Oct 24, 2025 Version 1

# IDEEL: UNC Implementation of Targeted PX1 Sequencing-MIP to Nanopore V.1

DOI
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IDEEL

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**Protocol status:** Working
**We use this protocol and it's working**

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**Last Modified:** October 24, 2025

**Protocol Integer ID:** 230584

**Keywords:** PCR, Barcoding, Nanopore Library preparation, Amplification, Adapter Ligation, targeted sequencing of the px1 locus, targeted sequencing, integrating molecular inversion probe, molecular inversion probe, nanopore the protocol, px1 locus, oxford nanopore, read sequencing, nanopore

## Abstract

The protocol describes a novel workflow integrating Molecular Inversion Probes (MIPs) with Oxford Nanopore long-read sequencing for targeted sequencing of the PX1 locus.

## Materials

**Required Materials:**

- Molecular Grade Water (MGW)
- Primers

P2_Px1Block3_v2_F: GACTCGCCAAGCTGAAGNNNNAGAGAACAGGAAAAAGTTTGTACGT
P2_Px1Frag3_v8_R: ACGTGTGCTCTTCCGATCTNNNNTCATCACTGTCGCTACTTTTGTC

- GoTaq® Long PCR Master Mix, 2X **Promega Catalog #**M402A
- Illumina FWD and REV barcode primers
- Gel electrophoresis reagents and equipment.
- 1X TE Low EDTA Buffer
- PromethION 2 solo and device specific flow cells
- ONT Ligation sequencing kit (SQK-LSK114 Kit)

**PCR Round 2 Primers**
**Forward Primers:**

| | Well Position | Name | Sequence |
|---|---|---|---|
| | A01 | hybrid_dual_fw_001 | AATGATACGGCGACCACCGAGATCTACACGTTAAGACACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A02 | hybrid_dual_fw_002 | AATGATACGGCGACCACCGAGATCTACACTCTAAGTTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A03 | hybrid_dual_fw_003 | AATGATACGGCGACCACCGAGATCTACACTGTACATTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A04 | hybrid_dual_fw_004 | AATGATACGGCGACCACCGAGATCTACACAATACCGCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A05 | hybrid_dual_fw_005 | AATGATACGGCGACCACCGAGATCTACACTGTCTATGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A06 | hybrid_dual_fw_006 | AATGATACGGCGACCACCGAGATCTACACTCACTGTTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A07 | hybrid_dual_fw_007 | AATGATACGGCGACCACCGAGATCTACACTTGTATTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A08 | hybrid_dual_fw_008 | AATGATACGGCGACCACCGAGATCTACACAGACTAACACACTCTTTCCCTACACGACTCGCCAAGCTGA |

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|---|---|---|---|
| | A09 | hybrid_dual_fw_009 | AATGATACGGCGACCACCGAGATCTACACATTGTCAAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A10 | hybrid_dual_fw_010 | AATGATACGGCGACCACCGAGATCTACACGTTCTACAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A11 | hybrid_dual_fw_011 | AATGATACGGCGACCACCGAGATCTACACTTCATTCGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | A12 | hybrid_dual_fw_012 | AATGATACGGCGACCACCGAGATCTACACAAGCGTCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B01 | hybrid_dual_fw_013 | AATGATACGGCGACCACCGAGATCTACACCGTAGGATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B02 | hybrid_dual_fw_014 | AATGATACGGCGACCACCGAGATCTACACAGTTAGCGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B03 | hybrid_dual_fw_015 | AATGATACGGCGACCACCGAGATCTACACGAACGTCTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B04 | hybrid_dual_fw_016 | AATGATACGGCGACCACCGAGATCTACACAGAACACTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B05 | hybrid_dual_fw_017 | AATGATACGGCGACCACCGAGATCTACACTGTTGGCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B06 | hybrid_dual_fw_018 | AATGATACGGCGACCACCGAGATCTACACGGCTAATAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B07 | hybrid_dual_fw_019 | AATGATACGGCGACCACCGAGATCTACACGCTAGAATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B08 | hybrid_dual_fw_020 | AATGATACGGCGACCACCGAGATCTACACTGTACTAAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B09 | hybrid_dual_fw_021 | AATGATACGGCGACCACCGAGATCTACACAGTTACACACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B10 | hybrid_dual_fw_022 | AATGATACGGCGACCACCGAGATCTACACGTACACGAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B11 | hybrid_dual_fw_023 | AATGATACGGCGACCACCGAGATCTACACACTGTAAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | B12 | hybrid_dual_fw_024 | AATGATACGGCGACCACCGAGATCTACACGATCATGAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C01 | hybrid_dual_fw_025 | AATGATACGGCGACCACCGAGATCTACACTGCTAACGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C02 | hybrid_dual_fw_026 | AATGATACGGCGACCACCGAGATCTACACTAATGCTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C03 | hybrid_dual_fw_027 | AATGATACGGCGACCACCGAGATCTACACCTTGTTGAACACTCTTTCCCTACACGACTCGCCAAGCTGA |

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|---|---|---|---|
| | C04 | hybrid_dual_fw_028 | AATGATACGGCGACCACCGAGATCTACACGATACAAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C05 | hybrid_dual_fw_029 | AATGATACGGCGACCACCGAGATCTACACATACAGCGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C06 | hybrid_dual_fw_030 | AATGATACGGCGACCACCGAGATCTACACCTGGATAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C07 | hybrid_dual_fw_031 | AATGATACGGCGACCACCGAGATCTACACTCATCAGCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C08 | hybrid_dual_fw_032 | AATGATACGGCGACCACCGAGATCTACACTTATACCGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C09 | hybrid_dual_fw_033 | AATGATACGGCGACCACCGAGATCTACACGCTGATTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C10 | hybrid_dual_fw_034 | AATGATACGGCGACCACCGAGATCTACACATAAGGCCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C11 | hybrid_dual_fw_035 | AATGATACGGCGACCACCGAGATCTACACAAGACTAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | C12 | hybrid_dual_fw_036 | AATGATACGGCGACCACCGAGATCTACACTAGCAACAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D01 | hybrid_dual_fw_037 | AATGATACGGCGACCACCGAGATCTACACCAAGTAATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D02 | hybrid_dual_fw_038 | AATGATACGGCGACCACCGAGATCTACACTCATGATGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D03 | hybrid_dual_fw_039 | AATGATACGGCGACCACCGAGATCTACACTAGCTCTTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D04 | hybrid_dual_fw_040 | AATGATACGGCGACCACCGAGATCTACACACTACATGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D05 | hybrid_dual_fw_041 | AATGATACGGCGACCACCGAGATCTACACTGTGCCATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D06 | hybrid_dual_fw_042 | AATGATACGGCGACCACCGAGATCTACACGACTTCCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D07 | hybrid_dual_fw_043 | AATGATACGGCGACCACCGAGATCTACACTATTCTCGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D08 | hybrid_dual_fw_044 | AATGATACGGCGACCACCGAGATCTACACCACTAGATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D09 | hybrid_dual_fw_045 | AATGATACGGCGACCACCGAGATCTACACCTTCGCATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D10 | hybrid_dual_fw_046 | AATGATACGGCGACCACCGAGATCTACACCTAGCGATACACTCTTTCCCTACACGACTCGCCAAGCTGA |

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|---|---|---|---|
| | D11 | hybrid_dual_fw_047 | AATGATACGGCGACCACCGAGATCTACACTACGTTAAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | D12 | hybrid_dual_fw_048 | AATGATACGGCGACCACCGAGATCTACACAAGTAGTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E01 | hybrid_dual_fw_049 | AATGATACGGCGACCACCGAGATCTACACGACGTATCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E02 | hybrid_dual_fw_050 | AATGATACGGCGACCACCGAGATCTACACACGTTCAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E03 | hybrid_dual_fw_051 | AATGATACGGCGACCACCGAGATCTACACAGCTATATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E04 | hybrid_dual_fw_052 | AATGATACGGCGACCACCGAGATCTACACAGTGCGAAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E05 | hybrid_dual_fw_053 | AATGATACGGCGACCACCGAGATCTACACCAATGGTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E06 | hybrid_dual_fw_054 | AATGATACGGCGACCACCGAGATCTACACATTCCGATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E07 | hybrid_dual_fw_055 | AATGATACGGCGACCACCGAGATCTACACAGCTGTTGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E08 | hybrid_dual_fw_056 | AATGATACGGCGACCACCGAGATCTACACTTCGACTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E09 | hybrid_dual_fw_057 | AATGATACGGCGACCACCGAGATCTACACCGAATGGTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E10 | hybrid_dual_fw_058 | AATGATACGGCGACCACCGAGATCTACACTAGTGTACACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E11 | hybrid_dual_fw_059 | AATGATACGGCGACCACCGAGATCTACACACGCATGAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | E12 | hybrid_dual_fw_060 | AATGATACGGCGACCACCGAGATCTACACTTCGTACAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F01 | hybrid_dual_fw_061 | AATGATACGGCGACCACCGAGATCTACACTTCTGCTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F02 | hybrid_dual_fw_062 | AATGATACGGCGACCACCGAGATCTACACTGGAAGCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F03 | hybrid_dual_fw_063 | AATGATACGGCGACCACCGAGATCTACACGATGCTCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F04 | hybrid_dual_fw_064 | AATGATACGGCGACCACCGAGATCTACACAGCCTGAAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F05 | hybrid_dual_fw_065 | AATGATACGGCGACCACCGAGATCTACACCACCAGTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |

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|---|---|---|---|
| | F06 | hybrid_dual_fw_066 | AATGATACGGCGACCACCGAGATCTACACGTTACACCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F07 | hybrid_dual_fw_067 | AATGATACGGCGACCACCGAGATCTACACGCGTTAGTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F08 | hybrid_dual_fw_068 | AATGATACGGCGACCACCGAGATCTACACTGACGTTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F09 | hybrid_dual_fw_069 | AATGATACGGCGACCACCGAGATCTACACTAGGCGTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F10 | hybrid_dual_fw_070 | AATGATACGGCGACCACCGAGATCTACACAAGTGAGCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F11 | hybrid_dual_fw_071 | AATGATACGGCGACCACCGAGATCTACACCCGGAATAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | F12 | hybrid_dual_fw_072 | AATGATACGGCGACCACCGAGATCTACACAATAGGCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G01 | hybrid_dual_fw_073 | AATGATACGGCGACCACCGAGATCTACACTTCAGGTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G02 | hybrid_dual_fw_074 | AATGATACGGCGACCACCGAGATCTACACTAATCGCTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G03 | hybrid_dual_fw_075 | AATGATACGGCGACCACCGAGATCTACACCTTAGGTGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G04 | hybrid_dual_fw_076 | AATGATACGGCGACCACCGAGATCTACACTTGATCTCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G05 | hybrid_dual_fw_077 | AATGATACGGCGACCACCGAGATCTACACTCTTACTGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G06 | hybrid_dual_fw_078 | AATGATACGGCGACCACCGAGATCTACACGAGTACACACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G07 | hybrid_dual_fw_079 | AATGATACGGCGACCACCGAGATCTACACACGTTACAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G08 | hybrid_dual_fw_080 | AATGATACGGCGACCACCGAGATCTACACTGGTAAGCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G09 | hybrid_dual_fw_081 | AATGATACGGCGACCACCGAGATCTACACGATAGCACACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G10 | hybrid_dual_fw_082 | AATGATACGGCGACCACCGAGATCTACACGACTTAGGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G11 | hybrid_dual_fw_083 | AATGATACGGCGACCACCGAGATCTACACAGTCGTTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | G12 | hybrid_dual_fw_084 | AATGATACGGCGACCACCGAGATCTACACCGTGATTAACACTCTTTCCCTACACGACTCGCCAAGCTGA |

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|---|---|---|---|
| | H01 | hybrid_dual_fw_085 | AATGATACGGCGACCACCGAGATCTACACTCAGTAGAACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H02 | hybrid_dual_fw_086 | AATGATACGGCGACCACCGAGATCTACACATGTGTCTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H03 | hybrid_dual_fw_087 | AATGATACGGCGACCACCGAGATCTACACCAGAATATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H04 | hybrid_dual_fw_088 | AATGATACGGCGACCACCGAGATCTACACTAAGTCTGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H05 | hybrid_dual_fw_089 | AATGATACGGCGACCACCGAGATCTACACATCGACATACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H06 | hybrid_dual_fw_090 | AATGATACGGCGACCACCGAGATCTACACACAGCTGTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H07 | hybrid_dual_fw_091 | AATGATACGGCGACCACCGAGATCTACACATCACAAGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H08 | hybrid_dual_fw_092 | AATGATACGGCGACCACCGAGATCTACACGTCGAATTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H09 | hybrid_dual_fw_093 | AATGATACGGCGACCACCGAGATCTACACTGCCGATTACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H10 | hybrid_dual_fw_094 | AATGATACGGCGACCACCGAGATCTACACACTAATGCACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H11 | hybrid_dual_fw_095 | AATGATACGGCGACCACCGAGATCTACACCTAGTAGGACACTCTTTCCCTACACGACTCGCCAAGCTGA |
| | H12 | hybrid_dual_fw_096 | AATGATACGGCGACCACCGAGATCTACACTTAAGCCAACACTCTTTCCCTACACGACTCGCCAAGCTGA |

**Reverse Primers**

| | Well Position | Name | Sequence |
|---|---|---|---|
| | A01 | hybrid_dual_rev_001 | CAAGCAGAAGACGGCATACGAGATGTTAAGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A02 | hybrid_dual_rev_002 | CAAGCAGAAGACGGCATACGAGATTCTAAGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A03 | hybrid_dual_rev_003 | CAAGCAGAAGACGGCATACGAGATTGTACATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A04 | hybrid_dual_rev_004 | CAAGCAGAAGACGGCATACGAGATAATACCGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

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|---|---|---|---|
| | A05 | hybrid_dual_rev_005 | CAAGCAGAAGACGGCATACGAGATTGTCTATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A06 | hybrid_dual_rev_006 | CAAGCAGAAGACGGCATACGAGATTCACTGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A07 | hybrid_dual_rev_007 | CAAGCAGAAGACGGCATACGAGATTTGTATTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A08 | hybrid_dual_rev_008 | CAAGCAGAAGACGGCATACGAGATAGACTAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A09 | hybrid_dual_rev_009 | CAAGCAGAAGACGGCATACGAGATATTGTCAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A10 | hybrid_dual_rev_010 | CAAGCAGAAGACGGCATACGAGATGTTCTACAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A11 | hybrid_dual_rev_011 | CAAGCAGAAGACGGCATACGAGATTTCATTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | A12 | hybrid_dual_rev_012 | CAAGCAGAAGACGGCATACGAGATAAGCGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B01 | hybrid_dual_rev_013 | CAAGCAGAAGACGGCATACGAGATCGTAGGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B02 | hybrid_dual_rev_014 | CAAGCAGAAGACGGCATACGAGATAGTTAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B03 | hybrid_dual_rev_015 | CAAGCAGAAGACGGCATACGAGATGAACGTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B04 | hybrid_dual_rev_016 | CAAGCAGAAGACGGCATACGAGATAGAACACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B05 | hybrid_dual_rev_017 | CAAGCAGAAGACGGCATACGAGATTGTTGGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B06 | hybrid_dual_rev_018 | CAAGCAGAAGACGGCATACGAGATGGCTAATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B07 | hybrid_dual_rev_019 | CAAGCAGAAGACGGCATACGAGATGCTAGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B08 | hybrid_dual_rev_020 | CAAGCAGAAGACGGCATACGAGATTGTACTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B09 | hybrid_dual_rev_021 | CAAGCAGAAGACGGCATACGAGATAGTTACACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B10 | hybrid_dual_rev_022 | CAAGCAGAAGACGGCATACGAGATGTACACGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | B11 | hybrid_dual_rev_023 | CAAGCAGAAGACGGCATACGAGATACTGTAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

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|---|---|---|---|
| | B12 | hybrid_dual_rev_024 | CAAGCAGAAGACGGCATACGAGATGATCATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C01 | hybrid_dual_rev_025 | CAAGCAGAAGACGGCATACGAGATTGCTAACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C02 | hybrid_dual_rev_026 | CAAGCAGAAGACGGCATACGAGATTAATGCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C03 | hybrid_dual_rev_027 | CAAGCAGAAGACGGCATACGAGATCTTGTTGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C04 | hybrid_dual_rev_028 | CAAGCAGAAGACGGCATACGAGATGATACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C05 | hybrid_dual_rev_029 | CAAGCAGAAGACGGCATACGAGATATACAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C06 | hybrid_dual_rev_030 | CAAGCAGAAGACGGCATACGAGATCTGGATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C07 | hybrid_dual_rev_031 | CAAGCAGAAGACGGCATACGAGATTCATCAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C08 | hybrid_dual_rev_032 | CAAGCAGAAGACGGCATACGAGATTTATACCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C09 | hybrid_dual_rev_033 | CAAGCAGAAGACGGCATACGAGATGCTGATTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C10 | hybrid_dual_rev_034 | CAAGCAGAAGACGGCATACGAGATATAAGGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C11 | hybrid_dual_rev_035 | CAAGCAGAAGACGGCATACGAGATAAGACTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | C12 | hybrid_dual_rev_036 | CAAGCAGAAGACGGCATACGAGATTAGCAACAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D01 | hybrid_dual_rev_037 | CAAGCAGAAGACGGCATACGAGATCAAGTAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D02 | hybrid_dual_rev_038 | CAAGCAGAAGACGGCATACGAGATTCATGATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D03 | hybrid_dual_rev_039 | CAAGCAGAAGACGGCATACGAGATTAGCTCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D04 | hybrid_dual_rev_040 | CAAGCAGAAGACGGCATACGAGATACTACATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D05 | hybrid_dual_rev_041 | CAAGCAGAAGACGGCATACGAGATTGTGCCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D06 | hybrid_dual_rev_042 | CAAGCAGAAGACGGCATACGAGATGACTTCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

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|---|---|---|---|
| | D07 | hybrid_dual_rev_043 | CAAGCAGAAGACGGCATACGAGATTATTCTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D08 | hybrid_dual_rev_044 | CAAGCAGAAGACGGCATACGAGATCACTAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D09 | hybrid_dual_rev_045 | CAAGCAGAAGACGGCATACGAGATCTTCGCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D10 | hybrid_dual_rev_046 | CAAGCAGAAGACGGCATACGAGATCTAGCGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D11 | hybrid_dual_rev_047 | CAAGCAGAAGACGGCATACGAGATTACGTTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | D12 | hybrid_dual_rev_048 | CAAGCAGAAGACGGCATACGAGATAAGTAGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E01 | hybrid_dual_rev_049 | CAAGCAGAAGACGGCATACGAGATGACGTATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E02 | hybrid_dual_rev_050 | CAAGCAGAAGACGGCATACGAGATACGTTCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E03 | hybrid_dual_rev_051 | CAAGCAGAAGACGGCATACGAGATAGCTATATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E04 | hybrid_dual_rev_052 | CAAGCAGAAGACGGCATACGAGATAGTGCGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E05 | hybrid_dual_rev_053 | CAAGCAGAAGACGGCATACGAGATCAATGGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E06 | hybrid_dual_rev_054 | CAAGCAGAAGACGGCATACGAGATATTCCGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E07 | hybrid_dual_rev_055 | CAAGCAGAAGACGGCATACGAGATAGCTGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E08 | hybrid_dual_rev_056 | CAAGCAGAAGACGGCATACGAGATTTCGACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E09 | hybrid_dual_rev_057 | CAAGCAGAAGACGGCATACGAGATCGAATGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E10 | hybrid_dual_rev_058 | CAAGCAGAAGACGGCATACGAGATTAGTGTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E11 | hybrid_dual_rev_059 | CAAGCAGAAGACGGCATACGAGATACGCATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | E12 | hybrid_dual_rev_060 | CAAGCAGAAGACGGCATACGAGATTTCGTACAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F01 | hybrid_dual_rev_061 | CAAGCAGAAGACGGCATACGAGATTTCTGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

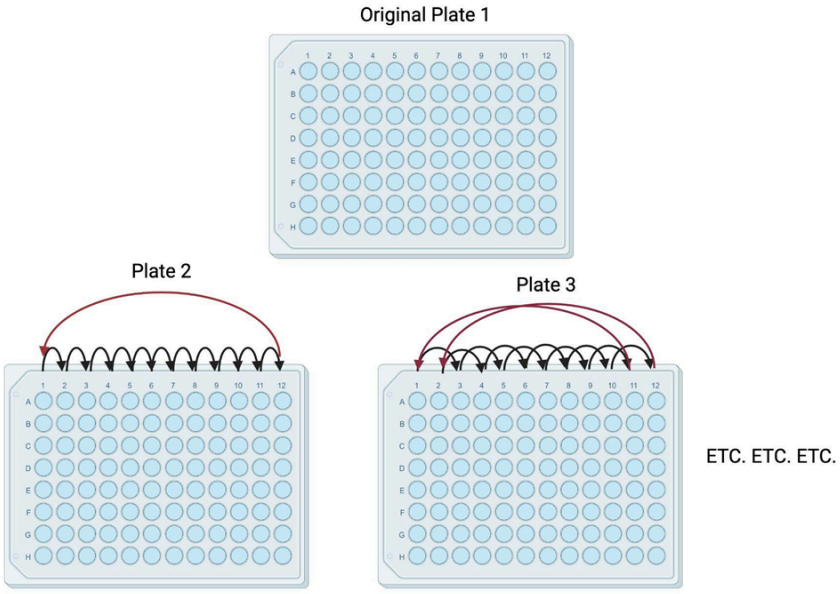
| | | | |
|---|---|---|---|
| | F02 | hybrid_dual_rev_062 | CAAGCAGAAGACGGCATACGAGATTGGAAGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F03 | hybrid_dual_rev_063 | CAAGCAGAAGACGGCATACGAGATGATGCTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F04 | hybrid_dual_rev_064 | CAAGCAGAAGACGGCATACGAGATAGCCTGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F05 | hybrid_dual_rev_065 | CAAGCAGAAGACGGCATACGAGATCACCAGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F06 | hybrid_dual_rev_066 | CAAGCAGAAGACGGCATACGAGATGTTACACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F07 | hybrid_dual_rev_067 | CAAGCAGAAGACGGCATACGAGATGCGTTAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F08 | hybrid_dual_rev_068 | CAAGCAGAAGACGGCATACGAGATTGACGTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F09 | hybrid_dual_rev_069 | CAAGCAGAAGACGGCATACGAGATTAGGCGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F10 | hybrid_dual_rev_070 | CAAGCAGAAGACGGCATACGAGATAAGTGAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F11 | hybrid_dual_rev_071 | CAAGCAGAAGACGGCATACGAGATCCGGAATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | F12 | hybrid_dual_rev_072 | CAAGCAGAAGACGGCATACGAGATAATAGGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G01 | hybrid_dual_rev_073 | CAAGCAGAAGACGGCATACGAGATTTCAGGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G02 | hybrid_dual_rev_074 | CAAGCAGAAGACGGCATACGAGATTAATCGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G03 | hybrid_dual_rev_075 | CAAGCAGAAGACGGCATACGAGATCTTAGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G04 | hybrid_dual_rev_076 | CAAGCAGAAGACGGCATACGAGATTTGATCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G05 | hybrid_dual_rev_077 | CAAGCAGAAGACGGCATACGAGATTCTTACTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G06 | hybrid_dual_rev_078 | CAAGCAGAAGACGGCATACGAGATGAGTACACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G07 | hybrid_dual_rev_079 | CAAGCAGAAGACGGCATACGAGATACGTTACAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G08 | hybrid_dual_rev_080 | CAAGCAGAAGACGGCATACGAGATTGGTAAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

| | | | |
|---|---|---|---|
| | G09 | hybrid_dual_rev_081 | CAAGCAGAAGACGGCATACGAGATGATAGCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G10 | hybrid_dual_rev_082 | CAAGCAGAAGACGGCATACGAGATGACTTAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G11 | hybrid_dual_rev_083 | CAAGCAGAAGACGGCATACGAGATAGTCGTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | G12 | hybrid_dual_rev_084 | CAAGCAGAAGACGGCATACGAGATCGTGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H01 | hybrid_dual_rev_085 | CAAGCAGAAGACGGCATACGAGATTCAGTAGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H02 | hybrid_dual_rev_086 | CAAGCAGAAGACGGCATACGAGATATGTGTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H03 | hybrid_dual_rev_087 | CAAGCAGAAGACGGCATACGAGATCAGAATATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H04 | hybrid_dual_rev_088 | CAAGCAGAAGACGGCATACGAGATTAAGTCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H05 | hybrid_dual_rev_089 | CAAGCAGAAGACGGCATACGAGATATCGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H06 | hybrid_dual_rev_090 | CAAGCAGAAGACGGCATACGAGATACAGCTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H07 | hybrid_dual_rev_091 | CAAGCAGAAGACGGCATACGAGATATCACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H08 | hybrid_dual_rev_092 | CAAGCAGAAGACGGCATACGAGATGTCGAATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H09 | hybrid_dual_rev_093 | CAAGCAGAAGACGGCATACGAGATTGCCGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H10 | hybrid_dual_rev_094 | CAAGCAGAAGACGGCATACGAGATACTAATGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H11 | hybrid_dual_rev_095 | CAAGCAGAAGACGGCATACGAGATCTAGTAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |
| | H12 | hybrid_dual_rev_096 | CAAGCAGAAGACGGCATACGAGATTTAAGCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT |

## Section 1: Preparation of PCR2 Primer plates

1 To allow for high levels of combinations of indexing primers, we generate multiple forward primer plates (12) from a single 96-well plate. By using these 12 plates in combination with a single 96-well plate of reverse primers, we can index 1,152 samples. Additional primers can be designed to increase this multiplexing. Here we present one plate of forward and one plate of reverse primers to allow for this level of combination.

2 To generate forward primer plates, we shift the column to the right one column when replating to a new plate (e.g. column 1 becomes column 2 and column 12 becomes column 1). See the figure below. Be sure to uniquely label the plate so you know which primers are used in library preparation.




Primer plate

**Note**

We do NOT do this for reverse primers.

## Section 2: Generate Primers mix

10m

3 Spin lyophilized Primer.

4 Resuspend in MGW using the provided volume for a [M] 100 micromolar (µM) stock.

5 Vortex and spin down briefly.

6 Let the mixture sit for about 00:10:00 for complete dissolution. 10m

7 Prepare the stock primer pool ( [M] 100 micromolar (µM) ) by mixing 1 volume of P2_Px1Block3_v2_F with 1 volume of P2_Px1Frag3_v8_R.

8 Dilute primer pool with MGW to obtain [M] 25 micromolar (µM) working solution: 1 volume of  stock + 3 volumes of MGW.

## Section 3: PCR1

9 Pre-set the thermocycler before starting master mix preparation using the programme below.

| | A | B | C | D |
|---|---|---|---|---|
| | **Step** | **Temperature (°C)** | **Time (minute)** | **Number of cycles** |
| | Step1 (preheat) | 95 | hold | NA |
| | Step2 (Activation) | 95 | 02:00 | NA |
| | Step3 (Denaturation) | 94 | 00:30 | x32 |
| | Step4 (Annealing) | 65 | 08:00 | x32^ go to step 3 |
| | Step5 (final Extension) | 72 | 10:00 | NA |
| | Step6 | 4 | hold | NA |

10 All sample plates and controls are to be kept on cold blocks - switch out cold blocks if they are getting warm.

11 All reagents are to be kept On ice and the tube used to prepare the master mix should also remain On ice.

12 Centrifuge sample plates at 4000 rpm, 00:01:00. 1m

13 Prepare the master mix using the template (here 96 reactions) below and adjust it to the number of reactions.

| | A | B | C |
|---|---|---|---|
| | **Master Mix** | **x1** | **x96*1.1** |
| | Gotaq | 10 | 1056 |
| | Molecular grade water | 5.2 | 549.12 |
| | Primer pool (P2_Px1Block3_v2_F and P2_Px1Frag3_v8_R) | 0.8 | 84.48 |
| | template DNA | 4 | |

14 Divide the total master mix volume by 12 and aliquot the designated volume of into 12 tube strip on cold block to allow for multichannel pipetting.

15 Using a multichannel, pipette 16 µL of master mix into each well of 96 well plate..

16 Using a multichannel, plate 4 µL of sample into each well on the PCR1 plate.

Note

Add at least one positive control (lab strain, 4uL) and one PCR1 negative control (MGW, 4uL).

17 Centrifuge the plate at 4000 rpm, 00:01:00 . 1m

18 Place the plate in the thermocycler and start running the pre-set PCR program.

## Section 4: PCR2 or barcoding PCR

19 Once the first PCR is complete, pre-set the thermocycler before starting the master mix preparation for PCR2 using the programme below.

| | A | B | C | D |
|---|---|---|---|---|
| | **Step** | **Temperature (°C)** | **Time (minute)** | **Number of cycles** |
| | Step1 | 98 | hold | NA |
| | Step2 | 98 | 0:30 | NA |
| | Step3 | 95 | 0:10 | x10 |
| | Step4 | 62 | 8:0 | x10 |
| | Step5 | 72 | 0:20 | x10 |
| | Step6 | 72 | 1:0 | NA |
| | Step7 | 4 | hold | NA |

20 Prepare the master mix for PCR2 using the template (here 96 reactions) below and adjust it to the number of reactions.

| | A | B | C |
|---|---|---|---|
| | **Master mix** | **x1** | **x96*1.1** |
| | Gotaq | 10 | 1056 |
| | Molecular grade water | 6 | 633.6 |

21 Divide the total master mix volume by 12 and aliquot the designated volume ( 140.8 µL for 96 sample run) into 12 tube strip on cold block to allow for multichannel

pipetting.

22 Using a multichannel, pipette 16 µL of master mix into each well.

23 Spin down the forward and reverse PCR2 primer plates needed for the experiment at 4000 rpm, 00:01:00 and add 1 µL of forward primer and 1 µL of reverse primer into each well using the multichannel. 1m

> Note
>
> On your sample sheet, please denote the name of the forward and reverse primer plate used and the well of the sample to be sure you know the correct index for the sample you are genotyping.

24 In the post-PCR lab, add 2 µL of the PCR1 product to the master mix using a multichannel.

25 Optional: shake the plate gently on a shaker for 00:01:00 to mix the master mix added to the samples. 1m

26 Centrifuge the plate at 4000 rpm, 00:01:00 . 1m

27 Place the plate in the thermocycler and start running the pre-set PCR program.

> Note
>
> **Check a random set of 10 wells per plate for amplification by gel electrophoresis based on your lab protocol (including the NTC and positive control).**

## Section 5: PCR Product Pooling and Cleanup

28 Pool 5 µL of PCR2 product into a single Eppendorf tube. If the pool is very large, 3 µL is sufficient.

29 Bring Ampure Beads to Room temperature. The Bailey Lab makes their own bead solution with Spherotech beads (DIY).

30 Add an equal volume of beads to the pool (1X bead clean). Let it incubate for at least 00:10:00 at Room temperature. For a higher yield, place the tube on a Hula Mixer or invert it in your hand.

31 Place the pool on a magnet and wait until the beads have pelletted on the magnet and the solution is clear. Prepare enough fresh 80% ethanol to cover the beads for 2 washes.

32 Remove the supernatant and reserve it in another tube.

33 Wash with 80% ethanol for at least 00:00:30. Discard the ethanol.

34 Repeat the above step for a second wash.

35 Remove the ethanol and let the beads dry for around 00:01:00 (do not let the beads dry until cracking). 1m

36 Elute in 51 µL of 1X low EDTA TE Buffer. If the pool is very large, use your discretion and elute in a higher volume (we eluted in 150 µL with 6 96-well plates of sample). Incubate at Room temperature for 00:10:00. 10m

37 Pellet the beads on a magnet and remove the elution into another tube.

38 Quantify the elution with a Qubit fluorometer.

## Section 6: Nanopore Library preparation 36m 30s

39

Note

We use the SQK-LSK114 Kit (Ligation Sequencing) and have adapted this protocol from the Ligation Sequencing Amplicons V14 protocol from Nanopore. We use the P2 sequencing machine, but this protocol could be adapted for Minion.

40 **End Prep**

Transfer 200 fmol of cleaned DNA into a PCR tube or plate. Calculation for fmol can be done on this website: **https://www.promega.com/resources/tools/biomath/** (1 pmol = 1,000 fmol). Adjust the volume to 50 µL with nuclease-free water.

40.1 Add the following to a PCR tube:

| | A | B |
|---|---|---|
| | **Reagent** | **Volume** |
| | DNA Template | 50 uL |
| | Ultra II End Prep Reaction Buffer | 7 uL |
| | Ultra II End Prep Enzyme Mix | 3 uL |
| | Total | 60uL |

40.2 Mix thoroughly by pipetting.

40.3 Incubate at 20 °C for 00:05:00 and 65 °C for 00:05:00 . 10m

40.4 Spin down and transfer the sample to a clean 1.5ml Eppendorf tube

40.5 Bring beads to Room temperature and add 60 µL to the DNA sample.

40.6 Incubate for 00:10:00 at Room temperature. For increased yield, place the tube on a Hula Mixer or invert in your hand. Meanwhile, prepare 1 mL of 80% ethanol. 10m

40.7 Pellet on a magnet and remove the supernatant - keep the supernatant for extra security.

40.8 Wash with 500 µL of 80% ethanol. Remove after 00:00:30. 30s

40.9 Repeat the above step for a total of 2 washes.

40.10 Let the beads dry for around 00:01:00. Do not let the beads crack on the side of the tube. 1m

40.11 Elute in 61 µL of nuclease-free water. Let it incubate at Room temperature for 00:05:00. 5m

40.12 Pellet on the magnet and remove the elution.

40.13 Quantify the End-prepped DNA with a qubit fluorometer. This library can be stored at 4 °C for short-term storage, or at -20 °C or -80 °C for long-term storage.

41 **Adapter Ligation**

Thaw the Short Fragment Buffer (SFB) and the Elution Buffer (EB) at Room temperature. Place the Ligation Adapter (LA), Ligation Buffer (LNB), and Quick T4 Ligase On ice.

41.1 Mix the following in a 1.5 ml Eppendorf tube, mixing 10-20 times after each addition:

| | A | B |
|---|---|---|
| | **Reagent** | **Volume** |
| | End-prepped DNA | 60 uL |
| | Ligation Buffer (LNB) | 25 uL |
| | NEBNext Quick T4 Ligase | 10 uL |
| | Ligation Adapter (LA) | 5 uL |
| | Total | 100 uL |

41.2 Mix well by pipetting. Incubate at Room temperature for at least 00:10:00 . A longer incubation of 00:20:00 will increase the amount of ligated adapters.

41.3 Bring the beads to Room temperature and resuspend.

41.4 Add 60 µL of beads and mix by inverting. Incubate for 00:10:00 at Room temperature . For higher yield, invert in your hand or place on a Hula mixer. 10m

41.5 Pellet on a magnet and remove the supernatant.

41.6 Wash with 250 µL of the Short Fragment buffer. Remove after around 00:00:30 .

41.7 Repeat the above step for a total of 2 washes.

41.8 Allow beads to dry on the magnet, and pipette out any residual buffer. Do not let beads dry to the point of cracking.

41.9 Elute in 25 µL of the Elution Buffer (EB). Incubate for 00:10:00 at Room temperature .

41.10 Pellet on a magnet and pipette the elution into a tube (ideally a screw cap tube for best storage).

41.11 Quantify the library with a qubit fluorometer. Be sure that you have at least 120 fmol of DNA, which is around 25 ng of DNA for a 4CAST library. This library can be stored at 4 °C for short-term storage, or at -20 °C or -80 °C for long-term storage.

## Section 7: Nanopore sequencing

1h 15m

42 **Loading the Flow Cell**

42.1 Take the Promethion Flow Cell you plan to use out of the 4 °C refrigerator at least 00:20:00 before use. It is highly recommended to do a flow cell check before any sequencing.

42.2 Determine 100 - 120 fmols of library and add to an Eppendorf tube (Nanopore recommends 20 fmol for sequencing; however, this leads to underloading of the flow cell in most cases. If you have less than 100 fmols, add the entire library). 25 ng of 4CAST Library is around 120 fmols.

42.3 Bring up the 100 - 120 fmols of library to 32 µL with the elution buffer (EB).

42.4 After 00:20:00 at Room temperature, gently insert the flow cell into one of the ports of the P2. The lights will turn blue if inserted correctly, and red if inserted incorrectly.

42.5 In a 1.5 Eppendorf tube, mix the following to create the flow cell priming mix:

| | A | B |
|---|---|---|
| | **Reagent** | **Volume** |
| | Flow Cell Flush (FCF) | 1170 uL |

| | A | B |
|---|---|---|
| | Flow Cell Tether (FCT) | 30 uL |
| | Total | 1200 uL |

42.6 Set a P1000 pipette to 200. Open the inlet port and place the tip inside the port. Turn the dial until it shows 220-230. A small amount of buffer should enter the tip. Discard the tip and buffer.

42.7 Load 500 µL of the flow cell priming mix into the inlet port. Load by slowly twisting the dial in the counterclockwise direction. Wait 00:05:00 . 5m

42.8 While the priming mix is incubating, prepare the library:

| | A | B |
|---|---|---|
| | **Reagent** | **Volume** |
| | Sequencing Buffer (SB) | 100 uL |
| | Library Beads (LIB) | 68 uL |
| | DNA Library | 32 uL |
| | **Total** | **200 uL** |

42.9 After the 5-minute incubation, add another 500 µL of flow cell priming mix to the inlet port.

42.10 Immediately add the 200 µL of the library slowly into the inlet port.

42.11 Close the inlet port and wait for the library to incubate for 00:10:00 before starting any sequencing. 10m

## 43 Flow cell wash

43.1 A flow cell wash should be done as soon as possible after the sequencing run is complete.

43.2 Place the Wash Mix (WMX) On ice, and thaw the Wash Diluent (DIL) at Room temperature.

43.3 Vortex the Wash Diluent thoroughly.

43.4 Make the Flow Cell Wash Mix by adding the following to a 1.5ml Eppendorf tube:

| | A | B |
|---|---|---|
| | **Reagent** | **Volume** |
| | Wash Diluent (DIL) | 398 uL |
| | Wash Mix (WMX) | 2 uL |

43.5 Pipette to mix and place On ice.

43.6 Stop the sequencing run on Minknow, and leave the flow cell in the port.

43.7 Make sure the inlet port is closed, and remove waste from port 2 or port 3.

43.8 Open the inlet port and set the P1000 to 200. Remove the air bubble by turning the wheel until it reaches 220-230, and a small volume of buffer enters the pipette tip.

43.9 Load 400 µL of the Flow Cell Wash Mix into the inlet port. Incubate for 01:00:00. 1h

43.10 After 1 hour, remove the waste again from ports 2 or 3. Make sure the inlet port is closed.

43.11 Remove the air bubble if it is present after removing the waste. Then add 500 µL of Storage Buffer (S). The flow cell can now be placed back into a 4 °C refrigerator for long-term storage. Incubate the flow cell at least Overnight before doing another flow cell check.