Integrating the CETAC MVX-7100 Autosampler with the NGC Chromatography System

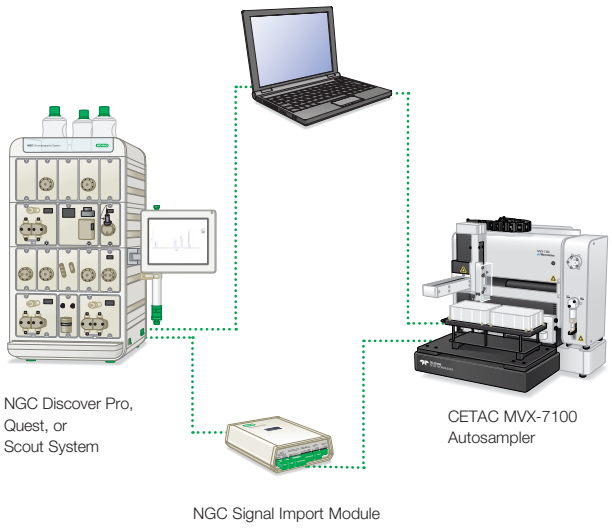
Protocol

Protein Purification

Bulletin 7194

Introduction

In protein purification, it is often necessary to analyze small protein aliquots acquired from large-scale purification. The NGC Chromatography System, along with the Teledyne CETAC Technologies MVX-7100, allows multiple small-scale injections for analysis. Here we describe how to integrate the CETAC MVX-7100 Autosampler with the NGC Quest System to automate sample injections, enabling high precision and accuracy with every run.



Materials

Bio-Rad Laboratories

- NGC Chromatography System with ChromLab Software, version 4.0 or higher (catalog #7880001)
- NGC Signal Import Module (SIM; catalog #7884016)

Additional Tools

Use Teledyne CETAC MVX-7100 Bio-Rad Configuration (part #A71-99-0016HR) or Teledyne CETAC MVX-7100 Bio-Rad Configuration with Temperature Control Block (part #A71-99-0015HR).

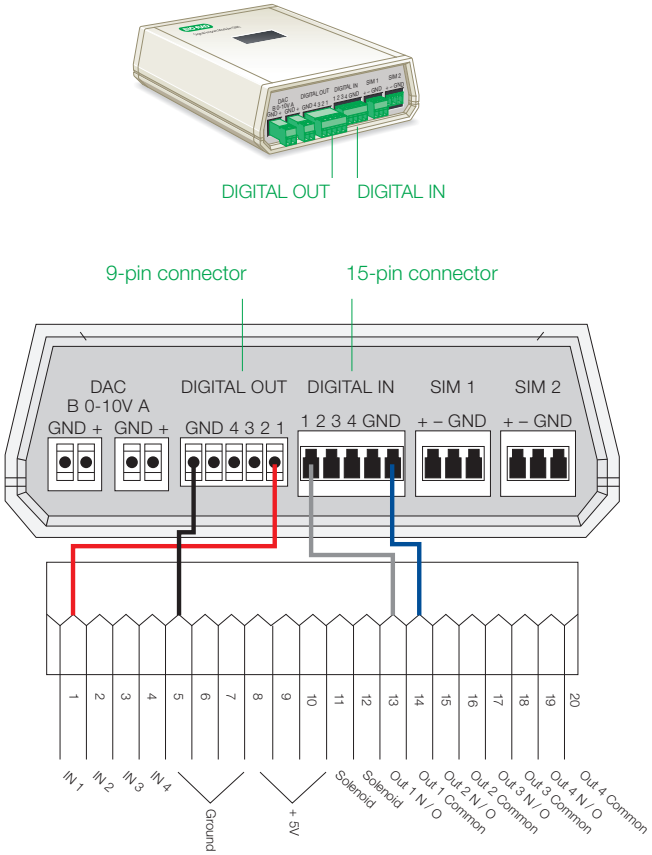
Procedure

1. Connecting the CETAC MVX-7100 Autosampler to the NGC Signal Import Module (SIM)

1.1. Connect the green ends of the provided cable to the NGC SIM DIGITAL IN and DIGITAL OUT.

1.2. Connect the Auxiliary I/O end of the provided cable to the connector on the MVX-7100 Autosampler.

1.3. Power up both instruments.



Connections between the NGC SIM and CETAC MVX-7100 Autosampler.

BIO-RAD

2. Plumbing Connections between the NGC System and CETAC MVX-7100 Autosampler

Note: The MVX-7100 sample injection valve should be placed as close as possible to the NGC injection valve.

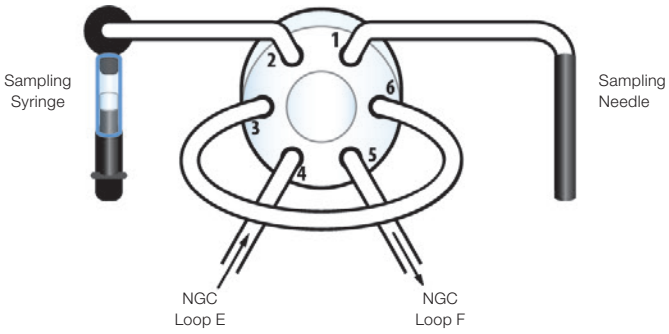
2.1. Use PEEK Tubing with same internal diameter as NGC System tubing to connect position 4 on Autosampler 6-port valve to Loop E (Empty) position on NGC Inject Valve.

2.2. Use PEEK Tubing with same internal diameter as NGC System tubing to connect position 5 on Autosampler 6-port valve to Loop F (Fill) position on NGC Inject Valve.

2.3. Attach an appropriately sized sample loop to positions 3 and 6 on Autosampler 6-port valve.

Note: Positions 1 and 2 on Autosampler 6-port valve should already be connected to the needle (1) and syringe pump (2), respectively.

2.4. After connecting all ports, take a syringe of equilibration buffer and insert into NGC Inject Valve syringe port, emptying the syringe to purge tubing of air. This volume should be five times the loop size.



MVX-7100 Autosampler and 6-port valve.

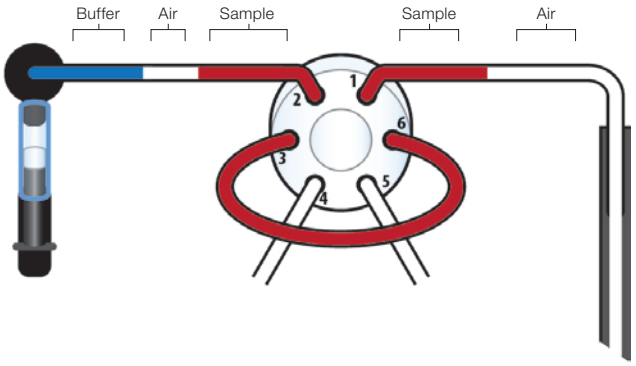
3. Programming the CETAC MVX-7100 Autosampler

The MVX-7100 Autosampler is controlled by CETAC Workstation, a step-based software with line-by-line commands. The following three methods allow sample loading onto the NGC System: Full Loop Pickup, Partial Loop Pickup, and Partial Loop Pickup Series.

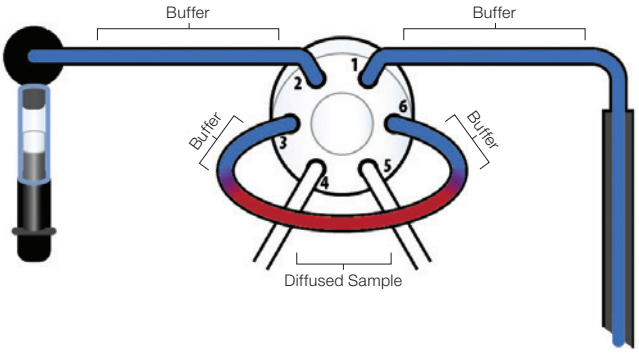
3.1. Full Loop Pickup is a method to inject sample volumes without dilution or diffusion by overfilling the sample loop on the autosampler. This method has the highest precision from sample to sample but does have minor sample loss.

3.2. Partial Loop Pickup is a method designed for loading with no sample loss. The autosampler picks up the designated amount of sample, moves the needle to the rinse station, and uses rinse station buffer to move the sample completely into the sample loop with an excess amount of chase buffer. This results in dilution of the sample but eliminates sample loss.

3.3. Partial Loop Pickup Series is a set of methods allowing for up to six different injection volumes to be used with a set of samples.



Full Loop Pickup sample loading. Air gaps on either side of the sample prevent sample diffusion and enable highest reproducibility with minimal sample loss.



Partial Loop Pickup sample loading. Buffer surrounds the sample volume, allowing the entire sample volume to be loaded with no sample loss but with some sample dilution due to diffusion with the loading buffer.

Full Loop Pickup Method

Step	Command	User Input
1	Select Sample Set	Variable
2	Digital Output	Port Number [1], State [Disabled], Duration (sec) [5]
3	Wait	Duration (sec) [2]
4	Trigger	Input Port# [1], State to wait on [Disabled]
5	Initialize Pump	Pump Address [16], Speed [Medium]
6	Wait for Syringe to Complete Previous Operation	Pump Address [16]
7	Valve to Load	Valve Address [14]
8	Wait	Duration (sec) [2]
9	Move to Rinse Port	Rinse Station # [1], Probe Depth [40]
10	Wait	Duration (sec) [2]
11	Raise Sample Probe	Raise Sample Probe
12	Aspirate	Syringe Address [16], Amount (µl) [5.000], Speed (µl/min) [2000.000], Valve Position [Output]
13	Wait for Syringe to Complete Previous Operation	Pump Address [16]
14	Wait	Duration (sec) [2]
15	Move to Sample Position	[Select] Next Sample Position
16	Wait	Duration (sec) [2]
17	Aspirate	Syringe Address [16], Amount (µl) [variable], Speed (µl/min) [2000.000], Valve Position [Output]
18	Wait for Syringe to Complete Previous Operation	Pump Address [16]
19	Wait	Duration (sec) [2]
20	Raise Sample Probe	Raise Sample Probe
21	Wait	Duration (sec) [2]
22	Aspirate	Syringe Address [16], Amount (µl) [345], Speed (µl/min) [2000.000], Valve Position [Output]
23	Wait for Syringe to Complete Previous Operation	Pump Address [16]
24	Wait	Duration (sec) [2]
25	Valve to Inject	Do not modify
26	Wait	Duration (sec) [2]
27	Digital Output	Port Number [1], State [Disabled], Duration (sec) [5]
28	Rinse Pump ON	Rinse Pump ON
29	Move to Rinse Port	Rinse Station # [1], Probe Depth [40]
30	Wait	Duration (sec) [2]
31	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
32	Wait for Syringe to Complete Previous Operation	Pump Address [16]
33	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
34	Wait for Syringe to Complete Previous Operation	Pump Address [16]
35	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
36	Wait for Syringe to Complete Previous Operation	Pump Address [16]
37	Rinse Pump OFF	Rinse Pump OFF
38	Raise Sample Probe	Raise Sample Probe
39	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
40	Wait for Syringe to Complete Previous Operation	Pump Address [16]
41	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
42	Wait for Syringe to Complete Previous Operation	Pump Address [16]
43	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
44	Wait for Syringe to Complete Previous Operation	Pump Address [16]
45	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
46	Wait for Syringe to Complete Previous Operation	Pump Address [16]
47	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
48	Wait for Syringe to Complete Previous Operation	Pump Address [16]
49	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
50	Wait for Syringe to Complete Previous Operation	Pump Address [16]
51	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
52	Wait for Syringe to Complete Previous Operation	Pump Address [16]
53	Wait	Duration (sec) [2]
54	Home/Initialize Autosampler	Home/Initialize Autosampler

Partial Loop Pickup Method

Step	Command	User Input
1	Select Sample Set	Variable
2	Digital Output	Port Number [1], State [Disabled], Duration (sec) [5]
3	Wait	Duration (sec) [2]
4	Trigger	Input Port# [1], State to wait on [Disabled]
5	Initialize Pump	Pump Address [16], Speed [Medium]
6	Wait for Syringe to Complete Previous Operation	Pump Address [16]
7	Valve to Load	Valve Address [14]
8	Wait	Duration (sec) [2]
9	Move to Rinse Port	Rinse Station # [1], Probe Depth [40]
10	Wait	Duration (sec) [2]
11	Raise Sample Probe	Raise Sample Probe
12	Wait	Duration (sec) [2]
13	Move to Sample Position	[Select] Next Sample Position
14	Wait	Duration (sec) [2]
15	Aspirate	Syringe Address [16], Amount (µl) [variable], Speed (µl/min) [2000.000], Valve Position [Output]
16	Wait for Syringe to Complete Previous Operation	Pump Address [16]
17	Wait	Duration (sec) [2]
18	Raise Sample Probe	Raise Sample Probe
19	Wait	Duration (sec) [2]
20	Rinse Pump ON	Rinse Pump ON
21	Wait	Duration (sec) [2]
22	Move to Rinse Port	Rinse Station # [1], Probe Depth [40]
23	Wait	Duration (sec) [2]
24	Aspirate	Syringe Address [16], Amount (µl) [500], Speed (µl/min) [5000.000], Valve Position [Output]
25	Wait for Syringe to Complete Previous Operation	Pump Address [16]
26	Wait	Duration (sec) [2]
27	Raise Sample Probe	Raise Sample Probe
28	Wait	Duration (sec) [2]
29	Valve to Inject	Do not modify
30	Wait	Duration (sec) [2]
31	Digital Output	Port Number [1], State [Disabled], Duration (sec) [5]
32	Move to Rinse Port	Rinse Station # [1], Probe Depth [40]
33	Wait	Duration (sec) [2]
34	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
35	Wait for Syringe to Complete Previous Operation	Pump Address [16]
36	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
37	Wait for Syringe to Complete Previous Operation	Pump Address [16]
38	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
39	Wait for Syringe to Complete Previous Operation	Pump Address [16]
40	Aspirate	Syringe Address [16], Amount (µl) [1250], Speed (µl/min) [5000.000], Valve Position [Input]
41	Wait for Syringe to Complete Previous Operation	Pump Address [16]
42	Empty Syringe	Syringe Address [16], Speed (µl/min) [5000.000], Valve Position [Output]
43	Wait for Syringe to Complete Previous Operation	Pump Address [16]
44	Raise Sample Probe	Raise Sample Probe
45	Wait	Duration (sec) [2]
46	Home/Initialize Autosampler	Home/Initialize Autosampler

These methods can be used for as many samples as the MVX-7100 Autosampler can hold without cross-contamination from sample to sample.

Note: For the Partial Loop Pickup Series, repeat the Partial Loop Pickup method for up to six different injection volumes to be used with a set of samples.

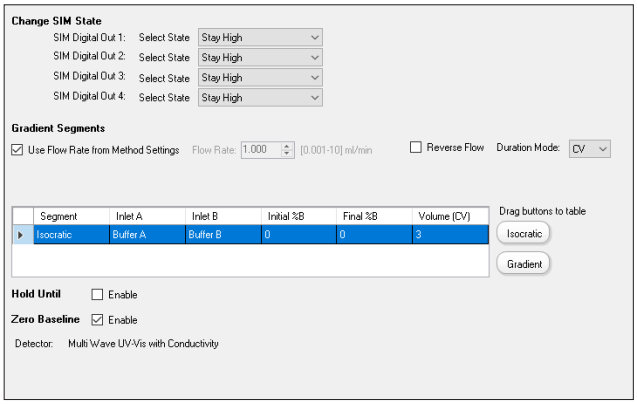
4. Programming the NGC System

The NGC System method software will need to be modified so that it can both send and receive SIM signals. In order to do this, the fluidic scheme should be changed to include a SIM (see the Fluidic Scheme Configurations section referencing the SIM in Chapter 3 of the NGC Chromatography Systems and ChromLab Software User Guide [#10000049092]). Once the SIM has been added to the fluidics scheme, navigate to the Method Editor window and add equilibration and sample application phases to your method. Customize them to provide reliable communication from the NGC System to the MVX-7100 Autosampler.

4.1. Add a SIM to your fluidics scheme in the Method Settings tab.

4.2. Create the MVX-7100 Equilibration Phase.

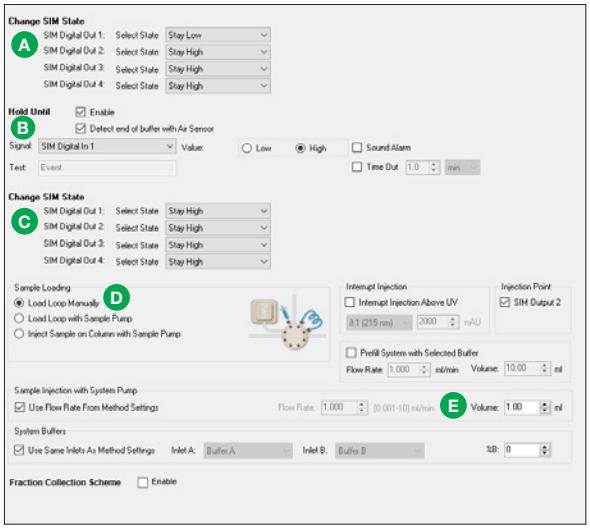
- In the Method Outline tab, add an Equilibration Phase from the Standard Phase Library to the Method Outline
- In the Method Steps tab, drag a SIM Control step from the Step Library to the top of the Equilibration Phase in the Method Steps table (it will be highlighted in green). The phase is automatically renamed Equilibration — Modified
- In the Method Outline tab, use the Change SIM State step to modify SIM Digital to designate all channels as Stay High



4.3. Create the MVX-7100 Sample Application Phase.

- In the Method Outline tab, add a Sample Application Phase from the Standard Phase Library to the method outline

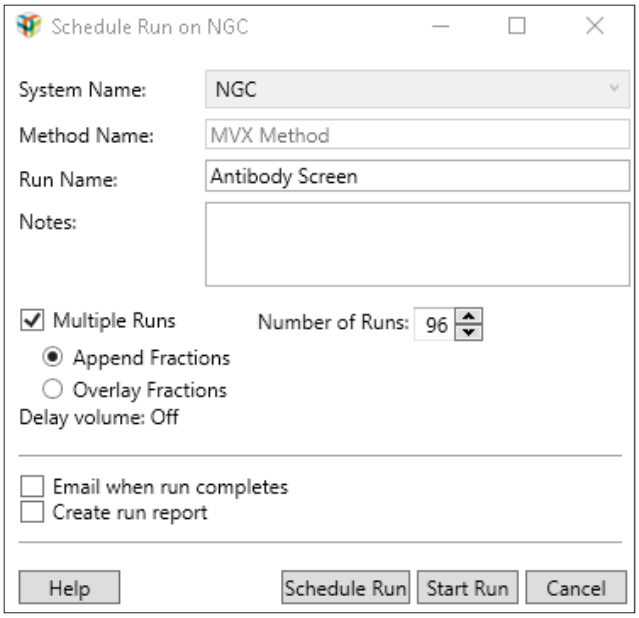
- In the Method Steps tab:
  - Drag a SIM Control step from the Step Library to the Sample Application Phase in the Method Steps table (it will be highlighted in blue). Place the step directly below Fraction Collection as the second step in the phase. The phase is automatically renamed Sample Application — Modified
  - Drag a Hold Until step from the Step Library to the Sample Application Phase directly below the SIM Control step. This will be the third step in the phase
  - Drag a SIM Control step from the Step Library to the Sample Application Phase in the Method Steps table. Place the step directly below Hold Until as the fourth step in the phase
- In the Method Outline tab:
  - **A** Modify the first Change SIM State to designate SIM Digital Out 1 as Stay Low. All other channels as Stay High
  - **B** Enable Hold Until step by selecting the checkbox and modifying the signal to be SIM Digital In 1, value High
  - **C** Modify the second Change SIM State to designate all channels as Stay High
  - **D** Under Sample Loading, select Load Loop Manually
  - **E** Under Sample Injection with System Pump, input a volume that is sufficient to properly load the sample onto the column. This volume should account for sample loop volume plus the volume required to move the sample to the NGC System. This volume should be at least two times the sample loop volume



4.4. Save your custom phases. After adding custom commands, rename and save these phases to the Custom Phase Library. For more information about using the Method Steps tab, see Chapter 5 Method Editor, Method Steps View, in the NGC Chromatography Systems and ChromLab Software User Guide (#10000049092).

4.5. Start a method using the CETAC MVX-7100 Autosampler and NGC System.

- Execute the method (created in step 3 of this protocol) for the number of samples to be run
- Start the NGC System method and match the number of multiple sequential runs to the number of samples in the autosampler



The CETAC MVX-7100 Autosampler and the NGC Chromatography System are now communicating and are able to perform sequential small-scale purifications. For more in-depth information about day-to-day maintenance of the MVX-7100 Autosampler and NGC System, as well as easy injection volume changes, see bulletin 7195, a quick guide for this application.

Visit **bio-rad.com/NGC-MVX7100** for more information.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 01 877 89019* ***Belgium*** *32 03 710 53 00* ***Brazil*** *55 11 3065 7550*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *36 01 459 6192* ***Denmark*** *45 04 452 10 00* ***Finland*** *35 08 980 422 00*
France *33 01 479 593 00* ***Germany*** *49 089 3188 4393* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 01 459 6190* ***India*** *91 124 4029300*
Israel *972 03 963 6050* ***Italy*** *39 02 49486600* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 0 318 540 666*
New Zealand *64 9 415 2280* ***Norway*** *47 0 233 841 30* ***Poland*** *36 01 459 6191* ***Portugal*** *351 21 4727717* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *36 01 459 6193* ***Spain*** *34 091 49 06 580* ***Sweden*** *46 08 555 127 00* ***Switzerland*** *41 0617 17 9555*
Taiwan *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 01923 47 1301*

Bulletin 7194 Ver A US/EG *19-0369 0719 Sig 0119*