EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR MISEQDX UNIVERSAL KIT 1.0

DECISION SUMMARY

A. 510(k) Number:	k133136
B. Purpose for Submission:	De novo request for evaluation of automatic class III designation of the MiSeqDx Universal Kit 1.0
C. Measurand:	Not applicable. Reagents for the creation of a Library specimen from a sample of human genomic DNA obtained from peripheral whole blood
D. Type of Test:	Not applicable: Reagents for preparation of a library specimen for use with high-throughput genomic sequence analyzer
E. Applicant:	Illumina Inc.
F. Proprietary and Established Name:	MiSeqDx Universal Kit 1.0

G. Regulatory Information:

FDA identifies this type of device as:

1. New regulation number: 21 CFR 862.3800
2. Classification: Class I
3. Product code: PFT – Reagents for molecular diagnostic test systems
4. Panel: Toxicology (91)

H. Intended Use:

1. Intended uses(s):

The MiSeqDx Universal Kit 1.0 is a set of reagents and consumables used in the processing of human genomic DNA samples derived from peripheral whole blood, and in the subsequent targeted re-sequencing of the resulting sample libraries. User-supplied analyte specific reagents are required for the preparation of libraries targeting specific genomic regions of interest. The MiSeqDx Universal Kit 1.0 is intended for use with the MiSeqDx instrument.

2. Indication for uses(s):

Same as Intended use above.

3. Special conditions for use statement(s):

For in vitro diagnostic use.

For prescription use only.

1. This product is limited to delivering:
   - Sequencing output >1 Gb
   - Reads >3 million
   - Read length (in paired end run) 2 x 150 bp
   - Bases higher than Q30 >75% (Greater than 75% of bases have Phred scale quality score greater than 30, indicating base call accuracy greater than 99.9%)
2. The system has been validated for the detection of SNVs and up to 3 base deletions. Evaluation of 1 base insertions was been limited to 3 different insertions on 3 separate chromosomes.
3. The system has problems detecting 1 base insertions or deletions in homopolymer tracts (e.g. polyA).
4. This MiSeqDx system is designed to deliver qualitative (i.e. genotype) results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made.
6. Recommended minimal coverage per amplicon needed for accurate variant calling (Q(max_gt | poly_site) >= 100) is 75x.

4. Special instrument requirements:

For use with the Illumina MiSeqDx platform (k123989).

I. Device Description:

The MiSeqDx Universal Kit 1.0 is intended for use with the MiSeqDx Platform.

The MiSeqDx Universal Kit 1.0 consists of 5 boxes :

Box 1A Pre-Amp reagents :

Component	**Active Ingredients**
Hybridization Buffer	Buffered aqueous solution containing salts and formamide
Extension-Ligation Mix	Buffered aqueous solution containing proprietary blend of DNA polymerases, DNA ligase, and dNTPs
Index Primers A (A501) - H (A508)	PCR primers with index sequences and sequencing adapters
Index Primers 1 (A701) - 12 (A712)	PCR primers with index sequences and sequencing adapters
PCR Polymerase	Proprietary DNA polymerase
PCR Master Mix	Buffered aqueous solution containing salts and dNTPs

Box 1B Post-Amp Reagents

Component	**Active Ingredients**
Library Normalization Diluent	Buffered aqueous solution containing salts, 2-Mercaptoethanol, and formamide
Library Dilution Buffer	Buffered aqueous solution
PhiX Internal Control	Buffered aqueous solution containing PhiX genomic DNA

Box 2 Post-Amp Reagents

Component	**Contents**
MiSeqDx Reagent Cartridge	Single-use cartridge that contains cluster generation and sequencing reagents for use with the MiSeqDx, including formamide, 2-Mercaptoethanol, and < 2% DMSO

Box 3A Pre-Amp Reagents

Component	Active Ingredients
Stringent Wash Buffer	Buffered aqueous solution containing salts, 2-Mercaptoethanol and formamide
Universal Wash Buffer	Buffered aqueous solution containing salts

Box 3B Post-Amp Reagents

Component	Active Ingredients
PCR Clean-Up Beads	Buffered aqueous solution containing solid phase paramagnetic beads and polyethylene glycol
Library Normalization Wash	Buffered aqueous solution containing salts, 2-Mercaptoethanol and formamide
Library Beads	Buffered aqueous solution containing solid phase paramagnetic beads
MiSeqDx Flow Cell	Glass substrate with covalently bound oligonucleotides

Box 4 Post-Amp Reagents

Component	Active Ingredients
MiSeqDx SBS Solution (PR2)	Buffered aqueous solution

Box 5 Pre-Amp Reagents

Component	Active Ingredients
Filter Plate	Polypropylene microtiter plate with a modified polyethersulfone membrane

Box 5 Post-Amp Reagents

Component	Active Ingredients
Elution Buffer	Buffered aqueous solution
Library Storage Buffer	Buffered aqueous solution

J. Substantial Equivalence Information:

1. Predicate Device Name(s):

   No predicate device exists.

2. Predicate 510(k) numbers:

   Not applicable.

2. Comparison with Predicate Device:

   Not applicable.

K. Special Control/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

Library Preparation using the MiSeqDx Universal Kit 1.0 includes the following steps:

1. Hybridization—The first step, Hybridization, hybridizes a pool of upstream and downstream analyte specific reagents (ASRs) specific to the regions of interest to the sample genomic DNA. At the end of this process, a three-step wash procedure with a filter capable of size selection removes unbound oligonucleotides from the genomic DNA.
2. Extension-Ligation—The second step, Extension-Ligation, connects the hybridized upstream and downstream oligonucleotides. A DNA polymerase extends from the upstream oligonucleotides through the targeted region, followed by ligation to the 5' end of the downstream oligonucleotide using a DNA ligase. The result is the formation of products that contain the oligonucleotides specific to the regions of interest flanked by sequences required for amplification.
3. PCR Amplification—The third step, PCR Amplification, amplifies the extension-ligation products using primers that add index sequences for sample multiplexing, as well as flow cell capture sequences required for cluster generation on the MiSeqDx. At the end of this process, a PCR clean-up procedure purifies the PCR products (referred to as a library).
4. Library Normalization—The final step, Library Normalization, normalizes the quantity of each library to ensure more equal library representation in the final pooled library.

M. Performance Characteristics*:*

1. Analytical Performance:

   a. Precision/Reproducibility:

   See k123989 for the studies used to establish the reproducibility of high throughput DNA targeted sequencing of human genomic DNA using the reagents found in the MiSeqDx Universal Kit 1.0 and the test system known as MiSeqDx Platform.

   b. Linearity/assay reportable range:

   Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Controls: A PhiX internal control (i.e. genomic DNA from the bacteriophage ΦX174) provided as a kit component is added to each pooled library prior to placement on the instrument. Successful sequencing of the PhiX genome indicates that the sequencing chemistry worked as expected. It is also recommended that a negative control, or no template control, (not provided in the kit) in used with every run in order to detect the presence of contamination in the environment or run.

Stability: Kit components have specific storage conditions (-15 to -25°C, 2 to 8°C, or 15 to 30°C). The shelf life of all components will be 12 months when stored at the appropriate conditions. Real time stability testing of the kit components has been performed to support 6 months of shelf life stability; stability testing is ongoing to support a 12 month shelf life claim.

Stability testing protocols and acceptance criteria for stability testing have been reviewed and found to be acceptable.

See k123989 for the studies used to verify the storage conditions and handling of blood samples for high throughput DNA targeted sequencing of human genomic DNA using the reagents found in the MiSeqDx Universal Kit 1.0 and the test system known as MiSeqDx Platform.

d. *Detection Limit:*

See k123989 for the studies used to establish the minimum DNA input for high throughput DNA targeted sequencing of human genomic DNA using the reagents found in the MiSeqDx Universal Kit 1.0 and the test system known as MiSeqDx Platform.

e. *Analytical specificity:*

See k123989 for the studies used to evaluate the impact of potential interferents on high throughput DNA targeted sequencing of human genomic DNA using the reagents found in the MiSeqDx Universal Kit 1.0 and the test system known as MiSeqDx Platform.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:
   a. *Method comparison with a predicate device:*

      Not applicable.

   b. *Matrix comparison:*

Not applicable. For use only with human genomic DNA obtained from peripheral whole blood.

3. Clinical studies:
   a. *Clinical Sensitivity:*

      Not applicable.

   b. *Clinical Specificity:*

      Not applicable.

   c. *Other clinical supportive data (when a. and b. are not applicable):*

      See k123989 for the studies used to establish the accuracy of high throughput DNA targeted sequencing of human genomic DNA using the reagents found in the MiSeqDx Universal Kit 1.0 and the test system known as MiSeqDx Platform.

4. Clinical cut-off:

   Not applicable.

5. Expected values/Reference range:

   Not applicable.

2. Other Supportive Instrument Performance Data Not Covered Above:

   None.

K. Proposed Labeling:

Labeling satisfies the requirements of 21 CFR 809.10, 21 CFR 801.109, including an appropriate prescription statement as required by 21 CFR 801.109(b).

L. Risks to Health and Mitigation Measures:

Identified Potential Risk	Required Mitigation Measure
Inaccurate test results due to inconsistently manufactured test system reagents	General controls, including current good manufacturing practices

M. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	• Well-characterized and reliable reagents are essential to the use of the test system. The test system is a tool for clinical laboratories that can be used in sequencing assays to provide high throughput genomic sequencing of genomic regions of interest at greater sequencing depth than current sequencing technology. • No other devices are available for generation of a genomic library of a region of genomic DNA to use with a high throughput DNA sequence analyzer. There is an unmet medical and public health need for a device that generates genomic libraries of a region of genomic DNA to use with a high throughput genomic sequence analyzer.

Summary of the Risk(s)	• Patients are subject to blood specimen collection, which is a standard procedure in clinical care and carries minimal risk. • Risk is related to inaccurate test results as follows: False positive: The risks to the individual of a false positive result obtained from this test system (library generation using the subject device followed by sequence analysis using a high throughput DNA sequence analyzer) could include unnecessary testing or treatment related to an inaccurate test result. Often, the result from this test system would be used with results from other diagnostic tests and clinical signs and symptoms to identify the genetic cause or contribution for a patient's disease or condition. False negative: The risks to the individual of a false negative result due to an inaccurate test result obtained from this test system (library generation using the subject device followed by sequence analysis using a high throughput DNA sequence analyzer) could delay further evaluation and appropriate therapy which will vary depending on the disease or condition. Public Health Risk from Incorrect Test Results: • The consequences to public health for both false positive and false negative results are similar. • This type of reagent is a component of the test that will ultimately be designed by a laboratory or other manufacturer. This type of reagent contributes only a small portion of the risk to the overall risk of the test. Therefore if the reagents are designed and validated for use with the instrument, they may be considered low risk by themselves. Requiring that reagents are subject to general controls (including the manufacture of the reagents under current Good Manufacturing Practices) is sufficient to provide reasonable assurance of the device's safety and effectiveness.
Summary of Other Factors	Not applicable

Conclusions Do the probable benefits outweigh the probable risks?
Given robust analytical performance characteristics and risk mitigation (i.e., general controls), the probable benefits to both the individual and public health outweigh the probable risks of this device.

N. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class I under regulation 21 CFR 862.3800. FDA believes that general controls provide reasonable assurance of the safety and effectiveness of the device type. This device, and similar devices, is classified under the following:

Product Code:	PFT
Device Type:	Reagents for molecular diagnostic test systems
Class:	I (general controls)
Regulation:	21 CFR 862.3800

(a)*Identification.* Reagents for molecular diagnostic test systems are reagents other than analyte specific reagents used as part of molecular diagnostic test systems, such as polymerases, nucleotides and nucleotide mixes, master mixes in which individual reagents are optimized to be used together, and labeled nucleic acid molecules.

(b)*Classification.* Class I (general controls). The device is exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to the limitations in § 862.9.