NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel

(For Illumina® Platforms)

Catalog #4224-01 (Kit contains 8 reactions)

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NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel - 4224-01

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Product Overview

The NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel produces barcoded amplicon libraries compatible with Illumina® platforms. Libraries are constructed using genomic DNA extracted from blood or cell samples. FFPE or cfDNA samples are not compatible with this kit. This panel contains a total of 186 primer pairs in two pools that allow for the amplification and sequencing of all coding exons of the *BRCA1*, *BRCA2, PALB2* and *CHEK2* genes. Amplicon regions of interest range in size between 92-235 bp. The regions of interest plus primer pad sites, which comprise the read portion of the libraries, range between 151-284 bp. These target regions are amplified in PCR I, followed by adapter ligation. PCR II then enriches for the product of interest, as well as introduces unique barcodes and sequences necessary for downstream sequencing (Fig. 1). NEXTflex™ Cleanup Beads are included, and have been validated with amplicon library preparation. NEXTflex™ BRCA Plus-1 Amplicon Primer Mixes are optimized to achieve high coverage uniformity and reduce off-target reads.

The NEXTflex BRCA1 & BRCA2 Plus-1 Amplicon Panel covers 30 kilobases comprising 80 coding *BRCA1*, *BRCA2, PALB2* and *CHEK2* exons. Libraries have 97% uniformity at ≥ 0.2x mean coverage, 100% uniformity at ≥ 0.1x mean coverage and > 94% on-target reads. Up to 60 samples can be multiplexed with at least 100x coverage on a single Illumina® 2x150 MiSeq® lane for detection of germline mutations. Up to 3 samples can be multiplexed at 2000x coverage for detection of somatic mutations. Standard Illumina® sequencing primers may be used with this kit.

Contents, Storage and Shelf Life

The NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel contains enough material to prepare 8 sample libraries. The shelf life of all reagents is 12 months when stored properly. All components should be stored at -20°C, except the Nuclease-free Water and Resuspension Buffer, which can be safely stored at room temperature, and NEXTflex™ Cleanup Beads, which should be stored at 4°C.

Kit Contents	Amount
PINK CAP	
NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 1	76 µL
BLUE CAP	
NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 2	76 µL
CLEAR CAP	
NEXTflex™ Hot Start PCR I Master Mix	192 µL
LIGHT PURPLE CAP	
NEXTflex™ Ligation Mix	336 µL
NEXTflex™ Amplicon DNA Adapter	20 µL

YELLOW CAP	
NEXTflex™ PCR II Barcoded Primer Mix	4 µL each

GREEN CAP	
NEXTflex™ PCR II Master Mix	80 µL

WHITE CAP	
Nuclease-free Water	1.5 mL
Resuspension Buffer	1.5 mL

BROWN CAP	
NEXTflex™ Cleanup Beads	(2) 1.5 mL

Required Materials not Provided

- 20 - 100 ng of extracted genomic DNA (two 10 - 50 ng aliquots in up to 28.5 µL nuclease-free water each)
- Ethanol 80% (room temperature)
- 96 well PCR Plate Non-skirted (Phenix Research™, Cat # MPS-499) or similar
- 96 well Library Storage and Pooling Plate (Fisher Scientific®, Cat # AB-0765) or similar
- Adhesive PCR Plate Seal (Bio-Rad®, Cat # MSB1001)
- Magnetic Stand -96 (Thermo Fisher Scientific®, Cat # AM10027) or similar
- Thermocycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Microcentrifuge
- 1.5 mL nuclease-free microcentrifuge tubes
- Vortex

Warnings and Precautions

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or Bioo Scientific at **nextgen@biooscientific.com**.

- Do not use the kit past the ePlus-1iration date.
- Ensure pipettes are properly calibrated, as library preparations are highly sensitive to pipetting error.
- Do not heat NEXTflex™ Adapters above room temperature.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality DNA. DNA that is heavily nicked or damaged may cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA, and 260 nm/280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. Other quantification methods using fluorescent dyes may also be used. The user should be aware that contaminating RNA, nucleotides, and single-stranded DNA may affect the amount of usable DNA in a sample preparation.
- NEXTflex™ BRCA Plus-1 Amplicon Primer Mixes are required for PCR I amplification.

NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel Preparation Flow Chart

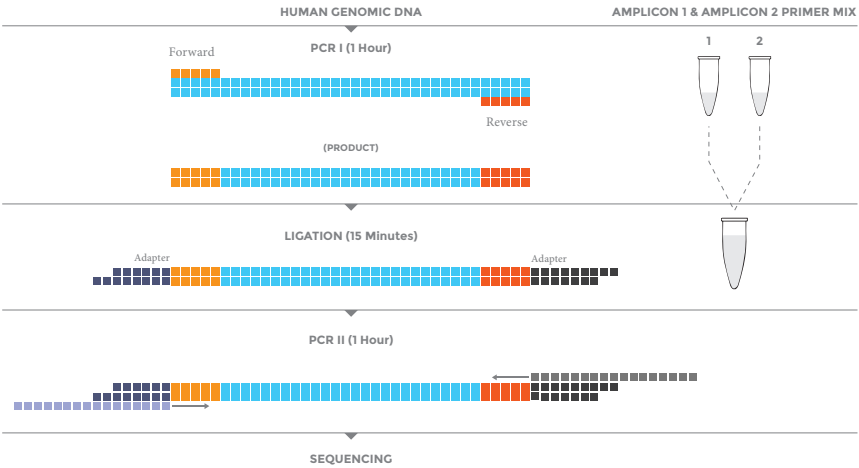

Figure 1: Sample flow chart with approximate times necessary for each step.

Starting Material

The NEXTflex™ BRCA1 & BRCA2 Plus-1 Amplicon Panel has been optimized and validated using 10 - 50 ng of high quality genomic DNA for each PCR I primer pool.

Reagent Preparation

1. Briefly spin down each component to ensure material has not lodged in the cap or side of tube. Keep on ice, and vortex each NEXTflex™ Mix just prior to use.
2. DTT in buffers may precipitate after freezing. If precipitate is seen in any mix, vortex for 1 minute or until the precipitate is in solution. The performance of the mix is not affected once precipitate is in solution.
3. Before every use, allow NEXTflex™ Cleanup Beads to come to room temperature and vortex until liquid appears homogenous.

STEP A: PCR I – BRCA1 and BRCA2 Plus-1 Amplification

Materials

Bioo Scientific Supplied

CLEAR CAP - NEXTflex™ Hot Start PCR I Master Mix
PINK CAP - NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 1
BLUE CAP - NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 2
WHITE CAP - Nuclease-Free Water

User Supplied

Thermocycler
96 Well PCR Plate
Adhesive PCR Plate Seal
For each reaction, 10 - 50 ng of genomic DNA in up to 28.5 µL

1. For each sample, prepare two separate reactions using BRCA Plus-1 Amplicon Primer Mix 1 and 2 by combining the following reagents in adjacent wells in a PCR plate. **Note: It is recommended to combine these reagents as a master mix if processing multiple samples.**

	Reaction 1
_ µL	Genomic DNA (10 - 50 ng in up to 28.5 µL)
_ µL	Nuclease-free Water
9.5 µL	NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 1
12 µL	NEXTflex™ Hot Start PCR I Master Mix
50 µL	TOTAL

	Reaction 2
_ µL	Genomic DNA (10 - 50 ng in up to 28.5 µL)
_ µL	Nuclease-free Water
9.5 µL	NEXTflex™ BRCA Plus-1 Amplicon Primer Mix 2
12 µL	NEXTflex™ Hot Start PCR I Master Mix
50 µL	TOTAL

2. Mix thoroughly by pipette.
3. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

2 min	98°C	
20 sec	98°C	} 18 cycles
4 min	62°C	
Hold	4°C	

4. Proceed immediately to Step B: PCR I Cleanup.

STEP B: PCR I Cleanup

Materials

Bioo Scientific Supplied

WHITE CAP - Resuspension Buffer
BROWN CAP - NEXTflex™ Cleanup Beads (room temperature)

User Supplied

80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
50 µL PCR I reactions (from Step A)

1. Add 30 µL of NEXTflex™ Cleanup Beads to each reaction. Mix thoroughly until homogenized.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes, or until the supernatant appears completely clear.
4. Do not discard the supernatant in this step. Transfer the clear supernatant to a new well. Be careful not to disrupt the magnetic bead pellet or transfer any magnetic beads with the sample.
5. Add 45 µL of NEXTflex™ Cleanup Beads to supernatant. Mix thoroughly until homogenized.
6. Incubate at room temperature for 5 minutes.
7. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes, or until the supernatant appears completely clear.
8. Remove and discard the supernatant. Do not disturb beads. Some liquid may remain in wells.
9. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
10. Repeat previous step, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
11. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes, or until bead pellet is visibly dry.
12. Resuspend dried beads with 16 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
13. Incubate resuspended beads at room temperature for 3 minutes.
14. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes, or until sample appears clear.
15. Transfer 14 µL of clear supernatant (purified PCR I Reaction) to new well.
16. Proceed immediately to Step C: Adapter Ligation.

STEP C: Adapter Ligation

Materials

Bioo Scientific Supplied

LIGHT PURPLE CAP - NEXTflex™ Amplicon DNA Adapter, NEXTflex™ Ligation Mix

User Supplied

Thermocycler
Adhesive PCR Plate Seal
Ice
14 µL Purified PCR I Reactions 1 & 2 (from Step B)

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:

14 µL	Purified PCR I Reaction 1
14 µL	Purified PCR I Reaction 2
2.5 µL	NEXTflex™ Amplicon DNA Adapter
42 µL	NEXTflex™ Ligation Mix
72.5 µL	TOTAL

2. Mix thoroughly by pipette.
3. Apply adhesive PCR plate seal and incubate in a thermocycler for 15 minutes at 22°C.
4. Proceed immediately to Step D: Cleanup.

STEP D: Cleanup

Materials

Bioo Scientific Supplied

WHITE CAP - Resuspension Buffer
BROWN CAP - NEXTflex™ Cleanup Beads (room temperature)

User Supplied

80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
72.5 µL Adapter Ligated DNA (from Step C)

1. Add 58 µL of NEXTflex™ Cleanup Beads to each sample. Mix thoroughly until homogenized.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes, or until the supernatant appears completely clear.
4. Remove and discard the supernatant. Do not disturb beads. Some liquid may remain in wells.
5. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
6. Repeat previous step, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes, or until bead pellet is visibly dry.
8. Resuspend dried beads with 40 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.
9. Incubate resuspended beads at room temperature for 3 minutes.
10. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes, or until sample appears clear.
11. Transfer 38 µL of clear supernatant to new well.
12. Proceed immediately to Step E: PCR II Amplification.

    STOPPING POINT: Alternatively, the procedure may be stopped at this point with samples stored at -20°C. To restart, thaw frozen samples on ice before proceeding to Step E: PCR II Amplification.

STEP E: PCR II Amplification

Materials

Bioo Scientific Supplied

YELLOW CAP - NEXTflex™ PCR II Barcoded Primer Mix
GREEN CAP - NEXTflex™ PCR II Master Mix
WHITE CAP - Resuspension Buffer
BROWN CAP - NEXTflex™ Cleanup Beads (room temperature)

User Supplied

Thermocycler
96 Well PCR Plate
Adhesive PCR Plate Seal
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand
38 µL Purified Adapter Ligated DNA (from Step D)

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:

38 µL	Purified Adapter Ligated DNA (from Step D)
2 µL	NEXTflex™ PCR II Barcoded Primer Mix
10 µL	NEXTflex™ PCR II Master Mix
50 µL	TOTAL

2. Mix thoroughly by pipette.
3. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

20 min	65°C	
2 min	98°C	
30 sec	98°C	} 9 cycles
30 sec	65°C	
60 sec	72°C	
4 min	72°C	

4. Remove PCR plate from the thermocycler. Add 40 µL of NEXTflex™ Cleanup Beads to each sample and mix thoroughly until homogenized.
5. Incubate at room temperature for 5 minutes.
6. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes, or until the supernatant appears completely clear.
7. Remove and discard the supernatant. Do not disturb beads. Some liquid may remain in wells.

8. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.

9. Repeat previous step, for a total of 2 ethanol washes. Ensure all ethanol has been removed.

10. Remove the plate from the magnetic stand and let dry at room temperature for 5 minutes or until bead pellet is visibly dry.

11. Resuspend dried beads with 22 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure beads are no longer attached to the side of the well.

12. Incubate resuspended beads at room temperature for 3 minutes.

13. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes, or until sample appears clear.

14. Gently transfer 20 µL of clear sample to a new well and proceed to library analysis or seal plate with adhesive PCR plate seal and store at -20°C. Qubit® (Thermo Fisher Scientific®) and Bioanalyzer® (Agilent®) are recommended to quantify and analyze quality of the library.

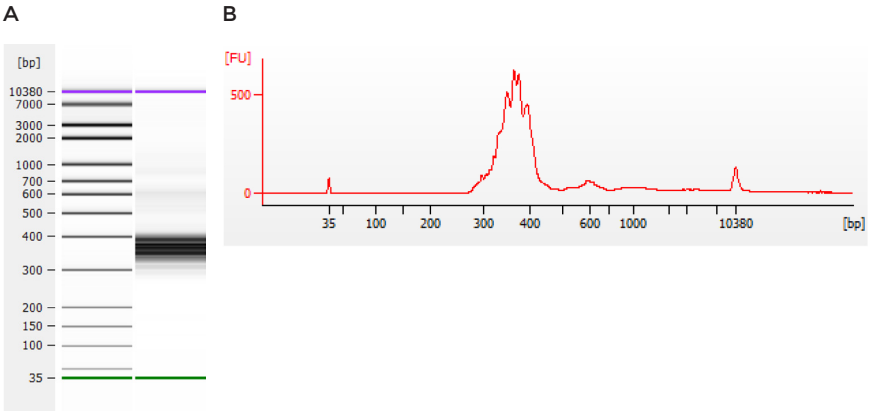

Figure 2. High Sensitivity DNA Chip Output:
A) NEXTflex BRCA 1 & BRCA2 Plus-1 Amplicon Panel Library - 20 ng input (Bioanalyzer® gel image)
B) NEXTflex BRCA 1 & BRCA2 Plus-1 Amplicon Panel Library - 20 ng input (electropherogram)

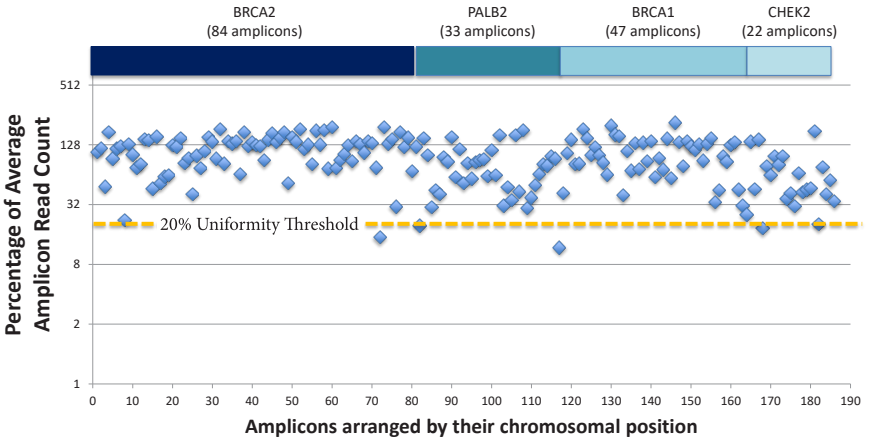

Figure 3. Performance of 186 amplicons from NEXTflex™ BRCA 1 & BRCA 2 Plus-1 Amplicon Panel for detection of germline mutations.

Oligonucleotide Sequences

NEXTflex™ PCR II Barcoded Primer Mix	
NEXTflex™	**Sequence 5' → 3'**
PCR II Forward Primer	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT CCGATCT
PCR II Reverse Barcoded Primer	CAAGCAGAAGACGGCATACGAGAT**XXXXXXXXXXXX**[1]GTGACTGGAGTT CAGACGTGTGCTCTTCCGATCT

[1]**XXXXXXXXXXXX** denotes the index region of the primer. The index sequences and the respective reverse complement sequences contained in each primer are listed below. The reverse complement is the sequence reported in the index read.

Reverse Primer Index Sequences and Reverse Complements

Barcoded Primer	Sequence 5' → 3'	Reverse Complement
1	GGCCGGCTAGAT	ATCTAGCCGGCC
2	AAGGAAGAGATA	TATCTCTTCCTT
3	GGACGGCATCTA	TAGATGCCGTCC
4	AAGGAAGGAGCG	CGCTCCTTCCTT
5	GGACGGCGCTCG	CGAGCGCCGTCC
6	CCGGACTCTCGA	TCGAGAGTCCGG
7	GGCCGGCCGAGC	GCTCGGCCGGCC
8	CCGGACTGAGCT	AGCTCAGTCCGG
9	GGACGCGGCAGT	ACTGCCGCGTCC
10	CCGGAGAAGTAA	TTACTTCTCCGG
11	GGCCGCGCGTCA	TGACGCGCGGCC
12	CCGGAGATCATT	AATGATCTCCGG
13	GGACGTACGCTT	AAGCGTACGTCC
14	AAGGACTGATAA	TTATCAGTCCTT
15	GGACGCGATGAC	GTCATCGCGTCC
16	CCGGAGAGACGG	CCGTCTCTCCGG
17	GGACGTAGCGAA	TTCGCTACGTCC
18	CCGGAAGAGCGT	ACGCTCTTCCGG
19	GGCCGCGTACTG	CAGTACGCGGCC
20	AAGGATCAGTAC	GTACTGATCCTT
21	GGCCGTATATCC	GGATATACGGCC
22	CCGGAAGCTATG	CATAGCTTCCGG
23	GGCCGATGCCTC	GAGGCATCGGCC
24	CCGGATCCTTAT	ATAAGGATCCGG
25	GGACGATCGGAG	CTCCGATCGTCC

26	CCGGATCGAATA	TATTCGATCCGG
27	GGACGATTAAGA	TCTTAATCGTCC
28	CCGGATCAGGCG	CGCCTGATCCGG
29	GGACGATATTCT	AGAATATCGTCC
30	CCGGATCTCCGC	GCGGAGATCCGG
31	GGACCGGCCATG	CATGGCCGGTCC
32	AAGGTACGTGAC	GTCACGTACCTT
33	GGACCGGTTGCA	TGCAACCGGTCC
34	CCGGTCAACAGG	CCTGTTGACCGG
35	GGACCTTGGGCT	AGCCCAAGGTCC
36	CCGGTACCAAGC	GCTTGGTACCGG
37	GGACCTTCCCGA	TCGGGAAGGTCC
38	CCGGTACGTTCG	CGAACGTACCGG
39	GGCCCTTAAATC	GATTTAAGGGCC
40	AAGGTCAGTTCT	AGAACTGACCTT
41	GGACCAAGGCGG	CCGCCTTGGTCC
42	CCGGTTGCATCA	TGATGCAACCGG
43	GGCCCAACCGCC	GGCGGTTGGGCC
44	CCGGTTGGTAGT	ACTACCAACCGG
45	GGACCAATTATT	AATAATTGGTCC
46	CCGGTTGACGAC	GTCGTCAACCGG
47	GGCCTGAGATTT	AAATCTCAGGCC
48	CCGGCCGCGCAC	GTGCGCGGCCGG
49	GGACTGACTAAA	TTTAGTCAGTCC
50	CCGGCCGGCGTG	CACGCCGGCCGG
51	GGACTGATCGGG	CCCGATCAGTCC
52	CCGGCCGATACA	TGTATCGGCCGG
53	GGACTCTGAAAG	CTTTCAGAGTCC
54	CCGGCGCCGGTA	TACCGGCGCCGG
55	GGACTCTCTTTC	GAAAGAGAGTCC
56	AAGGCTAGCCAG	CTGGCTAGCCTT
57	GGCCTCTTCCCT	AGGGAAGAGGCC
58	AAGGCTACGGTC	GACCGTAGCCTT
59	GGACTCTAGGGA	TCCCTAGAGTCC
60	AAGGCTATAACT	AGTTATAGCCTT
61	GGACTTCGAGGC	GCCTCGAAGTCC
62	AAGGCCGCGACG	CGTCGCGGCCTT
63	GGCCTTCCTCCG	CGGAGGAAGGCC
64	AAGGCCGGCTGC	GCAGCCGGCCTT
65	GGACTTCTCTTA	TAAGAGAAGTCC
66	AAGGCCGATCAT	ATGATCGGCCTT

67	GGACTTCAGAAT	ATTCTGAAGTCC
68	AAGGCCGTAGTA	TACTACGGCCTT
69	GGACTAGGACCA	TGGTCCTAGTCC
70	CCGGCTAATGTT	AACATTAGCCGG
71	GGACTAGCTGGT	ACCAGCTAGTCC
72	CCGGCTATACAA	TTGTATAGCCGG
73	GGACTAGTCAAC	GTTGACTAGTCC
74	CCGGCTACGTGG	CCACGTAGCCGG
75	GGACTAGAGTTG	CAACTCTAGTCC
76	AAGGCGCGCACA	TGTGCGCGCCTT
77	GGCCACAGTACC	GGTACTGTGGCC
78	AAGGGTTAATTT	AAATTAACCCTT
79	GGCCACATGCAA	TTGCATGTGGCC
80	AAGGGTTCCGGG	CCCGGAACCCTT
81	GGACACAACGTT	AACGTTGTGTCC
82	AAGGGTTGGCCC	GGGCCAACCCTT
83	GGACATGGTGTG	CACACCATGTCC
84	CCGGGAACCAAA	TTTGGTTCCCGG
85	GGACATGCACAC	GTGTGCATGTCC
86	CCGGGAATTGGG	CCCAATTCCCGG
87	GGACATGACACA	TGTGTCATGTCC
88	CCGGGAAGGTTT	AAACCTTCCCGG
89	GGACAACGTCAT	ATGACGTTGTCC
90	CCGGGTTAAGGA	TCCTTAACCCGG
91	GGACAACTGACG	CGTCAGTTGTCC
92	CCGGGTTCCTTC	GAAGGAACCCGG
93	GGCCAACACTGC	GCAGTGTTGGCC
94	CCGGGTTGGAAG	CTTCCAACCCGG
95	GGCTGGTCATAC	GTATGACCAGCC
96	CCGAACCTTAGG	CCTAAGGTTCGG
97	GGATGGTACGCA	TGCGTACCATCC
98	CCGAACCGGCTT	AAGCCGGTTCGG
99	GGATGCAGTTAT	ATAACTGCATCC
100	CCGAAGGCCCTC	GAGGGCCTTCGG
101	GGCTGCACAATA	TATTGTGCAGCC
102	CCGAAGGTTTCT	AGAAACCTTCGG
103	GGATGCATGGCG	CGCCATGCATCC
104	CCGAAGGAAAGA	TCTTTCCTTCGG
105	GGATGCAACCGC	GCGGTTGCATCC
106	AAGAATTGGGAT	ATCCCAATTCTT
107	GGCTGTGGTCGA	TCGACCACAGCC

108	AAGAACCAAGAG	CTCTTGGTTCTT
109	GGCTGTGCAGCT	AGCTGCACAGCC
110	AAGAACCGGAGA	TCTCCGGTTCTT
111	GGCTGTGACTAG	CTAGTCACAGCC
112	AAGAACCTTCTC	GAGAAGGTTCTT
113	GGATGACCACGG	CCGTGGTCATCC
114	CCGAATTGGTCA	TGACCAATTCGG
115	GGATGACTGTAA	TTACAGTCATCC
116	CCGAATTAACTG	CAGTTAATTCGG
117	GGCTGACACATT	AATGTGTCAGCC
118	AAGAAGGTTGAA	TTCAACCTTCTT
119	GGATCGAGAAGC	GCTTCTCGATCC
120	AAGATATATTAT	ATAATATATCTT
121	GGATCGACTTCG	CGAAGTCGATCC
122	CCGATCGGCCGA	TCGGCCGATCGG
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124	CCGATCGATTAG	CTAATCGATCGG
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127	GGCTCCTGATCA	TGATCAGGAGCC
128	CCGATGCCGCGG	CCGCGGCATCGG
129	GGATCCTCTAGT	ACTAGAGGATCC
130	AAGATTATATAC	GTATATAATCTT
131	GGCTCCTTCGAC	GTCGAAGGAGCC
132	CCGATGCATATT	AATATGCATCGG
133	GGATCCTAGCTG	CAGCTAGGATCC
134	AAGATTAGCGCA	TGCGCTAATCTT
135	GGCTCTCCTGAA	TTCAGGAGAGCC
136	CCGATATTACGT	ACGTAATATCGG
137	GGATCTCTCAGG	CCTGAGAGATCC
138	AAGATCGCGTAA	TTACGCGATCTT
139	GGATCTCAGTCC	GGACTGAGATCC
140	CCGATATGCATG	CATGCATATCGG
141	GGATCAGGAGAG	CTCTCCTGATCC
142	AAGATGCCGATC	GATCGGCATCTT
143	GGCTCAGCTCTC	GAGAGCTGAGCC
144	CCGATTAGCTAT	ATAGCTAATCGG
145	GGATCAGTCTCT	AGAGACTGATCC
146	AAGATGCATCGA	TCGATGCATCTT
147	GGCTCAGAGAGA	TCTCTCTGAGCC
148	CCGATTATAGCG	CGCTATAATCGG

149	GGCTTGGCCTGA	TCAGGCCAAGCC
150	CCGACCAGTCCG	CGGACTGGTCGG
151	GGATTGGTTCAG	CTGAACCAATCC
152	CCGACCACAGGC	GCCTGTGGTCGG
153	GGCTTGGAAGTC	GACTTCCAAGCC
154	AAGACACTGAAG	CTTCAGTGTCTT
155	GGATTCCGGTGG	CCACCGGAATCC
156	CCGACGTCACCA	TGGTGACGTCGG
157	GGCTTCCTTGTT	AACAAGGAAGCC
158	CCGACGTACAAC	GTTGTACGTCGG
159	GGATTCCAACAA	TTGTTGGAATCC
160	AAGACTGTGTTT	AAACACAGTCTT
161	GGATTAACCCAT	ATGGGTTAATCC
162	CCGACTGGTTTC	GAAACCAGTCGG
163	GGGTTAATTTGC	GCAAATTAACCC
164	CCCACTGCAAAG	CTTTGCAGTGGG
165	GGATAGCGCAAA	TTTGCGCTATCC
166	AAGAGAGAGTGG	CCACTCTCTCTT
167	GGATAGCCGTTT	AAACGGCTATCC
168	CCGAGCTTCACA	TGTGAAGCTCGG
169	GGATAGCTACCC	GGGTAGCTATCC
170	AAGAGAGCTGTT	AACAGCTCTCTT
171	GGCTAGCATGGG	CCCATGCTAGCC
172	AAGAGAGGACAA	TTGTCCTCTCTT
173	GGATACGGCTTC	GAAGCCGTATCC
174	AAGAGTCCTCAG	CTGAGGACTCTT
175	GGCTACGCGAAG	CTTCGCGTAGCC
176	AAGAGTCGAGTC	GACTCGACTCTT
177	GGATACGTAGGA	TCCTACGTATCC
178	AAGAGTCAGACT	AGTCTGACTCTT
179	CCAGCGCGCCAT	ATGGCGCGCTGG
180	TTGCTAGAGGGC	GCCCTCTAGCAA
181	CCCGCGCTAACG	CGTTAGCGCGGG
182	TTGCTAGCTTTA	TAAAGCTAGCAA
183	CCAGCGCATTGC	GCAATGCGCTGG
184	TTGCTAGGAAAT	ATTTCCTAGCAA
185	CCAGCTAGCACC	GGTGCTAGCTGG
186	TTGCTCTCTGGG	CCCAGAGAGCAA
187	CCAGCATGCTGA	TCAGCATGCTGG
188	TTGCTGACTCCT	AGGAGTCAGCAA
189	CCAGCATCGACT	AGTCGATGCTGG

190	TTGCTGATCTTC	GAAGATCAGCAA
191	CCAGCATTAGTC	GACTAATGCTGG
192	TTGCTGAAGAAG	CTTCTTCAGCAA
193	CCAGCATATCAG	CTGATATGCTGG
194	TTGCTGAGAGGA	TCCTCTCAGCAA
195	CCCGTGTGTCTC	GAGACACACGGG
196	TTGCCAACCTAG	CTAGGTTGGCAA
197	CCAGTGTCAGAG	CTCTGACACTGG
198	TTGCCAATTCGA	TCGAATTGGCAA
199	CCAGTGTACTCT	AGAGTACACTGG
200	TTGCCAAGGATC	GATCCTTGGCAA
201	CCCGTCAGTGAA	TTCACTGACGGG
202	TTGCCTTAACGG	CCGTTAAGGCAA
203	CCAGTCACACTT	AAGTGTGACTGG
204	TTGCCTTGGTAA	TTACCAAGGCAA
205	CCCGTACTGGAT	ATCCAGTACGGG
206	TTGCCGGAAATA	TATTTCCGGCAA
207	CCGGTACACCTA	TAGGTGTACCGG
208	TTACCGGTTTAT	ATAAACCGGTAA
209	CCGGACTTCTAG	CTAGAAGTCCGG
210	TTACGTAATCTC	GAGATTACGTAA
211	CCGGACTAGATC	GATCTAGTCCGG
212	TTCCGTAGCTCT	AGAGCTACGGAA
213	CCACATGGTCAA	TTGACCATGTGG
214	TTGGGCCAAGGG	CCCTTGGCCCAA
215	CCACAGTCATGC	GCATGACTGTGG
216	TTGGGAATTAAT	ATTAATTCCCAA
217	CCACAGTGTACG	CGTACACTGTGG
218	TTGGGAACCGGC	GCCGGTTCCCAA
219	CCACTAGAGAAA	TTTCTCTAGTGG
220	TTGGCGCGCTGG	CCAGCGCGCCAA
221	CCACTAGTCTTT	AAAGACTAGTGG
222	TTGGCGCATCAA	TTGATGCGCCAA
223	CCACTTCAGTTC	GAACTGAAGTGG
224	TTGGCCGGCACT	AGTGCCGGCCAA
225	CCACTTCTCAAG	CTTGAGAAGTGG
226	TTGGCCGCGTGA	TCACGCGGCCAA
227	CCACTTCCTGGA	TCCAGGAAGTGG
228	TTGGCCGTACAG	CTGTACGGCCAA
229	CCACTTCGACCT	AGGTCGAAGTGG
230	TTGGCCGATGTC	GACATCGGCCAA

231	CCACTCTAGCCG	CGGCTAGAGTGG
232	TTGGCTAGCGTA	TACGCTAGCCAA
233	CCACTCTTCGGC	GCCGAAGAGTGG
234	TTGGCTAATACG	CGTATTAGCCAA
235	CCACTCTCTAAT	ATTAGAGAGTGG
236	TTGGCTATATGC	GCATATAGCCAA
237	CCACTCTGATTA	TAATCAGAGTGG
238	TTGGCTACGCAT	ATGCGTAGCCAA
239	CCACTGAAGGGT	ACCCTTCAGTGG
240	TTGGCATGCCAC	GTGGCATGCCAA
241	CCACTGATCCCA	TGGGATCAGTGG
242	TTGGCATCGGTG	CACCGATGCCAA
243	CCACTGACTTTG	CAAAGTCAGTGG
244	TTGGCATTAACA	TGTTAATGCCAA
245	CCACTGAGAAAC	GTTTCTCAGTGG
246	TTGGCATATTGT	ACAATATGCCAA
247	CCACCAATTTAC	GTAAATTGGTGG
248	TTGGTGTCAAGT	ACTTGACACCAA
249	CCACCAACCCGT	ACGGGTTGGTGG
250	TTGGTGTGTTCA	TGAACACACCAA
251	CCACCAAGGGCA	TGCCCTTGGTGG
252	TTGGTGTACCTG	CAGGTACACCAA
253	CCACCTTAATAT	ATATTAAGGTGG
254	TTGGTCAGTAGC	GCTACTGACCAA
255	CCACCTTCCGCG	CGCGGAAGGTGG
256	TTGGTCATGCTA	TAGCATGACCAA
257	CCACCTTGGCGC	GCGCCAAGGTGG
258	TTGGTCACATCG	CGATGTGACCAA
259	CCACCGGAAGCC	GGCTTCCGGTGG
260	TTGGTACTGAGG	CCTCAGTACCAA
261	CCACCGGCCTAA	TTAGGCCGGTGG
262	TTGGTACGTCTT	AAGACGTACCAA
263	CCACGATATAGC	GCTATATCGTGG
264	TTGGAGAGATAT	ATATCTCTCCAA
265	CCACGATTATCG	CGATAATCGTGG
266	TTGGAGACTATA	TATAGTCTCCAA
267	CCACGATCGCTA	TAGCGATCGTGG
268	TTGGAGATCGCG	CGCGATCTCCAA
269	CCACGATGCGAT	ATCGCATCGTGG
270	TTGGAGAAGCGC	GCGCTTCTCCAA
271	CCGCGTAATTCA	TGAATTACGCGG

272	TTAGACTGAATG	CATTCAGTCTAA
273	CCGTCCTCTTCC	GGAAGAGGACGG
274	GGAATGCGCCGT	ACGGCGCATTCC
275	CCCTCCTGAAGG	CCTTCAGGAGGG
276	GGGATGCATTAC	GTAATGCATCCC
277	CCGTCGAAGCTC	GAGCTTCGACGG
278	TTAATATTATAG	CTATAATATTAA
279	CCGTCGATCGAG	CTCGATCGACGG
280	GGCATCGATATA	TATATCGATGCC
281	CCATCGACTAGA	TCTAGTCGATGG
282	GGGATCGGCGCG	CGCGCCGATCCC
283	CCGTCGAGATCT	AGATCTCGACGG
284	GGCATCGCGCGC	GCGCGCGATGCC
285	CCATGACACTAC	GTAGTGTCATGG
286	GGGAATTGGAGG	CCTCCAATTCCC
287	CCCTGACTGATG	CATCAGTCAGGG
288	TTGAAGGAAGAC	GTCTTCCTTCAA
289	CCGTGACGTCGT	ACGACGTCACGG
290	GGCAATTAAGAA	TTCTTAATTGCC
291	CCATGTGACATA	TATGTCACATGG
292	TTGAACCTTGAT	ATCAAGGTTCAA
293	CCATGTGTGTAT	ATACACACATGG
294	TTGAACCAACTA	TAGTTGGTTCAA
295	CCGTGCAACGCT	AGCGTTGCACGG
296	TTCAATTGGCTC	GAGCCAATTGAA
297	CCATGCATGCGA	TCGCATGCATGG
298	TTGAATTCCGAG	CTCGGAATTCAA
299	CCGTGCACATAG	CTATGTGCACGG
300	GGAAAGGTTAGC	GCTAACCTTTCC
301	CCGTGCAGTATC	GATACTGCACGG
302	GGAAAGGCCGAT	ATCGGCCTTTCC
303	CCATGGTACCGG	CCGGTACCATGG
304	GGGAACCGGGAC	GTCCCGGTTCCC
305	CCGTGGTCAATT	AATTGACCACGG
306	GGAAACCTTTCA	TGAAAGGTTTCC
307	CCGCATGACTGG	CCAGTCATGCGG
308	TTCGGCCGGAAA	TTTCCGGCCGAA
309	TTGGGAAGGCCG	CGGCCTTCCCAA
310	AAACATGACGTC	GACGTCATGTTT
311	GGACATGTGTGT	ACACACATGTCC
312	AAGGGCCAACCA	TGGTTGGCCCTT

313	TTCCAGATTAGC	GCTAATCTGGAA
314	AATGGCGCATAG	CTATGCGCCATT
315	TTTCAGAAATCG	CGATTTCTGAAA
316	CCCGGATTGCGC	GCGCAATCCGGG
317	TTCCACTCCCTG	CAGGGAGTGGAA
318	CCTGGTATGGCA	TGCCATACCAGG
319	TTCCATCTTCTT	AAGAAGATGGAA
320	AATGGATACTAC	GTAGTATCCATT
321	TTTCATCAAGAA	TTCTTGATGAAA
322	AACGGATGTCGT	ACGACATCCGTT
323	TTTCAAGGGTCT	AGACCCTTGAAA
324	AACGGTAACATA	TATGTTACCGTT
325	TTTCAAGCCAGA	TCTGGCTTGAAA
326	AACGGTAGTGCG	CGCACTACCGTT
327	TTCCAAGAACTC	GAGTTCTTGGAA
328	AATGGTATGTAT	ATACATACCATT
329	TTCTGCTGGCCT	AGGCCAGCAGAA
330	AATAAGCACGTA	TACGTGCTTATT
331	TTCTGCTCCGGA	TCCGGAGCAGAA
332	AATAAGCTGCAT	ATGCAGCTTATT
333	TTCTGCTAATTC	GAATTAGCAGAA
334	AATAAGCGTACG	CGTACGCTTATT
335	TTATGTCGGTTA	TAACCGACATAA
336	AATAAATACACT	AGTGTATTTATT
337	TTCTGTCCCAAT	ATTGGGACAGAA
338	AATAAATTGTGA	TCACAATTTATT
339	TTCTGTCTTGGC	GCCAAGACAGAA
340	AATAAATCACAG	CTGTGATTTATT
341	TTTCACCGCAAT	ATTGCGGTGAAA
342	AAAGGGTCTGTC	GACAGACCCTTT
343	AAATTAGATATC	GATATCTAATTT
344	TTTACTATCGAT	ATCGATAGTAAA
345	AATTAGTCCTCA	TGAGGACTAATT
346	TTAAGCCTGATT	AATCAGGCTTAA
347	AAATAGTTTCTG	CAGAAACTATTT
348	GGTAGAACAGCA	TGCTGTTCTACC
349	AAATAGTAAGAC	GTCTTACTATTT
350	GGTAGAATGATG	CATCATTCTACC
351	AAATACAGGTCG	CGACCTGTATTT
352	GGGAGTTCACGC	GCGTGAACTCCC
353	AAATACACCAGC	GCTGGTGTATTT

354	TTTAGGGTGTAG	CTACACCCTAAA
355	AAATACATTGAT	ATCAATGTATTT
356	GGTAGTTACATA	TATGTAACTACC
357	AAATACAAACTA	TAGTTTGTATTT
358	GGGAGTTTGTAT	ATACAAACTCCC
359	AATTATGGGCTC	GAGCCCATAATT
360	TTAAGAAACGCG	CGCGTTTCTTAA
361	GGACGTAATAGG	CCTATTACGTCC
362	AAGGACTTCGCC	GGCGAAGTCCTT
363	GGACCGGAACGT	ACGTTCCGGTCC
364	CCGGTCATGTCC	GGACATGACCGG
365	GGCCTGAAGCCC	GGGCTTCAGGCC
366	CCGGCCGTATGT	ACATACGGCCGG
367	GGATGGTGTATG	CATACACCATCC
368	CCGAACCAATCC	GGATTGGTTCGG
369	GGCTGACGTGCC	GGCACGTCAGCC
370	CCGAATTCCAGT	ACTGGAATTCGG
371	GGCTCTCGACTT	AAGTCGAGAGCC
372	AAGATCGATGCC	GGCATCGATCTT
373	CCAGAGACTGCC	GGCAGTCTCTGG
374	TTGCGATGCAGG	CCTGCATCGCAA
375	CCACTAGCTCCC	GGGAGCTAGTGG
376	TTGGCGCTAGTT	AACTAGCGCCAA
377	CCACTAGGAGGG	CCCTCCTAGTGG
378	TTGGCGCCGACC	GGTCGGCGCCAA
379	CCACCGGTTCGG	CCGAACCGGTGG
380	TTGGTACACTCC	GGAGTGTACCAA
381	CCGTGGTTGGCC	GGCCAACCACGG
382	GGCAACCAAAGT	ACTTTGGTTGCC
383	CCGCATGCAGTT	AACTGCATGCGG
384	TTAGGCCTTCCC	GGGAAGGCCTAA

Low Level Multiplexing

Every combination of sequential odd and even numbered barcodes is fully color balanced at all positions of the index. For example, barcodes 5 and 6 offer opposite colors at every position, but barcodes 6 and 7 do not. Larger pools can be made by combining multiple sets of color balanced pairs. For pools of odd numbers of samples, any barcode can be added to a balanced pool. For example, for a pool of 3 samples, pooling barcodes 5, 6, and any other barcode is acceptable.

A BED file of the covered regions is available for download on our webpage.

To receive a complete electronic list of the BED and FASTA files for this kit, please follow the instructions on the label inside the kit box.

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- NEXTlfex™ Small RNA Barcode Primers

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- NEXTflex™ 16S V4 Amplicon-Seq Kit 2.0
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