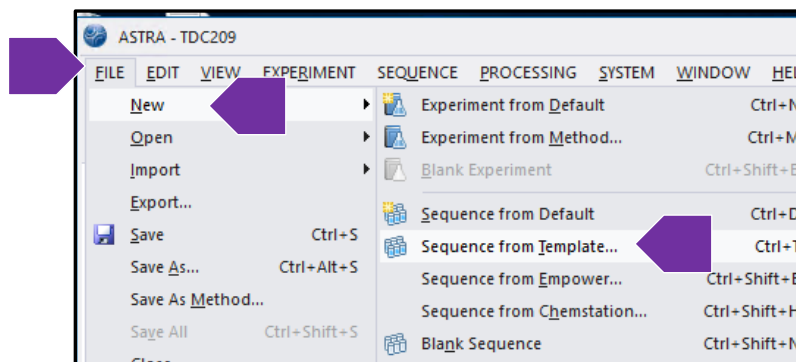
4 . The root directory opens. Select "UserSeqTemplate," Then click 'Create.'

    a . The default method in this template is MALS+dRI+VS final THF 1ml_min.

    b . Request help from IMSERC-MS staff for new methods if needed.
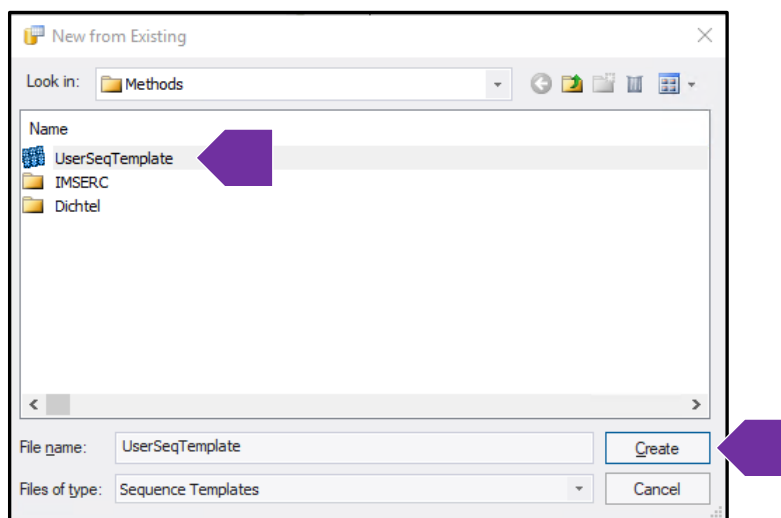


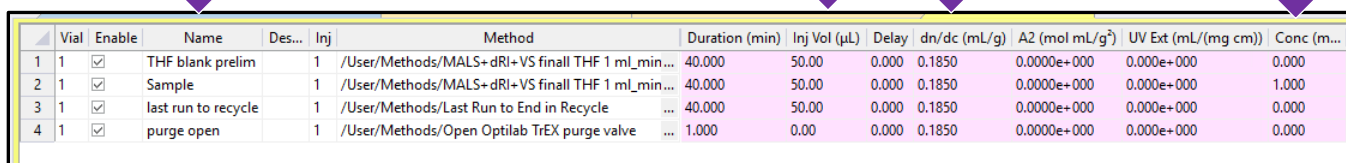5. Double click on 'Samples' to populate your sample list.



6. To add new samples to your sample list, *(a)* right click a previous sample that you would like to duplicate and/or amend, then click *(b)* "Add."
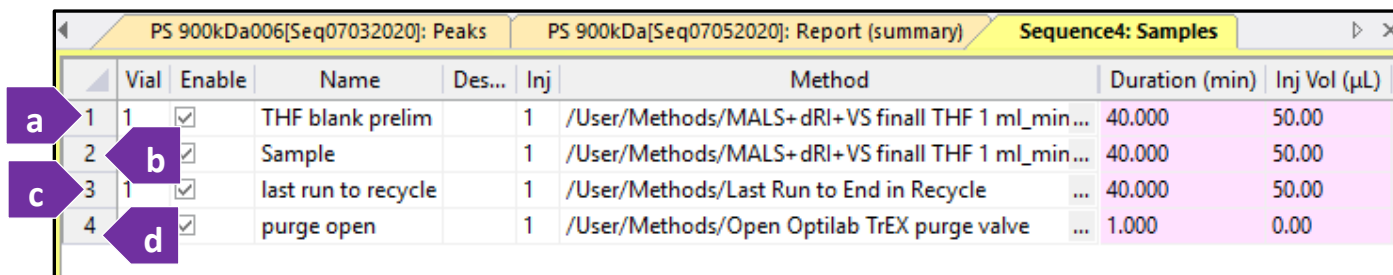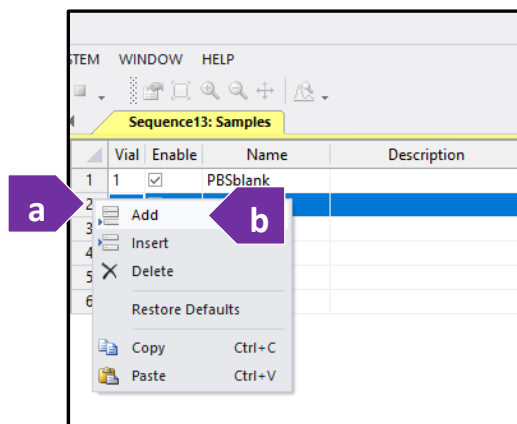
7. Make changes to your sample(s). At minimum change the a) Name b) injection volume c) dn/dc value d) Concentration.

| | Vial | Enable | Name | Des... | Inj | Method | Duration (min) | Inj Vol (µL) | Delay | dn/dc (mL/g) | A2 (mol mL/g²) | UV Ext (mL/(mg cm)) | Conc (m... |
|---|------|--------|------|--------|-----|--------|----------------|--------------|-------|--------------|-----------------|---------------------|-----------|
| 1 | 1 | ☑ | THF blank prelim | | 1 | /User/Methods/MALS+dRI+VS finall THF 1 ml_min... | 40.000 | 50.00 | 0.000 | 0.1850 | 0.0000e+000 | 0.000e+000 | 0.000 |
| 2 | 1 | ☑ | Sample | | 1 | /User/Methods/MALS+dRI+VS finall THF 1 ml_min... | 40.000 | 50.00 | 0.000 | 0.1850 | 0.0000e+000 | 0.000e+000 | 1.000 |
| 3 | 1 | ☑ | last run to recycle | | 1 | /User/Methods/Last Run to End in Recycle ... | 40.000 | 50.00 | 0.000 | 0.1850 | 0.0000e+000 | 0.000e+000 | 0.000 |
| 4 | 1 | ☑ | purge open | | 1 | /User/Methods/Open Optilab TrEX purge valve ... | 1.000 | 0.00 | 0.000 | 0.1850 | 0.0000e+000 | 0.000e+000 | 0.000 |

8. The sequence table includes:

   a. Line 1: A THF Blank

   b. Line 2: Your test samples

   c. Line 3: Last blank

      i. The purpose of the blank THF run is to 'clean out' the column to minimize the chance of contaminating the solvent reservoir when the solvent flow is switched from waste to recycle at the end of the method.

   d. Line 4: End of sequence switching the flow from waste to recycle.

| | PS 900kDa006[Seq07032020]: Peaks | PS 900kDa[Seq07052020]: Report (summary) | **Sequence4: Samples** | ▷ ✕ |

| | Vial | Enable | Name | Des... | Inj | Method | Duration (min) | Inj Vol (µL) |
|---|------|--------|------|--------|-----|--------|----------------|--------------|
| 1 | 1 | ☑ | THF blank prelim | | 1 | /User/Methods/MALS+dRI+VS finall THF 1 ml_min... | 40.000 | 50.00 |
| 2 | | ☑ | Sample | | 1 | /User/Methods/MALS+dRI+VS finall THF 1 ml_min... | 40.000 | 50.00 |
| 3 | 1 | ☑ | last run to recycle | | 1 | /User/Methods/Last Run to End in Recycle ... | 40.000 | 50.00 |
| 4 | | ☑ | purge open | | 1 | /User/Methods/Open Optilab TrEX purge valve ... | 1.000 | 0.00 |

9. Confirm your samples are correct. Confirm your methods are correct.

10. The injection volume maximum is 100 µL. The injection volume is dictated by the Agilent Open Lab (LC online) software.

    a. In Astra, the number is needed for some of the calculations. You can enter the injection volume during data processing. It is a good idea to include it in the ASTRA sequence for proper documentation.

11. Adjust your injection volume in Open Lab (LC online) software if the sample concentration is above 1 mg/mL.

12. To run, click the green arrow located at the top of the menu bar.
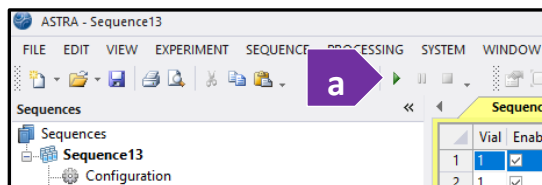
13. The sequence will be checked by the software, and you will be prompted to save your sequence by naming it. It is suggested to name the sequence with the date and something easily identifiable. Navigate to your annual data folder created during your initial training. It should always be located under your PI Name> Lastname_Firstname > YYYY. Then click 'Save.'

   a. This folder is also the location where your data will be saved by Astra.
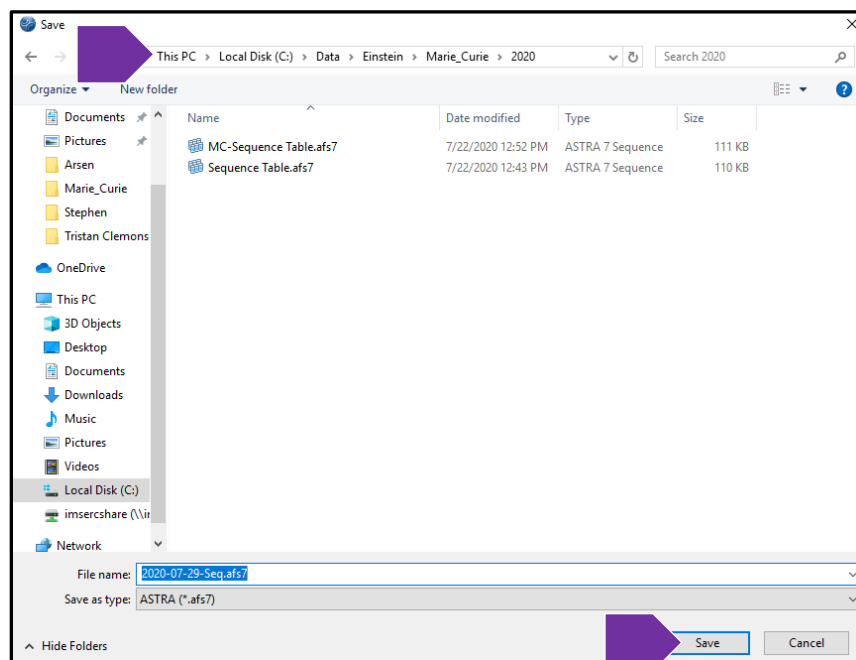
## WRITE AND RUN A SEQUENCE IN 'AGILENT OPEN LAB (LC ONLINE)

1.  The LC (online): Method and Run Control should be open and running. You can also access it by clicking on the icon .
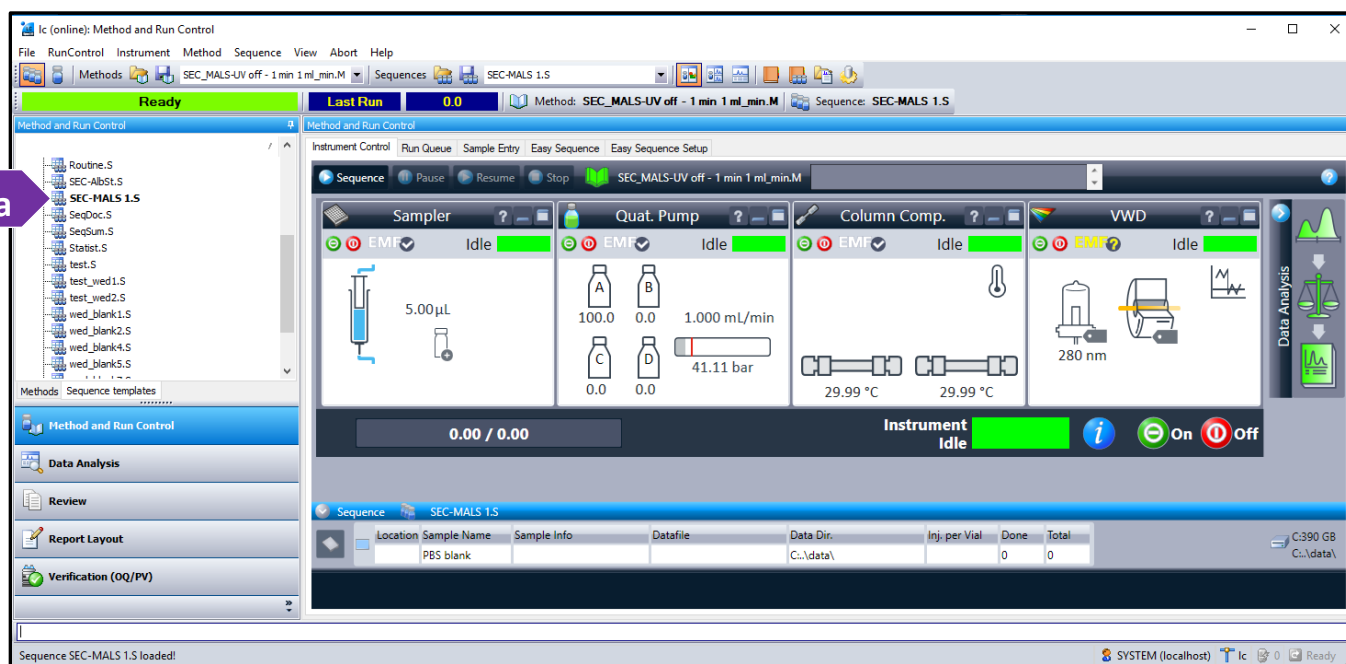
2.  Load your approved method established during your user training. Refer to the example below.

    a.  All method will start with SEC. SEC_MALS-40min 1 ml_min THF.M is selected.
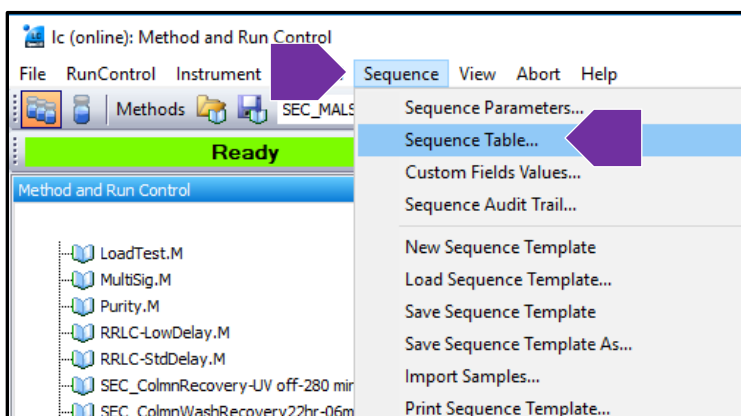
        i.  Request IMSERC to write new methods, if needed.

    b.  The same methods can be run for blank injections.

    c.  Test samples will be approximately around 40-42 minutes of runtime.



3.  Prepare a sequence table by clicking on 'Sequence,' then click 'Sequence table.'

4.  You can modify the sequence table as it appears and not pull up a saved file.

    a.  You are not saving any data in the LC Open Lab software.

5.  View the LC(online) Open Lab Sequence below.

    a.  Sample location has the format of Tray-vial. P1 is the left plate, P2 is the right plate. The image below shows a vial in position B5, in plate (tray) 1.

        i.   Columns of each tray are named A (left) through F (right).

        ii.  Each column has vial number 1 (front) through 11 (back).

    b.  Confirm that the sequence matches the sequence table in Astra. (number of samples, method duration, injection volume)

        i.    Line 1 is the PBS sample.

        ii.   Line 2 should be the test samples.

        iii.  Line 3 should include the blank run.

    c.  Note, Line 4 is needed since there are ASTRA method requiring for recycle mode. These are methods that tell the instrument to shut the laser off, open the purge and send the LC flow to recycle.

Sequence Table: lc

| Line | Sample Container | Sample Location | Sample Name | Method Name | Injection Source | Injection Volume | Inj/Loc | Sample Type |
|---|---|---|---|---|---|---|---|---|
| 1 | Use Current Configura | P2-C1 | THF blank prelim | SEC_MALS 40 min 1 ml_min THF | As Method | 50 | 1 | Blank |
| 2 | Use Current Configuration | P2-C8 | Sample | SEC_MALS 40 min 1 ml_min THF | As Method | 50 | 1 | Sample |
| 3 | Use Current Configuration | P2-C1 | last run to recycle | SEC_MALS 40 min 1 ml_min THF | As Method | 50 | 1 | Blank |
| 4 | Use Current Configuration | P2-C1 | purge open | SEC_MALS 2 min 1 ml_min THF | As Method | 1 | 1 | Blank |
| 5 | | | | | | | | |

Run    OK    Cancel    Apply

6. Click on (a) 'Apply' to apply sequence changes. And then (b) 'Run' at the bottom of the sequence table.



7. If you are asked to save as default sequence, click 'Yes.'

8. Once an injection is made, a TTL pulse will be sent to Astra to initiate data collection.

## DATA PROCESSING

Note that you MUST know the concentration of your sample to perform molecular weight calculations.

**PROCESSING YOUR GPC DATA**

1. Map the network drive, \\imsercdata.northwestern.edu\public
2. Open the Wyatt GPC Folder.
3. Copy all four files to your hard drive.
4. Run the setup file.
5. When prompted for keys, open the two file names as Astra 7 Keys.
    a. Copy and paste as requested.
6. Note that the tutorial Astra Quick Guide Rev A is compatible with Astra 7 and includes procedures that you will only do in case of a new method. You are using a method that has already contained some of those operations.
7. Note that one-on-one sessions are available to review data processing. Contact IMSERC-MS.

## PUBLICATION

**EXPERIMENTAL SECTION**

Data was collected with Organic GPC using Astra Version 7 data acquisition software and processed using Astra Version 7 for data analysis. The instrument was configured with an Agilent 1260 Series HPLC module equipped with a UV detector and coupled with Wyatt Heleos II MALS and RI detectors. The SEC analysis was run on ***the PSS Suprema column 7.6 x 250 mm at a flow rate of 1 mL/ min*** with Phosphate-Buffered Saline as a mobile phase.

*\*Default setup. User customized methods require information change, accordingly.*

**ACKNOWLEDGEMENT**

All results gained from the use of this instrument, and used in publication must use the following acknowledgement:

"This work made use of the IMSERC at Northwestern University, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the State of Illinois, and the International Institute for Nanotechnology (IIN)."

## TROUBLESHOOTING

**NUCORE / RESERVATION**

1. The computer Screen will not Turn On?

    a. Begin Your reservation in NUcore to initiate access to the instrument

1. There is an error with my reservation?

    a. If you have already started your reservation using NUCore, please logoff by selecting the error reporting option and a brief description about the issue.

        a. If you have not started your reservation using NUCore, please report problems with the instrument at http://imserc.northwestern.edu/contact-issue.html add place the 'Stop' sign near the instrument computer. 'Stop' signs are located at XXXX and online at the link above.

        b. Email or talk to a staff member.

**INSTRUMENT**

## APPENDICES

**APPENDIX A: ADVANCED OPERATIONS 1**

## REVISIONS

| V1.0 2020/07/ | • Release of original version of USER MANUAL for Organic GPC V1.0 |
|---|---|