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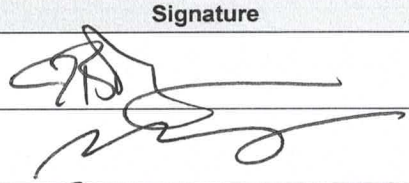
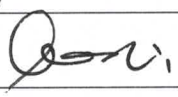
Owner	**John Randolph**	**05Feb2025**	
Operation Management	**Baozhong Zhao**	**05Feb2025**	
Quality Assurance	**Xibo Li**	**05Feb2025**	

Changes from previous version:

Section	Summary of Changes	Change Control Number
ALL	New document	

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1. PURPOSE

This SOP provides detailed guidelines for using Electrospray Ionization Mass Spectrometry (ESI-MS) to analyze oligonucleotides for quality control purposes using the Oligo HTCS Vanquish System. The goal is to verify the identity, purity, and integrity of the oligonucleotide sample by assessing its molecular weight and checking for any degradation or impurities.

2. SCOPE

This procedure applies to all laboratory personnel involved in the quality control (QC) of oligonucleotides using ESI-MS. It covers sample preparation, instrument setup and data acquisition.

3. INTERNAL REFERENCES

Document ID	Title
QUA004	Quality Policy

4. EXTERNAL REFERENCES

Document	Title
ISO9001	Quality management
	Oligo_HTCS_Vanquish_LTQ-XL_Users_Guide_revB_01-Dec-2020

5. RESPONSIBILITIES

Job Function and/or Department	Responsibility
All Personnel	Employees who use the Oligo HTCS Vanquish System for ESI MS characterization of oligonucleotides must be trained on this SOP. All staff are responsible for ensuring that equipment used in testing activities is qualified and approved for use, as appropriate.

6. DEFINITION

Term	Definition
Current Good Manufacturing Practices (cGMP)	Regulations enforced by the FDA and provided for systems that assure proper design, monitoring, and control of manufacturing processes and facilities used in storage, manufacture, testing, release, and distribution of regulated products.

7. Introduction

7.1. The Oligo HTCS Vanquish system consists of a Thermo ion trap mass spectrometer (LTQ series instruments) running on the Xcalibur PC data system (Thermo Scientific, Corp.), a Thermo Vanquish UHPLC, ProMass Deconvolution data processing software, and optimized analytical methods for high-throughput oligonucleotide LC/MS analysis.

8. Vanquish Oligo HTCS Methods and Set up

8.1. Vanquish Oligo HTCS Set Up

8.1.1. Fill solvent reservoirs and empty waste containers (Note if high-throughput desalting runs are only going to be used, Oligo Wash can be used in both left and right Pump Solvent A)

Left Pump Solvent A – Oligo Wash **(vol/L)**	0.075% HFIPA (750 uL)	0.0375% DIEA (375 uL)	10 uM EDTA (10 mL of 1 mM EDTA)	H2O (990 mL)
Solvent B –Oligo Elute **(vol/L)**	0.075% HFIPA (750 uL)	0.0375% DIEA (375 uL)	10 uM EDTA (10 mL of 1 mM EDTA)	65% ACN (650 mL ACN, 340 mL H2O)

Solvent C (vol/L) **Autosampler Wash**	40% ACN (400 mL)	40% MeOH (400 mL)	20% H2O (200 mL)	
HPLC Seal Wash	90% H2O (900 mL)	10% MeOH (100 mL)		

9. Vanquish Oligo Methods

9.1. Vanquish Instrument methods are normally saved in the **C:\Xcalibur\methods** directory. These methods are backed up in the **C:\Xcalibur\methods\backup** directory should you change something inadvertently and want to return to the original methods. Typically, the oligo_htcs method is exclusively used. However, for R&D purposes to verify the identity of unknown peaks, the LC/MS methods lcms_5min or lcms_20min may be used. The methods and their descriptions are shown below:

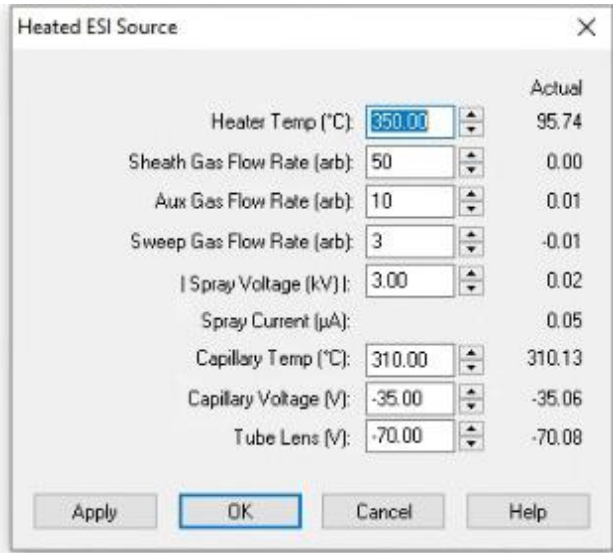
#	Instrument Method Name	Description
1	oligo_htcs	High-throughput injection and on-line desalting method using dual traps in the trap (left) column compartment. Both trap column valves are toggled for each run. The right column compartment upper valve is set to position 1_2 to bypass the analytical column. This method uses look ahead injection and no needle rinsing to minimize analysis time at ~40 sec/sample. The plumbing scheme is shown in Figure 1.5.1.
2	oligo_htcs_needle_wash	Same as the *oligo_htcs* method, except that a needle rinse is included to minimize carryover. Injection rate is ~52 sec/sample.
3	oligo_htcs_long_wash	Same as the *oligo_htcs_needle_wash* method, except that a longer trap wash time (additional 30 sec) is used to desalt samples that contain very high concentrations of salt. Injection rate is ~82 sec/sample.
3	lcms_5min	LCMS separation using the column in the right column compartment (mounted on the upper valve). Sample flows from the active trap in the trap compartment to the analytical column. The method utilizes a 5 min gradient and 9 min total run time. The plumbing scheme is shown in Figure 1.5.2.
4	lcms_20min	Same as *lcms_5min* method, but with a 20 min gradient, 24 min total run time. Plumbing scheme is shown in Figure 1.5.2.

10. LTQ Instrument Settings

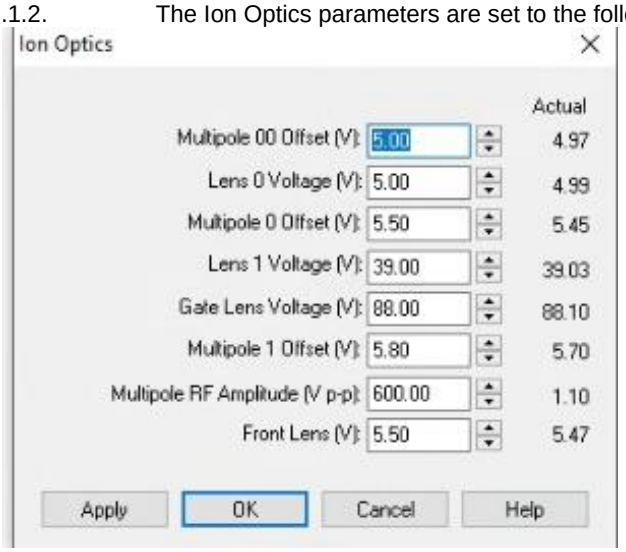
10.1. LTQ instrument settings are set from the **Thermo Tune Plus** application. Novatia has optimized your specific instrument settings for oligonucleotide applications and has saved the tune file as **oligo.LTQTUNE.** This tune file is referenced in all Oligo HTCS instrument methods. If you choose to retune the instrument and overwrite this file, you may want to first make a backup copy of the original oligo tune file.

10.1.1. ESI Source Conditions

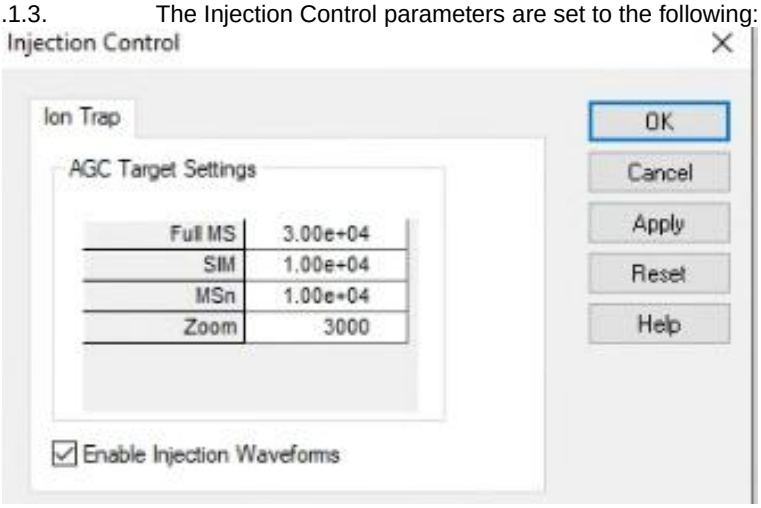
10.1.1.1. The Heated ESI Source parameters are set to the following:



10.1.1.2. The Ion Optics parameters are set to the following:



10.1.1.3. The Injection Control parameters are set to the following:

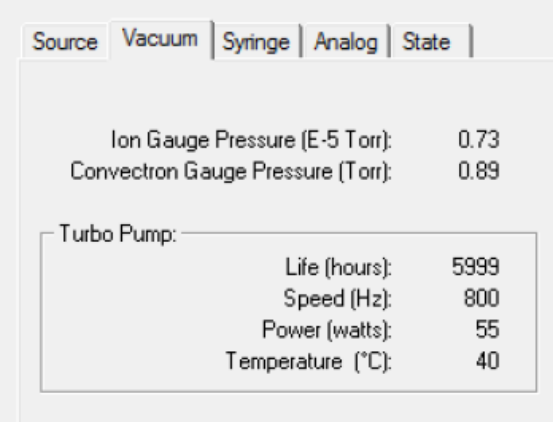


11. Preparation for Sample Acquisition

11.1. Check MS system vacuum

11.2. From the Xcalibur Home Page:

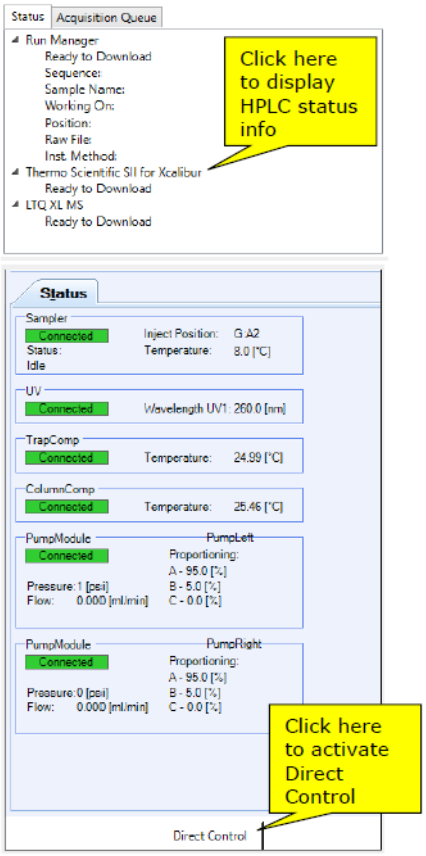
click on **LTQ XL MS** in the status window and the **Vacuum** tab



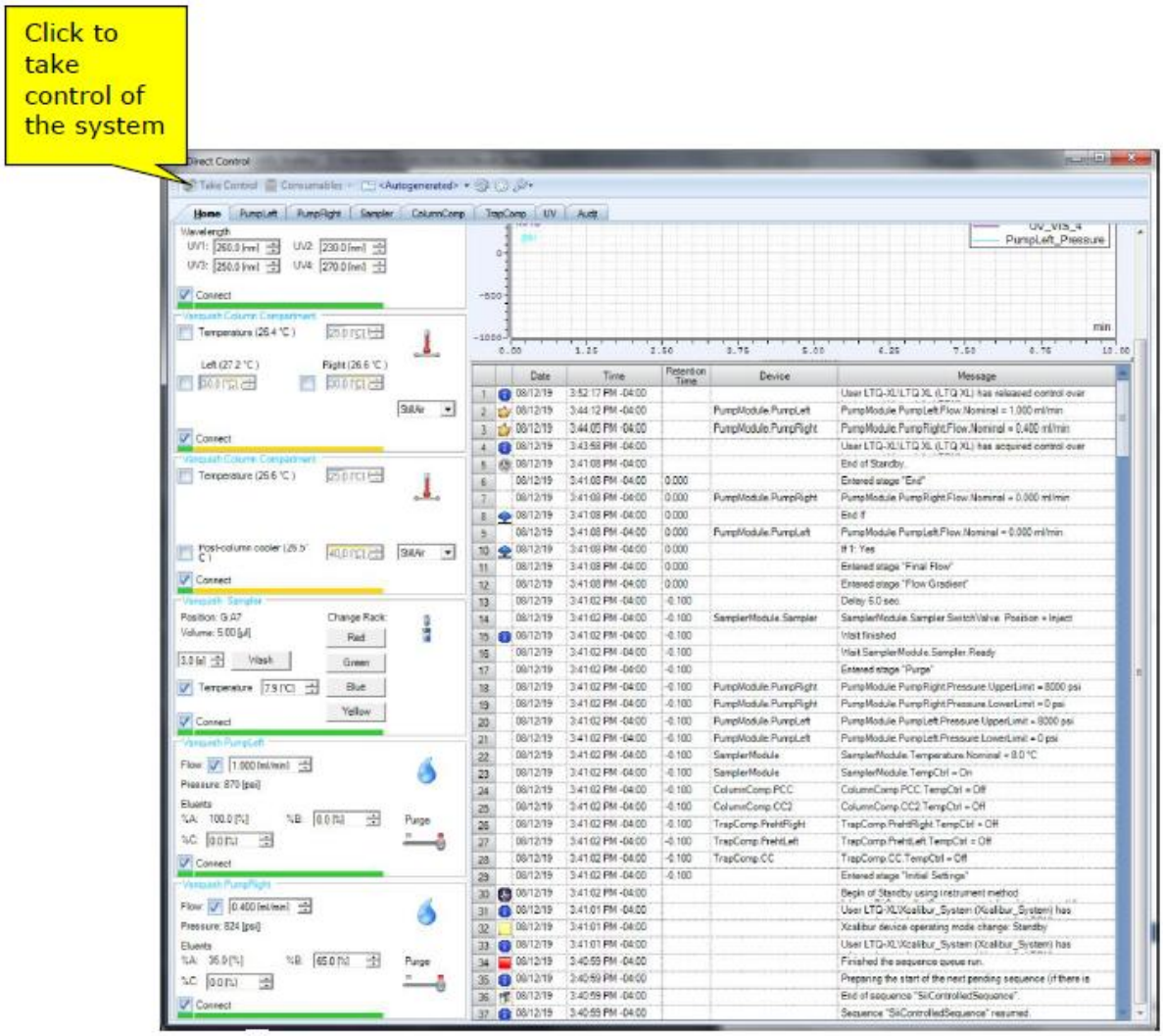
11.3. Verify the ion gauge (high vacuum) pressure is less than 1.5 x 10-5 Torr and the convectron (API) gauge pressure is less than 1 Torr.

11.4. Select Actions | Devices On**.** This will turn on the MS ion source gases and high voltages.

11.5. In the Xcalibur HomePage status area, click on the *Thermo Scientific SII for Xcalibur* item in the instrument status area of HomePage as shown below.



11.6. Click on Direct Control to open the Direct Control Application. The following window will be displayed:



11.7. Click on top left tab to Take Control of the system.

11.8. Set the PumpLeft pump to 1 mL/min 100%A and the PumpRight flow rate to 0.4 mL/min 65%B. If the UV lamp is not already on, the UV Lamp button can be used to turn on the lamp. It may be necessary to wait a few minutes for the lamp to warm up and perform a self-calibration before starting a run.

12. Sample Preparation

12.1. Prepare sample using HPLC grade water. 50 pmole injections are optimal - for example, a 10 uL injection volume of a 5 uM oligo solution. Place the sample plate in the carousel noting its position. Note for the first sample run for the day, include a standard of known mass to verify accuracy of the instrument. Note, if the observed mass differs from by target mass by >0.02%, the instrument should be recalibrated as described in sections 6.0-6.6 of the Oligo_HTCS_Vanquish_LTQ-XL_Users_Guide_revB_01-Dec-2020.

12.2. Use the Xcalibur Sequence Setup program to make the sample list file (.sld), filling in the File name, Sample position(s), Data path, Instrument method, Sample ID, Sample name, Injection volume, Process Method and Target Mass. The sample naming convention is: S20XXX-N (where S20XXX is the project number and N is the sequence number). Alternatively, for a large number of samples, a CSV template can be used to import the data using File | Import Sequence.

12.3. Verify that the state of LTQ XL MS and Thermo Scientific SII for Xcalibur are *Ready to Download* as indicated by the Status information tab of the Xcalibur Home Page.

12.4. Hit the icon  to begin acquisition of the entire list. Alternatively you can begin acquisition by selecting the **Actions | Run Sequence…**as a menu item. To run selected lines of the sample list, highlight sample list and hit the  button.

12.5. The raw ms spectra are processed automatically by Promass to generate the deconvoluted spectra.

13. Data Analysis

13.1. Using the defined data path – for example,

Lab_Data (Z:) › Instrument-data-backup › Vanquish-LTQ-1 › 20250128 › promass_results

Click on the html file Index. The summary of the results will be shown:

SynOligo BIOTECHNOLOGIES

Popup View

ProMass Sample Browser

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

Data File	Position	Sample ID	Sample Name	Sample Comments	Target Masses	Observed Masses	Intensity	Purity	Result Code
init	1	1	1	Template, DO NOT delete		8753.3	1.70E+004		
S20539-QT-1-1	1	2	2			3722.2	9.77E+005		

13.2. The summary of the results are color-coded as defined below:

Result Code	Indication
	Target mass found in chromatogram as the most abundant component within mass tolerance of 0.02 %, with minimum tolerance of 3 Da
	Target mass found as a major component or as a minor component with other target masses, but NOT as most abundant component in chromatogram
	Target mass found in chromatogram with either or all of the following: (a) other non-target components present in spectrum > 30% abundance (b) low spectral quality (low intensity and/or score)
	Target mass found in chromatogram, but NOT as the most abundant in any of the chromatographic peaks
	Target mass NOT found in chromatogram within mass tolerance of 0.02 %, with minimum tolerance of 3 Da
	No target masses specified

13.3. The scientist operating the instrument will do the initial review of the results based upon the customer's requirements and whether this is a final QC or initial QC result prior to downstream purification.

13.4. Initial QC

13.4.1. Samples with a green code result are considered passing.

13.4.2. Samples with a blue or purple code result are considered a tentative pass. Consult director to determine if there is **sufficient** purity for downstream purification and yield requirements or if, given the length of the oligo, it is considered passing and is reported as such.

13.4.3. Samples with an orange code result will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.4.4. Samples with a red code result 🔴 will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.4.4.1. If there are two oligos on the same plate that both failed, verify the oligos were not accidentally swapped.

13.4.5. For samples with a pale blue color code result 🔵 are typically initial results for research purposes. Notify the results to the appropriate chemist.

13.5. Final QC

13.5.1. For all final QC results, the QC analyst reviews the customer's required specifications for the oligos, paying special attention to the maximum allowed impurities and minimum yield requirements. Note all final QC data – ESI MS, HPLC, yield and concentration - is given a final review by the appropriate director or manager to generate the Certificate of Analysis and approval for shipping.

13.5.2. Salt Free oligos

13.5.2.1. Samples with a green code result 🟢 are considered passing.

13.5.2.2. Samples with a blue or purple code result 🔵 🟣 are considered a tentative pass. Consult director to determine if, given the length of the oligo or its complexity, it is considered passing and is reported as such.

13.5.2.3. Samples with an orange code result 🟠 will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.5.2.4. Samples with a red code result 🔴 will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.5.2.4.1. If there are two oligos on the same plate that both failed, verify the oligos were not accidentally swapped.

13.5.2.5. For samples with a pale blue color code result 🔵 are typically initial results for research purposes. Notify the results to the appropriate chemist.

13.5.3. HPLC purified oligos

13.5.3.1. Samples with a green code result 🟢 are considered tentatively passing until the customer specifications are reviewed.

13.5.3.2. Samples with a blue or purple code result 🔵 🟣 are considered a tentative fail. Consult director or manager to determine if special ESI conditions are required for the modifications found in the oligo to minimize MS artifacts.

13.5.3.3. Samples with a red code result 🟠 will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.5.3.4. Samples with a red code result 🔴 will be inspected to insure there was enough sample in the well or vial and if so, the sample is run again. If the same result is obtained, the oligo is failed and the results are reported to the director.

13.5.3.4.1. If there are two oligos on the same plate that both failed, verify the oligos were not accidentally swapped.

13.5.3.5. For samples with a pale blue color code result 🔵 are typically initial results for research purposes. Notify the results to the appropriate chemist.

14. Maintenance

14.1. Maintenance carried out on the Oligo HTCS Vanquish System must be recorded in the associated Instrument Logbook.

14.2. Installation, operation, and performance qualification documents associated with the instruments are to be stored with QA.

14.3. Refer to the Oligo_HTCS_Vanquish_LTQ-XL_Users_Guide_revB_01-Dec-2020.or more information or detailed instructions regarding instrument maintenance, refer to the LTQ manuals for detailed maintenance schedules and instructions. A full set of instrument manuals is available under **Start | Programs | Thermo Instruments | Manuals | LTQ XL**.

14.4. The maintenance schedule is shown below:

Period	Maintenance
Daily/Weekly	• Empty solvent waste containers. • Refill mobile phase reservoirs, HPLC seal wash solvent, and autosampler wash solvent. • Monitor pressure (with new trap columns the pressure should be 700-1100 psi). • Replace the column traps if loss in sensitivity, or excessive system pressure is observed.
3-6 months	• Change MS vacuum pump oil every 6 months. • Clean the fan filter on the rear of the LTQ card cage. • Run the LTQ calibration after cleaning the instrument or every 3-6 months. • Check the flow rate of the HPLC pumps periodically to ensure proper operation of the check valves and pump head seals. • Measure the flow from each pump at 300 uL/min collected in an Eppendorf tube over one minute. Make the measurement with at least 200 psi backpressure by inserting a column in line if necessary. The measured flow rate should be within 5-10% of the flow setting.
6 months – 1 year	• Clean interior high vacuum side of API source (tube lens and skimmer). • Clean LTQ MS analyzer as necessary (i.e. if there's a loss in performance) by service engineer or highly trained user. • Service HPLC • Replace pump head seals annually. • Inspect and/or replace pump pistons and check valves on an annual basis, or as needed.