N-to-C 5.0 and C-to-N 5.0 Protein Sequencer Methods

Technical Note

HP 241 Protein Sequencer (N+C)

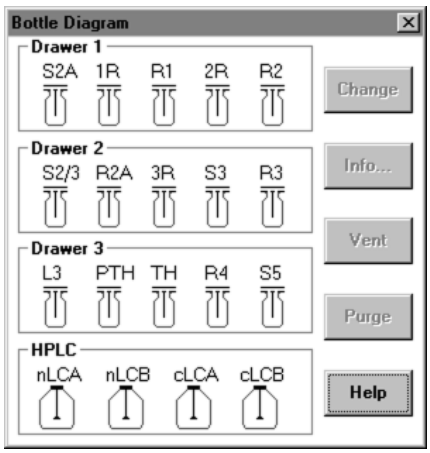
The N-to-C 5.0 and C-to-N 5.0 sequencer methods are responsible for the switching between the N-terminal and C-terminal sequencing chemistry methods on the Hewlett-Packard column-based protein sequencer.

The N-to-C 5.0 method is run when switching from the N-terminal to the C-terminal sequencing chemistry. The column method pauses the column, while the flask method prepares a coordinated injection with the on-line HPLC to place the proper HPLC solvents in the HPLC binary pumps for the C-terminal chromatography.

The C-to-N 5.0 method is run when switching from the C-terminal to the N-terminal sequencing chemistry. The column method washes the column, while the flask method, in addition to washing the flask, prepares a coordinated injection with the on-line HPLC to place the proper HPLC solvents in the HPLC binary pumps for the N-terminal chromatography. These methods are generally run with a Zitex strip in an empty RP/SAX biphasic column, but any column configuration is applicable. No column preparation is required with this method.

The methods are:
- N-to-C 5.0 (column)
- N-to-C 5.0 (flask)
- Column wash 5.0
- Flask wash 5.0

The sequence program controls all of the column and flask methods, as well as any cycle exception methods. The N-to-C 5.0 and C-to-N 5.0 sequencer methods do not use an exception method.



The Routine 5.0 sequencer methods require the bottle configuration shown. The reagents and solvents are purchased from Hewlett-Packard. These methods produce less than 1mL/cycle of liquid waste.

S2A	Neat ethyl acetate
1R	Diphenylphosphorylisothiocyanate (DPP-ITC) in toluene/heptane (23:27:50)
R1	Phenylisothiocyanate (PITC) in heptane (3:97)
2R	Pyridine in ethylacetate (2:98)
R2	Diisopropylethylamine (DIEA) in 1-propanol/water (1:6:3)
S2/3	Acetonitrile/toluene (23:77)
R2A	Ocylamine in heptane (3:97)
3R	Potassium trimethylsilanolate (0.1M) in methanol/t-butanol (50:50)
S3	Acetonitrile/toluene (15:85)
R3	Neat trifluoroacetic acid (TFA)
L3	Acetic acid in methanol/water (1:74:25)
PTH	Mixture of PTH amino acids (10 pmol/100 µl) in acetonitrile with DPTU as a marker
TH	Mixture of TH amino acids (50 pmol/100 µl) in acetonitrile
R4	Trifluoroacetic acid (TFA) in water (1:3)
S5	Phosphate buffer (pH 2.9), 0.2% ion pairing reagent

Method Details

The steps for the N-to-C 5.0 and C-to-N 5.0 methods are described below. The steps indicate the ranges in volume that are appropriate for the various reagents/solvent deliveries. All sequencer methods are accessed by choosing the top menu item Edit/Method in the Protein Sequencer window. The sequencer column configuration used with the N-to-C 5.0 and C-to-N 5.0 methods consists of an empty reverse-phase (RP) sample column (top) mated with an empty strong anion exchange (SAX) column (bottom).

Method (Column): Column wash 5.0
Total time: 45.5 min

All metering steps deliver the specified reagent or solvent to waste.

Steps 2, 3: "Flush with S2A, S3, S2/3 flushes the delivery lines and should not wet the column

Steps 5, 7, 8, 9, 10, 11, 12, 27, 28: The delivery of S2A should completely fill the column, followed by drying.

Steps 14, 15, 25, 33, 34, 38, 39: The delivery of S3 should completely fill the column, followed by drying.

Steps 17, 18, 30, 31, 41, 42: The delivery of S2/3 should completely fill the column, followed by drying.

Steps 19, 21, 23: The volume of R3 (approximately 1/2 column volume) delivered during this step should completely wet the membrane.

Step	Description	Primary Time	Temperature
1:	Wash: Dry column DOWN	30.0	55
2:	Wash: Flush with S2A, S3, S2/3	14.0	55
3:	Wash: Flush with S2A, S3, S2/3	14.0	55
4:	Wash: Meter S2A UP	60.0	55
5:	Wash: Deliver and DRY UP (closed)	30.0	55
6:	Wash: Meter S2A DOWN	60.0	55
7:	Wash: Deliver and DRY DOWN (closed)	30.0	55
8:	Wash: Flush with S2A DOWN	14.0	55
9:	Wash: Flush with S2A UP	14.0	55
10:	Wash: Flush with S2A DOWN	14.0	55
11:	Wash: Flush with S2A UP	14.0	55
12:	Wash: Flush with S2A DOWN	14.0	55
13:	Wash: Dry Column DOWN	30.0	55
14:	Wash: Flush with S3 UP	15.0	55
15:	Wash: Flush with S3 DOWN	15.0	55
16:	Wash: Dry column UP	30.0	55
17:	Wash: Flush with S2/3 DOWN	15.0	55
18:	Wash: Flush with S2/3 UP	15.0	55
19:	Wash: Flush with R3 UP	30.0	55
20:	Wash: Dry column UP	30.0	55
21:	Wash: Flush with R3 DOWN to waste	30.0	55
22:	Wash: Dry column DOWN	30.0	55
23:	!Wash: Flush with R3 DOWN to flask	30.0	55
24:	!Wash: Flush with S3	15.0	55
25:	!Wash: Dry column DOWN to flask	60.0	55
26:	!Wash: Dry column and flask	30.0	55
27:	!Wash: Flush with S2A DOWN to flask	14.0	55
28:	!Wash: Flush with S2A UP to flask	14.0	55
29:	!Wash: Dry column and flask	30.0	55
30:	!Wash: Flush with S2/3 DOWN to flask	15.0	55
31:	!Wash: Flush with S2/3 UP to flask	15.0	55
32:	!Wash: Dry column and flask	30.0	55
33:	!Wash: Flush with S3 UP to flask	15.0	55
34:	!Wash: Flush with S3 DOWN to flask	15.0	55
35:	!Wash: Dry column and flask	30.0	55
36:	!Wash: Flush with L3 and R4	20.0	55
37:	!Wash: Flush with L3 and R4	20.0	55
38:	!Wash: Flush with S3 UP	15.0	55
39:	!Wash: Flush with S3 DOWN	15.0	55
40:	Wash: Dry column DOWN	30.0	55
41:	Wash: Flush with S2/3 DOWN	15.0	55
42:	Wash: Flush with S2/3 UP	15.0	55
43:	Wash: Dry column DOWN	30.0	55
44:	Wash: Flush with S2/3 and S2A	15.0	55
45:	Wash: Dry column UP	60.0	55

! indicates system step

Method (Column): N-to-C 5.0
Total time: 20.7 min

The column pauses during this method while the N-to-C 5.0 flask method runs

Step	Description	Primary Time	Temperature
1:	wait	20.0	30
2:	!wait system	20.0	30
3:	wait	600.0	30
4:	wait	600.0	30

! indicates system step

Method (Flask): Flask wash 5.0
Total time: 67.1 min

Step 3: After Step 3, the volume of R4 delivered to the flask should be approximately 70 µl (+/- 5 µl).

Step 5: By the end of Step 5, the flask will have been dry for 100-150 seconds.

Step 6: By the end of Step 6, 70 µl (+/-5 µl) of L3 will have been delivered to the flask.

Step 7: By the end of Step 7, the flask will have been dry for 75-100 seconds.

Step 8: The delivery of L3 may not be visible.

Step 9: After Step 9, the volume of L3 and S5 in the flask should be 75-80 µl. If adjustment is needed, it should be done by adjusting the metering time of S5, leaving L3 unchanged.

TEST Delivery protocol:
To check the delivery volume of L3 and S5, copy Steps 8, 9, 10 and 11 to the clipboard and run from the clipboard. At the end of Step 11, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask.

Step 12: Before injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 12 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Wash: Deliver R4 rinse	80.0	50
2:	Wash: Empty flask	60.0	50
3:	Convert: Deliver R4	29.0	70
4:	Convert: React	400.0	70
5:	Convert: Evaporate dry	430.0	70
6:	Convert: Flush with L3	15.0	70
7:	Convert: Evaporate dry L3	180.0	70
8:	Convert: Deliver L3 dripwise	2.0	60
9:	Convert: Deliver S5 dripwise	12.0	60
10:	Convert: Mix and equilibrate	15.0	60
11:	Convert: Solubilize	20.0	60
12:	Convert: Fill loop and inject	1.8	60
13:	Convert: Empty flask	30.0	60
14:	Convert: Flush with L3	85.0	45
15:	Convert: Mix	20.0	45
16:	Convert: Empty flask	30.0	45
17:	Convert: Deliver R4 wash	40.0	45
18:	Convert: Empty flask	30.0	45
19:	Convert: Deliver R4 wash	40.0	45
20:	Convert: Empty flask	30.0	45
21:	Convert: Dry waste line	60.0	45
22:	Convert: Dry vent line	60.0	45

Method (Flask): N-to-C 5.0
Total time: 11.2 min

Step 5: After Step 5, the volume of S5 in the flask should be 75-80 µl.

TEST delivery protocol:
To check the delivery volume of S5, copy Step 5 to the clipboard and run from the clipboard. At the end of Step 5, remove the flask from the heating chamber. Carefully unscrew the flask cap and measure the delivered volume using a syringe. Be careful that some of the delivered volume is not retained on the tube sides or top of the flask

Step 8: Before the injection the injector loop should be filled, leaving the solvent visible in both the inlet and outlet lines of the injector loop. The time for Step 8 may have to be adjusted in order to optimize the delivery.

Step	Description	Primary Time	Temperature
1:	Wash: Empty flask	30.0	50
2:	Wash: Flush with L3	85.0	50
3:	Wash: Mix	20.0	50
4:	Wash: Empty flask	30.0	50
5:	Wash: Deliver S5 dripwise	12.5	50
6:	Wash: Mix and equilibrate	15.0	50
7:	Wash: Solubilize	20.0	50
8:	Wash: Fill loop and inject	2.0	50
9:	Wash: Empty flask	20.0	50
10:	Wash: Flush with L3	85.0	50
11:	Wash: Mix	20.0	50
12:	Wash: Empty flask	30.0	50
13:	Wash: Dry waste line	60.0	50
14:	Wash: Dry vent line	60.0	50

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