

Vantage 3D DNA SNV Qualification Kit

The nCounter® Vantage 3D DNA SNV Qualification Kit is designed to assess whether the nCounter MAX, FLEX, or SPRINT instrument system is operating at its optimal performance level to yield accurate SNV detection calls. The unique nature of SNV probe chemistry is sensitive to temperature fluctuations during the elevated-temperature sample processing steps. To ensure accurate DNA SNV panel calls, NanoString requires that a Qualification Run be performed on each Prep Station or SPRINT Profiler that is to be used for SNV assays prior to use of the nCounter Vantage 3D SNV Panels. The DNA SNV Qualification Kit uses mixtures of synthetic reference and variant DNA targets to ensure accurate variant detection. The results from this Qualification Run, in the form of Log Files, must be provided to NanoString (snvqualify@nanosttring.com) to determine whether adjustment is required prior to use with any DNA SNV Panels. Please contact NanoString Support (snvqualify@nanosttring.com) for additional assistance with this qualification procedure.

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Materials and Equipment

Table 1. Materials provided with the Vantage 3D DNA SNV Qualification Kit

Kit	Reagents	Storage
nCounter Vantage 3D DNA SNV Qualification Kit (CSO) Catalog #: VDXC-QualK-12	SNV TagSet	-80°C
	DNA SNV Reference Target Pool	-80°C
	DNA SNV Variant Target Pool (2 tubes)	-80°C
	SNV Solid Tumor Probe S Pool	-80°C
	SNV Solid Tumor Probe T Pool	-80°C
	Reporter Library File (RLF)	NA

*An nCounter Master Kit (for MAX/FLEX) or a SPRINT Cartridge (for SPRINT) will be provided by NanoString Technologies with the Qualification Kit.

Table 2. Additional materials required to use the DNA SNV Qualification Kit (not provided)

Material	Part Number	Manufacturer
Nuclease-free water or TE buffer, pH 8.0	Various	Various
Thin-wall PCR strip tubes	Various	Various
1.7 mL microfuge tubes	Various	Various

Table 3. Equipment required for use with the DNA SNV Qualification Kit

Equipment
nCounter SPRINT Profiler, MAX Analysis System, or FLEX Analysis System
Microfuge or picofuge
Pipettes (p1000, p100, p20, p10)
Calibrated thermal cycler with heated lid

Thermal Cycler Guidelines

Please note that a thermal cycler **with a heated lid** is required for this protocol. NanoString recommends using a model with a programmable heated lid. Models without programmable lids may reach a very high temperature that causes tubes to melt or deform. However, programmable lids may offer different levels of control.

- Ideally, NanoString recommends a thermal cycler with a heated lid that can adjust throughout the protocol. The heated lid should be set to 5°C greater than the current incubation temperature at any moment.
- Some heated lids cannot be assigned a floating temperature. In this situation, program the heated lid to be 5°C greater than the maximum temperature during the protocol. The heated lid should not exceed 110°C.

Important Probe Handling Instructions

- During setup, do not vortex or pipette vigorously to mix.
- Mixing should be done by flicking or inverting the tubes.
- If using a microfuge to spin down tubes, do not spin any faster than 1,000xg for more than 30 seconds.
- Do not “pulse” to spin because that will cause the centrifuge to go to the maximum speed and may spin the probes out of solution.

Hybridization Prior to Qualification Run

IMPORTANT: Instructions for preparing the Probe S Pool, Probe T Pool, Reference Target Pool, and Variant Target pool working stocks are provided in steps 3–6 below. **When making these working stocks, mix gently but thoroughly, such as by flicking the tubes or performing several cycles of gentle up-and-down pipetting to ensure that the solutions are homogeneous.**

Each hybridization reaction will contain the following components:

Table 4. Hybridization reaction components for a single tube

Component	Volume (μL)
SNV TagSet	2
Hybridization Buffer*	5
SNV Solid Tumor Probe T Pool working stock	0.5
SNV Solid Tumor Probe S Pool working stock	0.5
DNA SNV Reference Target Pool working stock	2
TE buffer or nuclease-free water (for Reference Target Pool) or DNA SNV Variant Target Pool working stock (for Variant Target Pool)	5
Total Volume	15

*Hybridization Buffer is provided with the Master Kit and comes as part of the SPRINT Reagent Pack.

nCounter MAX or FLEX Analysis Systems

The order of addition is important, so please follow the protocol **exactly as presented**.

1. Pre-heat thermal cycler to 65°C with heated lid turned on and “forever” time setting.
2. Remove the tubes of SNV TagSet, SNV Solid Tumor Probe T Pool, SNV Solid Tumor Probe S Pool, DNA SNV Reference Target Pool, and DNA SNV Variant Target Pool (only one tube of the Variant pool is needed when running on the MAX/FLEX) from the freezer and thaw at room temperature. Invert or flick tubes several times to mix well and briefly spin down reagents.

NOTE: Inspect the thawed tubes of SNV TagSet to make sure no colored precipitate is present. If you see a colored precipitate, heat the entire tube to 75°C for 10 minutes and cool at room temperature before using. **Do not heat the Probe S Pool above room temperature.**

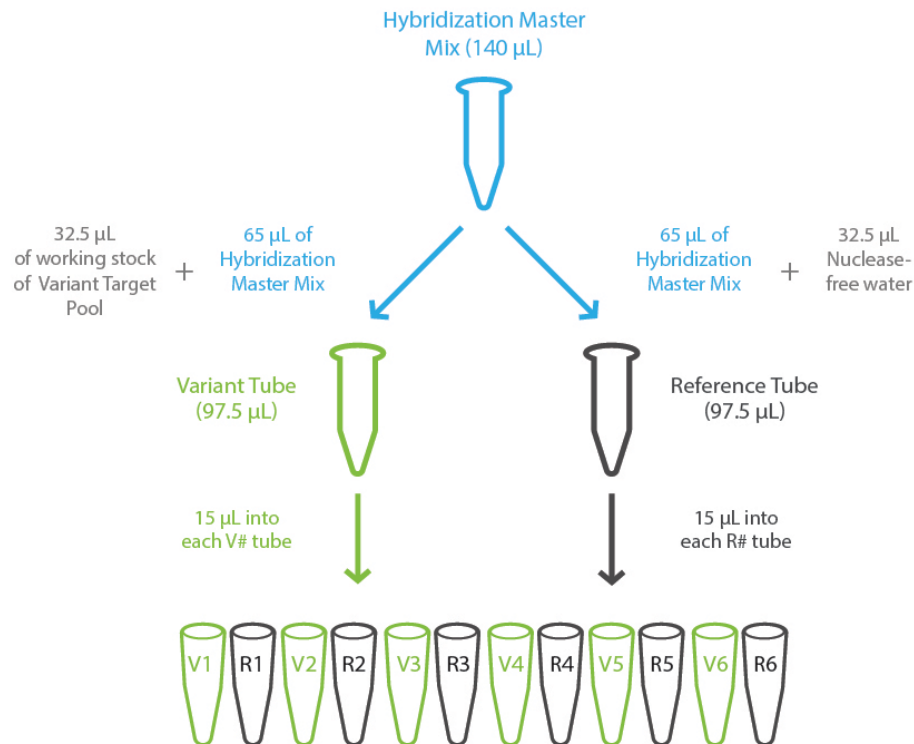
3. Create a 30X working stock of Probe T Pool by adding 22 µL of nuclease-free water or TE buffer to the tube of SNV Solid Tumor Probe T Pool.
4. Create a 30X working stock of Probe S Pool by adding 22 µL of nuclease-free water or TE buffer to the tube of SNV Solid Tumor Probe S Pool.
5. Create a 7.5X working stock of Reference Target Pool by adding 22 µL of nuclease-free water or TE buffer to the tube of DNA SNV Reference Target Pool.
6. Create a 3X working stock of Variant Target Pool by adding 22 µL of nuclease-free water or TE buffer to the one of the tubes of DNA SNV Variant Target Pool (as mentioned, the other tube is only required for SPRINT).
7. Create a Hybridization Master Mix sufficient for 14 reactions by adding the following directly into the thawed tube of SNV TagSet, in the **exact order as presented**.
 - 28 µL of SNV TagSet (already in the tube)
 - 70 µL of Hybridization Buffer,
 - 7 µL of 30X working stock of SNV Solid Tumor Probe T Pool
 - 7 µL of 30X working stock of SNV Solid Tumor Probe S Pool
 - 28 µL of 7.5X working stock of DNA SNV Reference Target Pool

Flick the tube repeatedly to mix, then spin down the Hybridization Master Mix.

8. Aliquot the Hybridization Master Mix by dispensing 65 µL each into two clean microfuge tubes, using a fresh pipet tip for each pipetting step. Label one tube “Reference” and the other tube “Variant”. The total volume in step 7 adds up to 140 µL to ensure that there is more than enough solution to sub-aliquot into two tubes of 65 µL each.
9. To the “Reference” tube, add 32.5 µL of nuclease-free water or TE buffer to make the Reference Hybridization Reaction.
10. To the “Variant” tube, add 32.5 µL of the 3X working stock of DNA SNV Variant Target Pool to make the Variant Hybridization Reaction.

11. Label the 12-strip hybridization PCR tubes supplied with the nCounter Master Kit. Ensure that the tubes will fit in a microfuge or picofuge (cut the strip in half if necessary while taking care not to crack the tubes).
12. Add 15 μL of the appropriate type (either “Reference” or “Variant”) of Hybridization Reaction in an alternating pattern to each of the 12-strip tubes as indicated in [Figure 1](#).

NOTE: Use a fresh tip for each pipetting step to accurately pipette the correct volume. The SNV TagSet has components that can wick up into the tip. You may not dispense a consistent and correct amount if you use the same tip to dispense reaction mix into all the hybridization tubes.



Tube Number	Type of Hybridization Reaction Mix
Tubes 1,3,5,7,9,11	Variant Hybridization Reaction Mix
Tubes 2,4,6,8,10,12	Reference Hybridization Reaction Mix

Figure 1. MAX/FLEX: Assembly of Hybridization Reaction Mixes and contents of 12-strip hybridization tubes.

13. Cap tubes tightly, mix the reagents by flicking with a finger to ensure complete mixing and perform a final spin down.
14. Incubate hybridization reactions for 16–20 hours at 65°C with the heated lid set at 70°C. Maximum hybridization time should not exceed 20 hours. Program the thermal cycler to ramp down and hold at 4°C after 20 hours at 65°C.

15. Once the hybridization reactions have been removed from the thermal cycler, proceed immediately to post-hybridization processing as described in the [nCounter Analysis System user manual \(MAN-C0035\)](#). If storage is necessary, store hybridizations in a dark place at 4°C for up to 6 hours. Hybridization reactions may be stored frozen (at -20°C or below for up to one week, or at -80°C for longer) prior to processing.

Sending Logs from the Qualification Run

After imaging on the nCounter Digital Analyzer, retrieve the log files (see Chapter 6 of [nCounter Analysis System user manual \(MAN-C0035\)](#)).

1. On the touch screen in the front of the Digital Analyzer, select Maintenance → Troubleshoot → Download Logs.
2. Select the sub-folder for the qualification run from the appropriate date folder and download it to a USB drive.
3. The files will be downloaded as a *_LOG.ZIP folder on the USB drive.
4. Email the *_LOG.ZIP folder and the Prep Station's and Digital Analyzer's serial numbers to snvqualify@nanosttring.com.

NOTE: The Log Files are from the Digital Analyzer; however, the serial numbers for both the Prep Station and Digital Analyzer are needed.

5. A NanoString representative will communicate results of the qualification assay and indicate whether an adjustment is required.

If you do not hear back from snvqualify@nanosttring.com within **two** business days, it is possible your local email security systems would not allow for the transfer of a ZIP folder. In this case, please send a second email without attachments asking for confirmation of receipt of the log files. If the initial email with attachments was not received by snvqualify@nanosttring.com, a NanoString Technical Services representative will set up an alternative mechanism for file transfer and will verify subsequent receipt of the data.

nCounter SPRINT Profiler

The order of addition is important, so please follow the protocol **exactly as presented**.

1. Pre-heat thermal cycler to 65°C with heated lid turned on and “forever” time setting.
2. Remove the tubes of SNV TagSet, SNV Solid Tumor Probe T Pool, SNV Solid Tumor Probe S Pool, DNA SNV Reference Target Pool, and DNA SNV Variant Target Pool from the freezer and thaw at room temperature. Invert or flick tubes several times to mix well and briefly spin down reagents.

NOTE: Inspect the thawed tubes of SNV TagSet to make sure no colored precipitate is present. If you see a colored precipitate, heat the entire tube to 75°C for 10 minutes and cool at room temperature before using. **Do not heat the Probe S Pool above room temperature.**

3. Create a working stock of Probe T by adding 22 µL of nuclease-free water or TE buffer to the tube of SNV Solid Tumor Probe T Pool.
4. Create a working stock of Probe S by adding 22 µL of nuclease-free water or TE buffer to the tube of SNV Solid Tumor Probe S Pool.
5. Create a working stock of Reference Target Pool by adding 22 µL of nuclease-free water or TE buffer to the tube of DNA SNV Reference Target Pool.
6. Create a working stock of Variant Target Pool by adding 22 µL of nuclease-free water or TE buffer to each of the two tubes of DNA SNV Variant Target Pool. Then combine the contents into one of the tubes, using a pipette set at 40 µL (to ensure you get all of the expected 38.5 µL volume).
7. Create a Hybridization Master Mix sufficient for 14 reactions by adding the following to a thawed tube of SNV TagSet, in the **exact order as presented**.
 - 28 µL of SNV TagSet (already in the tube)
 - 70 µL of Hybridization Buffer
 - 7 µL SNV Solid Tumor Probe T working stock
 - 7 µL of SNV Solid Tumor Probe S working stock
 - 28 µL of DNA SNV Reference Target working stock
 - 70 µL of DNA SNV Variant Target working stock, obtained from the combined vials
 - 28 µL of Nuclease-free water or TE buffer, pH 8.

Flick the tube repeatedly to mix, then spin down the Hybridization Master Mix.

8. Label 12-strip PCR tubes. Ensure that the tubes will fit in a microfuge or picofuge (cut the strip in half if necessary).
9. Add 15 µL of the Hybridization Master Mix to each of the 12-strip tubes.

NOTE: Use a fresh tip for each pipetting step to accurately pipette the correct volume. The TagSet has components that can wick up into the tip. You may not dispense the correct amount if you use the same tip to dispense master mix into all the hybridization tubes.

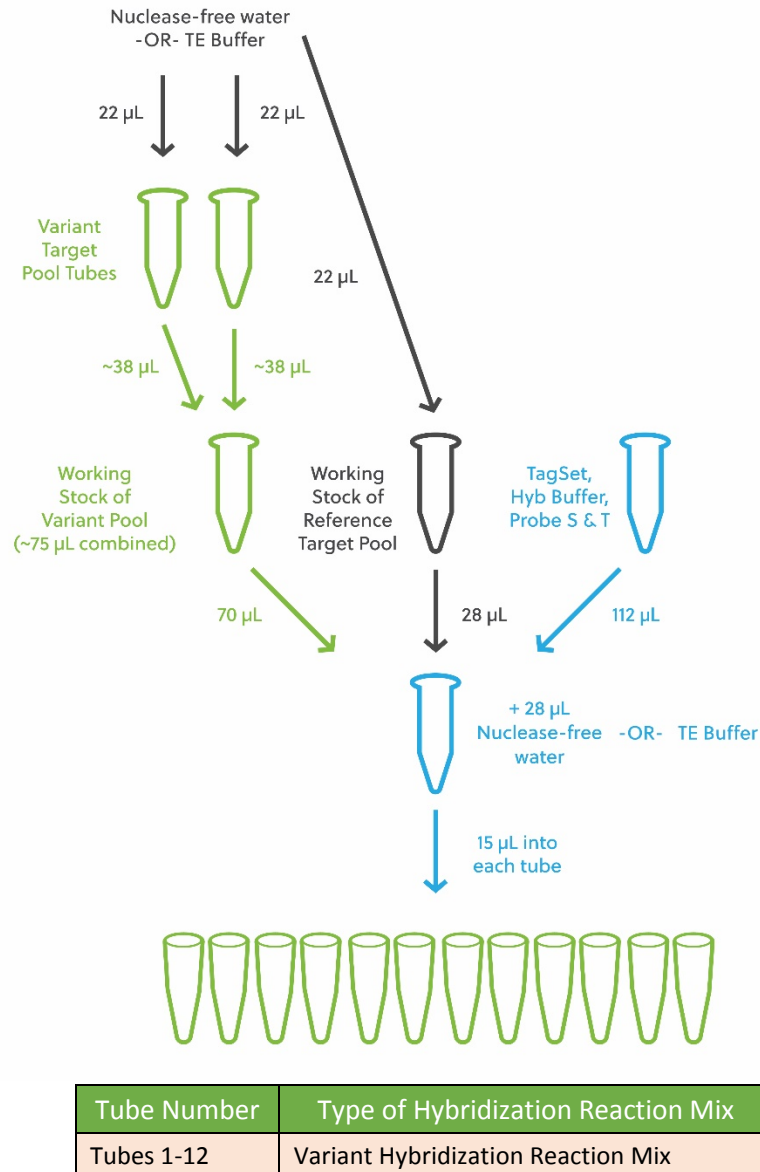


Figure 2. SPRINT: Assembly of Hybridization Reaction Mixes and contents of 12-strip hybridization tubes.

10. Cap tubes tightly, mix the reagents by flicking with a finger to ensure complete mixing and perform a final spin down.
11. Incubate hybridization reactions for 16–20 hours at 65°C with the heated lid set at 70°C. Maximum hybridization time should not exceed 20 hours. Program the thermal cycler to ramp down and hold at 4°C after 20 hours at 65°C are complete.
12. Once the hybridization reactions have been removed from the thermal cycler, add 15 µL of nuclease-free water or TE, pH 8, to each of the 12 wells.
13. Proceed immediately to post-hybridization processing as described in the [nCounter SPRINT Profiler User Manual \(MAN-10017\)](#): loading the samples from the 12-strip tubes to the cartridge, as described in Section 5, and setting up a run, as described in Section 6. If storage is necessary, store hybridizations

in a dark place at 4°C for up to 6 hours. Hybridization reactions may be stored frozen (at -20°C or below for up to one week, or at -80°C for longer) prior to processing.

Sending Logs from the Qualification Run

1. Following completion of imaging, retrieve the log files (see Section 8 of the [nCounter SPRINT Profiler User Manual \(MAN-10017\)](#)).
 - a. On the touch screen in the front of the SPRINT Profiler, select Administration → Support → Logs
 - b. Select the sub-folder for the qualification run from the appropriate date folder and download it to a USB drive.
 - c. The files will be downloaded as a *_LOG.ZIP folder on the USB drive.

or

If the SPRINT Profiler is networked, from the Control Center home screen, click Download Data.

- a. Select the run name for the Qualification Assay run performed.
 - b. Scroll down to Log Information, and click Download Logs.
2. Email the compressed *_LOG.ZIP folder and the SPRINT Profiler's serial number to snvqualify@nanosttring.com.
3. A NanoString representative will communicate results of the Qualification Assay and indicate whether adjustment is required.

If you do not hear back from snvqualify@nanosttring.com within **two** business days, it is possible you're your local email security systems would not allow for the transfer of a ZIP folder. In this case, please send a second email without attachments asking for confirmation of receipt of the log files. If the initial email with attachments was not received by snvqualify@nanosttring.com, a NanoString Technical Services representative will set up an alternative mechanism for file transfer and will verify subsequent receipt of the data.

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