PureBIND Blood Genomic DNA Isolation kit

USER MANUAL

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Version 0.1

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About this User Manual

NOTE The information in this user manual is subject to change without notice.

- It is strongly recommended that first-time users of the *PureBIND Blood Genomic DNA Isolation Kit* completely read the detailed sections of this user manual before using this product.
- The *PureBIND Blood Genomic DNA Isolation Kit User Manual* is an intrinsic part of the product and provides detailed procedures, reference information and troubleshooting for this kit.

Components

Product number:	B0711-096
Number of preps*	96
Proteinase K Solution	5 mL
PureBIND Beads	3 mL
Lysis Buffer	40 mL
Wash Buffer 1 *Customer adds 44 mL isopropanol*	2 x 66 mL
Wash Buffer 2 *Customer adds 77 mL ethanol*	2 x 33 mL
Elution Buffer	11 mL

*Based on 350 µL blood sample per prep.

**Please see "Before you begin – Prepare Working Buffer"

Materials to be Supplied by User

Chemical Reagents	Suggested Supplier and Product	
Isopropanol (≥99.5%)	Sigma-Aldrich	Cat. no. I9516
Ethanol, absolute (96-100%)	Fisher Scientific	Cat. no. BP281814
Consumables		
Sterile, DNase/RNase-free 1.5-2 mL microcentrifuge tubes.		
Pipettor (1-1000 µL volume range) with associated tips		
Equipment	Suggested Supplier and Model	
Magnetic Separator	Thermo Fisher	DynaMag-2
Heat block	Labnet	D-1000
Vortex Mixer	VWR International	VM-3000
Sample Mixer	Thermo Fisher	HulaMixer®

Introduction

Product Description

The Pur*eBIND Blood Genomic DNA Isolation Kit* is designed to isolate genomic DNA from human whole blood with high yield and purity. The kit utilizes automation-ready magnetic bead technology and solutions that enable efficient, consistent, and scalable isolation of DNA. Each standard prep using 25 µL beads will typically yield about 10 µg of DNA from 350 µL of blood sample. The resulting DNA can be used in a wide range of downstream applications such as restriction enzyme digestion, PCR, qPCR, and NGS.

Highlights

- No RNA contamination.
- High molecular weight gDNA & mitochondrial DNA (>20 kb).
- High Purity gDNA from whole blood, does not require differential lysis of RBCs and WBCs.
- Isolates > 9 micrograms of DNA/350 uL of input blood sample.
- Compatibility with commercial stabilizer tubes (e.g. PAXgene Blood DNA tubes, BioMatrica DNAgard Blood).
- Automation-friendly, KingFisher (Duo, Duo Primer, and Flex) script available.

Product Principle

The magnetic microsphere technology and solutions in this kit are used in a simple separation protocol to isolate gDNA from human blood (*Figure 1*): a chemo-enzymatic lysis of white blood cells is followed by incubation with magnetic beads in the presence of isopropanol. During this incubation, the beads bind to DNA and are thereafter easily separated from the lysate using a magnet. Magnetic separation facilitates simple washing and elution of the isolated DNA.

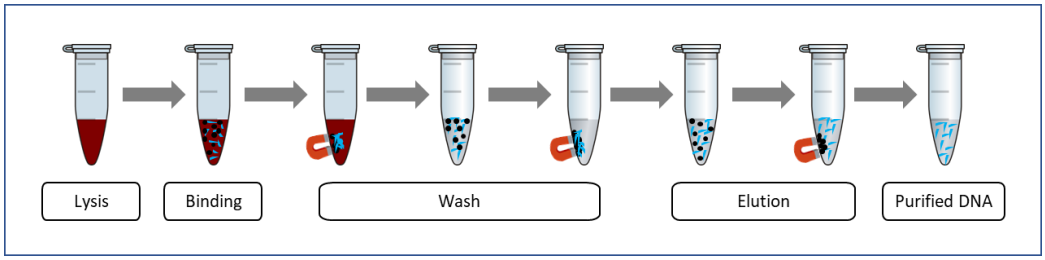


Figure 1: Illustration showing magnetic separation of genomic DNA from human blood using the PureBind Blood Genomic DNA Isolation Kit.

Before you begin

General guidelines

- Read the user manual; make sure all the directions are followed as indicated.
- Use a mixer that ensures that beads do not settle in the tube.
- Avoid air bubbles during pipetting.
- Never use less than recommended volume of magnetic beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Do not add Proteinase K directly to the PureBind Blood gDNA Lysis Solution.
- All vortex steps should be performed at maximum speed to ensure mixing.
- PureBIND Beads should be resuspended to a homogenous suspension prior to use.

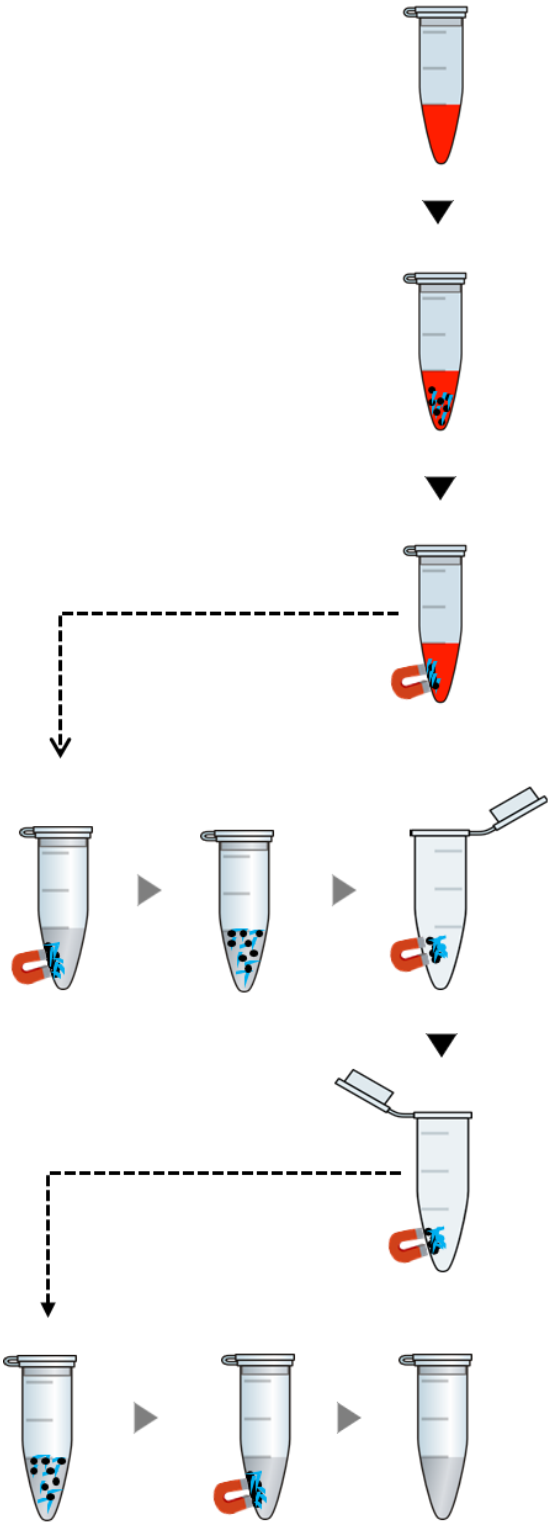
Prepare working buffers

- Prior to first time use, add 44 mL **isopropanol** (>99%) to each of the two bottles **Wash Buffer 1** to obtain a working solution. Check the box on label and mix well by inverting 10 times.
- Prior to first time use, add 77 mL **ethanol** (>99%) to each of the two bottles of **Wash Buffer 2** to obtain a working solution. Check the box on label and mix well by inverting 10 times.

CAUTION Wash Buffer 1 contain chaotropic salt! Wear appropriate personal protective equipment!

Isolate genomic DNA manually

At-a-glance



Step 1.
Add Proteinase K to blood sample. Mix sample and incubate at room temperature for 2 mins.

Step 2.
Add Lysis buffer, Magnetic Beads and isopropanol to blood sample. Mix sample and incubate on a roller for 3 mins.

Step 3.
Collect beads on magnet and discard supernatant.

Step 4.
Wash beads twice with Wash Buffer 1 and then two times with Wash Buffer 2.

Step 5.
Air dry beads for 5 mins.

Step 6.
Add Elution Buffer and resuspend beads to elute purified gDNA. Transfer supernatant to new microcentrifuge tube.

Detailed protocol

The following protocol is for genomic DNA isolation from 350 µL human blood. The protocol has been validated to scale up to a 10 mL and down to a 10 µL blood sample. **If the amount of blood used is between 10-350 µL, follow the procedures below without changing the amount of solutions. However, the amount of proteinase K, magnetic beads, lysis buffer, washing buffers, and elution buffer will need to be proportionally increased if the volume of blood exceeds 350 µL.**

Lyse sample and bind genomic DNA to PureBIND Beads

1. Add 50 µL **Proteinase K Solution** to a 350 µL blood sample. Vortex for 30 secs. Then incubate for 2 mins at room temperature.
2. Add 350 µL **Lysis Buffer** to the sample. Vortex for 30 secs to mix. Incubate for 10 mins at 56°C followed by a final vortex for 30 secs.
3. Add 25 µL **PureBind Blood gDNA Magnetic Beads** and 400 µL of **isopropanol** (>99%) to each sample. Vortex for 30 secs and incubate on a roller for 3 mins at room temperature followed by a final vortex for 30 secs.
4. Place the tube on the magnetic separator for 2 mins so beads are pelleted against the magnet. Then carefully discard the supernatant with a pipette by placing the pipette tip at the point furthest from the magnet.

CAUTION Avoid touching the magnetic bead pellet when removing the supernatant

Wash with Wash Buffers

5. Remove the tube from the magnetic separator and add 1 mL **Wash Buffer 1**. Vortex for 30 secs at room temperature.
6. Place tube on the magnetic separator for 2 mins or until the solution clears and beads are pelleted against the magnet, then carefully discard the supernatant with a pipette.
7. Repeat step **Error! Reference source not found.**-**Error! Reference source not found.** for a second wash with **Wash Buffer 1.**
8. Remove the tube from the magnetic separator and add 1 mL **Wash Buffer 2**. Vortex for 30 secs at room temperature. Then transfer the resuspended beads solution to a clean tube.
9. Place tube on the magnetic separator for 1 min or until the solution clears and beads are pelleted against the magnet, then carefully discard the supernatant with a pipette.
10. Remove the tube from the magnetic separator and add 1 mL **Wash Buffer 2**. Vortex for 30 secs at room temperature.
11. Place tube on the magnetic separator for 1 min or until the solution clears and beads are pelleted against the magnet, then carefully discard the supernatant with a pipette.
12. Leave the tube on the magnetic separator with the tube lid open. Air dry the beads for 5 mins.

NOTE Remove any visible supernatant with a small-bore pipette tip.

CAUTION Do not over dry beads as it may result in loss of DNA.

Elute the genomic DNA

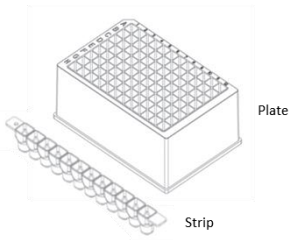
13. Remove the tube from the magnetic separator and add 100 µL **PureBind Blood gDNA Elution Buffer**. Completely resuspend beads by vortexing and/or pipetting for 2 mins at room temperature.
14. Place tube on the magnetic separator for 1 min or until the solution clears and beads are pelleted against the magnet.
15. Transfer the supernatant containing the purified gDNA, without disturbing the pellet, to a clean microcentrifuge tube.

CAUTION Purified genomic DNA is ready for immediate use. Alternatively, you can store the DNA at 4˚C for up to 24 hours or -20˚C for long-term storage.

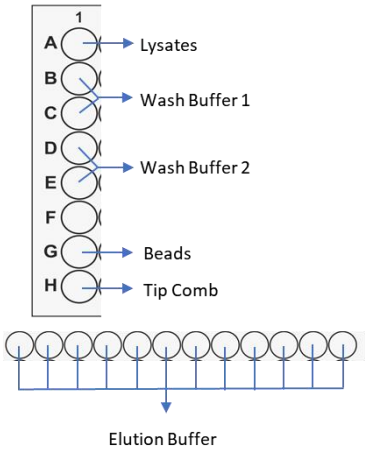
Isolate genomic DNA using the KingFisher™ Duo Prime System

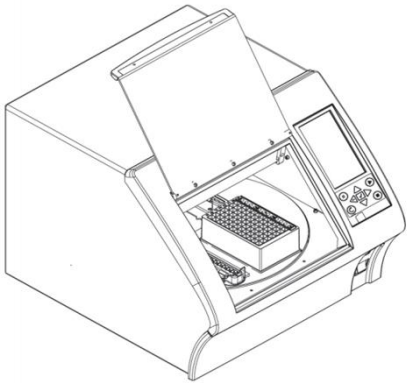
At-a-glace

Step 1. Prepare sample and remove reagents from kit

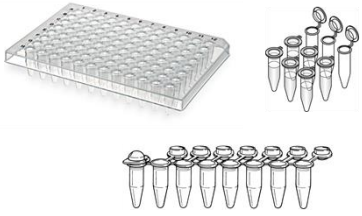


Step 2. Aliquot sample and reagents to appropriate wells:





Step 3. Place plate and elution strip on platform. Download program script on website and start run.



Step 4. After run is done remove plate and strip from instrument. Transfer eluted purified DNA to appropriate vessel.

Detailed Protocol

The following protocol is for genomic DNA isolation from 200 µL human blood sample.

Digest and lyse sample

1. Add 50 µL of **Proteinase K Solution** to a 200 µL blood sample. Vortex for 30 secs to mix. Then incubate for 2 mins at room temperature.
2. Add 350 µL **Lysis Buffer** to the sample. Vortex for 30 secs to mix. Then incubate for 10 mins at 56°C followed by a final vortex for 30 secs.

   NOTE For preparation in 96 well blocks increase the incubation time to 20 minutes to allow for proper heat transfer to the sample.
3. Add 400 µL of **isopropanol** (>99%) to each sample. Pipet 10 times or vortex for 30 secs to mix.
4. Set aside the lysates and prepare the appropriate plate(s) specific to your platform.

Setup the processing plate(s)

IMPORTANT! Setup the processing plate outside the instrument and make sure to add the appropriate volumes as described in the following order:

5. To **Plate Row B** (Wash 1) add 1 mL of **Wash Buffer 1**.
6. To **Plate Row C** (Wash 2) add 1 mL of **Wash Buffer 1**.
7. To **Plate Row D** (Wash 3) add 1 mL of **Wash Buffer 2**.
8. To **Plate Row E** (Wash 4) add 1 mL of **Wash Buffer 2**.
9. To the **Elution Strip** (Elution) add 25-100 µL of **Elution Buffer**.
10. To **Plate Row G** (Beads) add 25 µL of PureBIND Beads.

    NOTE Vortex beads to ensure a homogeneous mix prior to aliquoting to the wells.
11. To **Plate Row H** place a **12-Tip Comb**.
12. After the plates(s) have been pre-loaded with reagents and Tip-Comb, add the lysate from step 4 to **Plate Row A** (Lysis/Binding) wells.

Bind, wash, and elute the genomic DNA

13. Ensure that instrument is set up for processing with the appropriate program and magnetic head. Select the program on the instrument. The appropriate program script can be downloaded at www.oceannanotech.com.
14. Load the prepared processing plates.
15. Start the run.
16. At the end of the run, remove the plate and Elution Strip from the instrument.
17. Seal the Elution Strip or transfer the eluted genomic DNA to individual microcentrifuge tubes. The purified genomic DNA is ready for immediate use. Alternatively, you can store the DNA at 4°C for up to 24 hours or at -20°C or -80°C for long-term storage in an appropriate storage strip or tubes. (Ask your Thermo Fisher representative for the appropriate consumables that will meet this need).

Safety Instructions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective eyewear. For more information, please consult the appropriate safety data sheets (SDSs).

Lysis Buffer and Wash Buffer 1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is split, clean with suitable laboratory detergent and water.

Example Data and Applications

Inhibitor-free genomic DNA from human whole blood

Donor/Ctrl	Biological Replicate	Mean Cq*	Stdev Cq*
A	1	24.92	0.08
	2	25.10	0.13
	3	24.78	0.41
B	1	24.82	0.03
	2	24.78	0.19
	3	24.66	0.21
SPUD Pos Ctrl		25.01	1.41
Elution Buffer		24.82	0.03

*The mean Cqs and their standard deviations were derived from technical replicates.

Figure 2

SPUD assay analysis of isolated genomic DNA demonstrates absence of co-purified inhibitors. Whole blood was processed with the PureBIND Blood Genomic DNA Isolation Kit and eluted in 100 µL. Then 100 ng of eluted DNA were assayed for the ability to inhibit amplification of the SPUD amplicon as detailed in Analytical Biochemistry, 2006 Apr 15’351(2):308-10. Uniformity of the observed Cq with the isolated DNA templates relative to the SPUD positive control indicates that the isolated DAN is free of PCR/qPCR inhibitors.

High purity genomic DNA from human whole blood (no RNase required)

		Purity Ratios			
	[DNA], ng/µL	260/280	260/230	Total DNA Yield (µg)	ng DNA/µL blood
Donor 1	135	1.89	2.13	13.50	38.57
	126	1.90	2.04	12.64	36.11
	117	1.91	2.10	11.86	33.78
Donor 2	98	1.92	2.22	9.77	27.91
	108	1.91	2.12	10.83	30.94
	133	1.91	2.17	13.44	38.30

Figure 3

High purity genomic DNA isolated from whole blood with the PureBIND Blood Genomic DNA Isolation Kit and eluted in 100 µL. Eluted DNA was analyzed by spectrophotometric analysis to determine purity ratios and DNA concentration.

High molecular weight genomic DNA

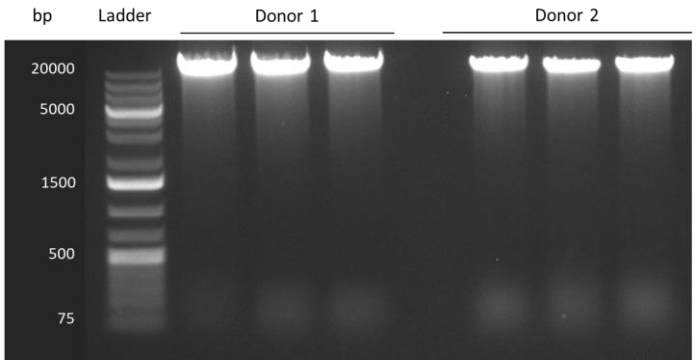


Figure 4

High yields of high molecular weight genomic DNA isolated from whole blood PureBIND Blood Genomic DNA Isolation Kit and eluted in 100 µL. For analysis 500 ng of purified DNA was electrophoresed on a 1% agarose gel/TAE alongside the GeneRuler™ 1 kb Plus ladder (Thermo Scientific).

Long Range PCR from Isolated Genomic & Mitochondrial DNA

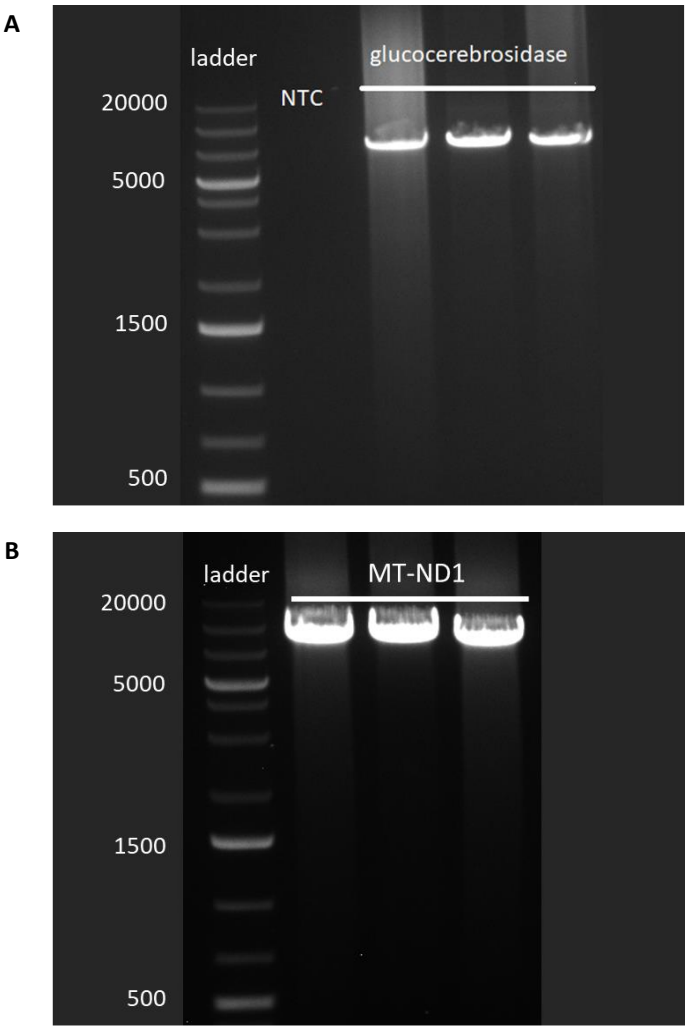


Figure 5

Long range PCR of genomic DNA isolated from whole blood with PureBIND Blood Genomic DNA Isolation Kit and eluted in 100 µL. Then 20 ng of purified DNA was amplified via PCR using TaKaRa LA Taq® and primers for:

(**A**) 7.3 kb fragment of human glucocerebrosidase and

(**B**) 8.2 kb fragment of mitochondrial NADH-ubiquinone oxidoreductase chain 1 (MT-ND1) (right).

Thereafter the PCR products were electrophoresed on a 1% agarose gel/TAE for 1 hour.

Successful PCR amplification of these high molecular weight targets highlights the structural integrity of the isolated DNA and our kit's capacity to isolate mitochondrial DNA.

Performance against other magnetic bead-based kits

		NanoDrop			Qubit	
Supplier	**Blood Input µL**	**Sample Conc. ng/µL**	**A260/280**	**A260/230**	**Sample Conc. ng/µL**	**Total Conc. ng/µL blood input**
Ocean NanoTech PureBIND Blood DNA	350	128.3 ± 9.6	1.9 ± 0.0	2.0 ± 0.0	157.3 ± 8.5	44.95 ± 2.43
Chemagen Chemagic DNA Bood	250	20.9 ± 1.7	2.1 ± 0.1	1.4 ± 0.1	10.1 ± 2.2	4.03 ± 0.88
Zymo Research Genomic DNA MagPrep	70	18.1 ± 1.5	1.8 ± 0.0	0.2 ± 0.1	20.5 ± 1.2	29.24 ± 1.73
Promega Maxwell HT 96 gDNA	350	75.8 ± 5.6	1.9 ± 0.0	1.4 ± 0.1	32.9 ± 5.0	9.4 ± 1.44

Figure 6

PureBIND Blood Genomic DNA Isolation Kit significantly outperforms other magnetic bead-based blood gDNA isolation kits. Blood from the same donor was drawn into K_2-EDTA tubes and processed through each kit using the kit's requisite input blood volume. Isolated DNA was eluted in 100 µL and assessed for purity via NanoDrop analysis for concentration. Total yield (based on Qubit measurements) and the yield as a function of blood input volume were calculated for each kit. All values were derived from three biological replicates.

Troubleshooting

Problem	Possible cause and suggestions
Poor DNA/RNA yield and quality	**Wrong bottle reagents used in during procedures.** Check all bottle reagents before use. Viral nucleic acid eluate too dilute. Use recommended 20-50 µL of Elution Buffer.
Decrease in performance of Nucleic Acid in downstream applications	**Ethanol carryover.** Allow ethanol to evaporate during air drying step. Salt carryover during elution. Ensure Wash Buffers are at room temperature and that they are prepared prior to use.
Degraded RNA	**RNase contamination.** To avoid RNase contamination, wear gloves during all procedure and change gloves frequently. Use sterile, disposable RNase free pipette tips and remove RNase contamination from items and work surfaces. Poor quality of samples. Always use fresh samples.

Ordering Information

Related Products

Product Name	Number of Preps	Product No.
PureBIND Viral Nucleic Acid Isolation Kit	96	P1122-096

Other Suggested Products

Product Name	Number of Preps	Product No.
SiDIRECT Cell-Free DNA Isolation Kit	48	C0411-048

Technical Support

For more information about Ocean NanoTech product and to download manuals in PDF format, please visit our web site www.oceannanotech.com

For additional information or technical assistance, please call or email us at:

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Revision History

Revision	Date	Author	Description
0.0	10-31-2018	Luis M	Issued
0.1	12-06-2018	Marinelle	Reviewed