DyNAbind DEL Kit User Guide

On behalf of our entire team at DyNAbind, we thank you for purchasing this DNA-Encoded Library kit! Inside, over 370,000 fragment structure combinations are ready to be deployed against your target protein. This kit has been designed to be usable by nearly anyone with common biochemistry lab experience. However, you will need the following skills and equipment:

- Benchtop centrifuge
- Standard PCR
- Realtime PCR
- Gel Electrophoresis and Purification
- Nanodrop or other spectrophotometer for optical DNA quantification

In addition, certain PCR primers are required for working up the results of the selections. **Please carefully read this entire document before beginning to ensure that the necessary reagents, primers and equipment are available.**

Here is an overview of the process. First, protein will be immobilized on solid support beads and selection will be carried out with the library. After completion of selection, the eluted and ligated DEL samples must be prepared for sequencing to reveal the identities of the top binding compounds. First, real-time PCR is used to determine the appropriate number of PCR cycles for workup. Next, traditional PCR is used to amplify the selected DNA codes and add some necessary DNA for Illumina sequencing, including the Molecular Identifier (MID) DNA tags which will identify each experiment (e.g. single- or dual-fragment, condition X or condition Y, etc.) in the sequencing data later. Then after gel electrophoresis, DNA is purified and a final PCR step is performed to prepare samples for sequencing. After a last gel electrophoresis and extraction, samples can be submitted for sequencing. Let's get started! We have provided two options in the protocol to allow for working with either a His-tagged (1a) or a biotinylated (1b) protein. Other methods of attaching a protein to beads can be used but may require additional optimization.

For each target and condition set used, a library aliquot should also be run against non-functionalized beads as a negative control. The software for analyzing the single-fragment data requires that a negative control be entered to filter the enrichment scores for significant matches. With a dual-fragment analysis, there are so many possible combinations that the recommended sequencing depth will not produce significant results for a negative control. Analysis of dual-fragment data will calculate the enrichment relative to the assumption of rare but uniform binding of the pairs. In this case, the negative control can be run as a sample to check excessive pair binding to your beads. It is recommended to collect both the single-fragment and the dual-fragment data, as having each dataset can help to validate the other dataset.

1a) Selection with the DEL using a His-tag protein

In this first stage, we'll immobilize a His-tagged protein on His-tag pulldown solid support beads and screen the library to select binding structures.

His-tag pulldown Bead Protocol

Prepare the following stocks of working buffers:

Important: these are neutral starting points for buffer composition. Adjust them to suitable pH and salt values for your protein. Also consider where possible ideal temperatures for stability of your protein during the selection process. Critical buffer components are indicated with bold text.

Note: When working with solid support beads, consult the manufacturer's specifications and adapt the protocol accordingly for, e.g. loading beads and pulling down with a magnet rack.

Note: While we strongly recommend acquiring both single- and dual-fragment data for your target, in the event that only single-fragment data is desired, omit steps 9-11 in the protocol below, as well as any dual-fragment indicated PCR steps later in the protocol.

- PBS: 50 mM sodium phosphate, 100 mM NaCl, pH 7.4
- Washing buffer (PBS-T): PBS supplemented with **0.05% TWEEN-20**
- DEL buffer (PBS-T-HS): PBS supplemented with **0.05% TWEEN-20** and **0.01 mg/ml herring sperm DNA**
- Elution buffer: **360 mM Imidazole**, dissolved in 50 mM Tris-HCl pH 8.0, 150 mM NaCl and 0.05% Tween-20

1. Prepare DEL working solution by dissolving the DEL pellet in 100 µL DEL Buffer, vortexing thoroughly to mix.
2. Place 10 to 25 µL of His-tag pulldown beads (for example holding up to 40 µg of 20 to 50 kDa protein) in a 1.5 mL protein low-binding microcentrifuge tube.
3. Wash with 1 mL Washing Buffer, pull the beads down in a magnetic rack and carefully remove the supernatant without touching the beads. Repeat two more times.
4. Prepare 100 µL of His-tagged protein solution in PBS. Protein amount should be at 1.5 times excess the bead loading capacity.
5. Resuspend beads in the protein solution in a protein low-binding microcentrifuge tube and incubate for 30 minutes with gentle rocking in a mixer, then carefully remove the supernatant without touching the beads.
6. Add 200 µl Washing Buffer, carefully transfer the beads to a 1.5 mL DNA low binding microcentrifuge tube, pull the beads down in a magnetic rack and carefully remove the supernatant.
7. Resuspend the beads in 100 µL DEL solution and incubate the tube for 2 hours with gentle rocking in a mixer.
8. Remove DEL solution and wash three times with 200 µL Washing Buffer, pulling the beads down in a magnetic rack and carefully removing supernatant each time. Change the pipette tip and transfer to a new DNA low binding tube for each washing step.
9. Add T4 DNA ligase (400 units final volume) with 1 mM ATP and NEB2 buffer to a final volume of 20 µl, then allow to ligate overnight at 16°C with gentle rocking in a mixer.
   Important: do not deactivate the ligase after this step

10. Add Klenow that has 5'→3' polymerase activity but lacks both 5'→3' and 3'→5' exonuclease activity to a final concentration of 10 Units and dNTPs to a final concentration of 40 µM and incubate for 3 hours at 25° C with gentle rocking in a mixer (Do not use Klenow that has 5'→3' polymerase activity but only lacks 5'→3' exonuclease activity and still retains 3'→5' exonuclease activity).
11. Remove the enzyme solution via a magnetic rack.
12. Resuspend in of 100 µl Elution Buffer, then transfer to a new DNA low binding microcentrifuge tube.
13. Incubate for 10 minutes with gentle rocking in a mixer.
14. Pull down the beads down in a magnetic rack and transfer the supernatant containing the DNA-tags to a new DNA low binding tube.
15. Continue to section 2.

1b) Selection with the DEL using a biotinylated protein

In this first stage, we'll immobilize a biotinylated protein on streptavidin solid support beads and screen the library to select binding structures.

Streptavidin Sepharose Bead Protocol

Prepare the following stocks of working buffers:

Important: these are neutral starting points for buffer composition. Adjust them to suitable pH and salt values for your protein. Also consider where possible ideal temperatures for stability of your protein during the selection process. Critical buffer components are indicated with bold text.

Note: When working with solid support beads, consult the manufacturer's specifications and adapt the protocol accordingly for, e.g. loading beads and spinning down in a centrifuge.

Note: While we strongly recommend acquiring both single- and dual-fragment data for your target, in the event that only single-fragment data is desired, omit steps 10-12 in the protocol below, as well as any dual-fragment indicated PCR steps later in the protocol.

- PBS: 50 mM sodium phosphate, 100 mM NaCl, pH 7.4
- Washing buffer (PBS-T): PBS supplemented with **0.05% TWEEN-20**
- Blocking buffer: PBS supplemented with **0.05% TWEEN-20** and **100 µM D-biotin**
- DEL buffer (PBS-T-HS): PBS supplemented with **0.05% TWEEN-20** and **0.01 mg/ml herring sperm DNA**
- Elution buffer: **10 mM Tris**, **pH 8.5**, supplemented with **0.05% TWEEN-20**

1. Prepare DEL working solution by dissolving the DEL pellet in 100 µL DEL Buffer, vortexing thoroughly to mix and then briefly spinning down in a benchtop centrifuge.
2. Place 10 to 30 µL of streptavidin sepharose beads (for example holding up to 40 µg of 20 to 50 kDa protein) in a 1.5 mL protein low-binding microcentrifuge tube.
3. Wash with 1 mL Washing Buffer, centrifuge the beads down and carefully remove the supernatant without touching the beads. Repeat two more times.
4. Prepare 100 µL of biotinylated protein solution in PBS. Protein amount should be at 1.5 times excess the bead loading capacity.
5. Resuspend beads in the protein solution and incubate 30 minutes with gentle rocking in a mixer, then carefully remove the supernatant without touching the beads.

6. Add 200 µl Blocking Buffer, incubate for 5 minutes with gentle rocking in a mixer and then carefully remove the supernatant without touching the beads. Repeat once more.
7. Add 200 µl Washing Buffer, carefully transfer the beads to a 1.5 mL DNA low binding microcentrifuge tube, spin down in a centrifuge and carefully remove the supernatant.
8. Resuspend the beads in 100 µL DEL solution and incubate for 2 hours with gentle rocking in a mixer.
9. Remove DEL solution and wash three times with 200 µL Washing Buffer, spinning down in a benchtop centrifuge and carefully removing supernatant each time. Change the pipette tip and transfer to a new DNA low binding tube for each washing step.
10. Add T4 DNA ligase (400 units final volume) with 1 mM ATP and NEB2 buffer to a final volume of 20 µl, then allow to ligate overnight at 16°C with gentle rocking in a mixer.
    Important: do not deactivate the ligase after this step
11. Add Klenow that has 5'→3' polymerase activity but lacks both 5'→3' and 3'→5' exonuclease activity to a final concentration of 10 Units and dNTPs to a final concentration of 40 µM and incubate for 3 hours @ 25°C with gentle rocking in a mixer (Do not use Klenow that has 5'→3' polymerase activity but only lacks 5'→3' exonuclease activity and still retains 3'→5' exonuclease activity).
12. Remove the enzyme solutions and resuspend in 100 µl Elution Buffer, then transfer to a new DNA low binding microcentrifuge tube.
13. Heat at 95°C for 10 minutes.
14. Briefly spin the beads down in a benchtop centrifuge and transfer the supernatant containing the DNA-tags to a new DNA low binding tube.

2) Realtime PCR

As the amount of bound structures and recovered DNA can vary depending on the target and selection conditions, it's important to first quantify the DNA present so the following workup steps can be performed correctly.

For each condition tested the 3' single-fragment, 5' single-fragment and dual-fragment DNA codes must be analyzed separately, meaning three real-time PCR runs are performed per condition. For a list of all the primers used in the protocol, see Appendix B.

The following primers are required:

Primer	**Sequence**
A1	GGAGGTGTAGACGACAGAGT
A2	CCGACCGAATCCAGAAGG
A3	CAGATCGAGCAACTCCAC
A4	TGGTCTCAGCCGCCCTAT

Set up the first PCR mixture (Single Fragment - 5') according to the following table:

Component	**10 µl Reaction**
qPCR Master mix 2x	1x
Primer A1 5 µM	0.5 µM
Primer A2 5 µM	0.5 µM
Nuclease-free water	2 µL
Eluted selection DNA 1:10 diluted	1 µL

Set up the second PCR mixture (Single Fragment - 3') according to the following table:

Component	**10 µl Reaction**
qPCR Master mix 2x	1x
Primer A3 5 µM	0.5 µM
Primer A4 5 µM	0.5 µM
Nuclease-free water	2 µL
Eluted selection DNA 1:10 diluted	1 µL

Set up the third PCR mixture (Dual-Fragment) according to the following table:

(If only single-fragment data is desired, this PCR mixture can be omitted)

Component	**10 µl Reaction**
qPCR Master mix 2x	1x
Primer A2 5 µM	0.5 µM
Primer A4 5 µM	0.5 µM
Nuclease-free water	2 µL
Eluted selection DNA 1:10 diluted	1 µL

Run the real-time PCR according to the following program:

STEP	**TEMP**	**TIME**
1x	95°C	60 seconds
35 Cycles	98°C 62°C 72°C	15 seconds 30 seconds 30 seconds
(Optional meltcurve)	60-95°C	
10 min	72°C	

Once the real-time PCR is complete, the readout is used to determine the optimal cycle number for preparative PCR. The ideal cycle number is the highest one still clearly in the exponential PCR phase, as indicated in Figure 1 below.

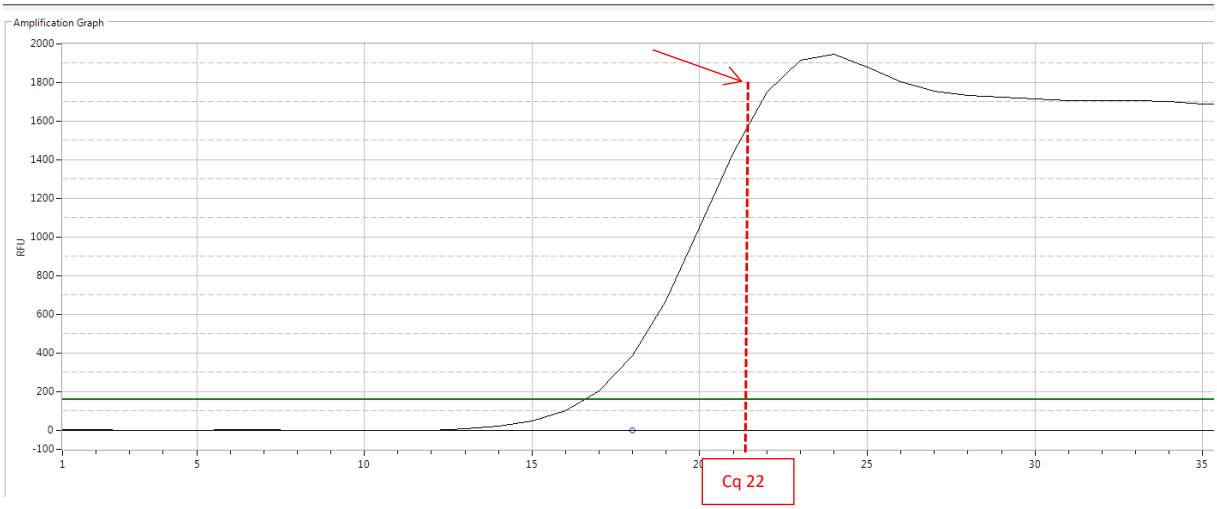

Figure 1. Determining the cycle number from qPCR for preparative PCR amplification.

3) Preparative PCR 1

Next, we'll perform preparative PCR on each selection elution to amplify the contents and introduce the MID (Molecular Identifier) tags which will be used to mark and separate the different experiments during analysis of the Illumina sequencing.

As in the real-time PCR step, three PCR runs will be performed, one for each selection condition and sublibrary (3' single-fragment, 5' single-fragment and dual-fragment DNA). Primers B1 and B2 need to be used as listed in the table below. They introduce one of the sequences for the flow cell binding (italics).

Primers B3, B4, and B5 introduce the multiplexing Read 1 Illumina sequence (underlined) and the MIDs (bold). Appendix A lists 15 MID sequences that are predesigned and can be used. For a list of all the primers used in the protocol including the MIDs, see Appendix B. After collecting the Illumina sequencing data, the MIDs will be entered in the software portal on the Sigma-Aldrich site for decoding the associated sequences. Using 15 MIDs would allow combining these samples for a single sequencing run depending on the Illumina instrument and depth of sequencing desired. It is recommended to collect a sequence length of 150 bp and a depth of at least 1 million reads per MID with the single read option. Having greater depth may be helpful for distinguishing hits but would increase the cost of the sequencing. Please do not introduce additional MIDs or barcodes between the Read 1 primer and the MID as this would decrease the reach of the sequencing and could make it difficult for the software to distinguish the fragment barcodes.

The following primers are required:

Primer	Sequence
B1	*CAAGCAGAAGACGGCATACGAGAT*GGAGGTGTAGACGACAGAGTATTTGAC
B2	*CAAGCAGAAGACGGCATACGAGAT*TGGTCTCAGCCGCCCTAT
B3	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**[MID1]**CCGACCGAATCCAGAAGG
B4	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**[MID2]**GGAGGTTGGGATCGCAG
B5	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**[MID3]**CCGACCGAATCCAGAAGG

For each condition tested, set up the following three PCR mixtures:

Set up the first PCR mixture (Single-Fragment - 5') according to the following table:

Component	50 µl Reaction
Nuclease-free water	to 50 µl
5X HF PCR Buffer	1x
10 mM dNTPs	250 µM
Primer B1 5 µM	0.6 µM
Primer B3 (w/ MID1) 5 µM	0.6 µM
Eluted Selection DNA 1:10 diluted	1 µL
High Fidelity Polymerase	1 U

Set up the second PCR mixture (Single-Fragment - 3') according to the following table:

Component	**50 µl Reaction**
Nuclease-free water	to 50 µl
5X HF PCR Buffer	1x
10 mM dNTPs	250 µM
Primer B2 5 µM	0.6 µM
Primer B4 (w/ MID2) 5 µM	0.6 µM
Eluted Selection DNA 1:10 diluted	1 µL
High Fidelity Polymerase	1 U

Set up the third PCR mixture (Dual-Fragment) according to the following table:

(If only single-fragment data is desired, this PCR mixture can be omitted)

Component	**50 µl Reaction**
Nuclease-free water	to 50 µl
5X HF PCR Buffer	1x
10 mM dNTPs	250 µM
Primer B2 5 µM	0.6 µM
Primer B5 (w/ MID3) 5 µM	0.6 µM
Eluted Selection DNA 1:10 diluted	1 µL
High Fidelity Polymerase	1 U

Run the PCR according to the following program, using the cycle number determined previously by the real-time PCR experiment:

STEP	TEMP	TIME
Initial Denaturation	98°C	60 seconds
15-25 Cycles	98°C 62°C 72°C	30 seconds 60 seconds 30 seconds
Final Extension	72°C	10 minutes
Hold	4°C	

4) Agarose Gel Electrophoresis

Next, the PCR product is purified via agarose gel electrophoresis.

A 2.0% agarose gel should be used with either TAE or TBE buffer. Use a gel that can accommodate all 50 µL of the PCR amplification product. Use a DNA ladder covering a 100 to 500 bp range to track the product bands.

Expected product sizes are:

- 3’ single-fragment DNA – 144 nt
- 5’ single-fragment DNA – 146 nt
- Ligated dual-fragment DNA – 209 nt

5) Agarose Gel Purification

The next step is to extract the purified PCR products from the agarose gel. This step can be performed with any agarose purification kit you prefer.

Tip: After dissolving the agarose gel in your kit’s dissolving buffer, mix the dissolved sample with isopropanol 1:1 for better DNA yield.

Afterward, quantify each sample via Nanodrop or equivalent spectrophotometer. You may need to dilute the sample if it is outside the linear range.

6) Preparative PCR 2

A final PCR is now performed to introduce the other Illumina sequence for flow cell binding (italicized) with primer C2. The underlined sequence in C2 overlaps with the Read 1 sequence in primers B3, B4, and B5. Primer C1 contains the other sequence for flow cell binding (italics) that was introduced with primers B1 and B2. The quantified 1st PCR product groups should be pooled together at equimolar concentration to a final concentration of 15 nM, e.g. all 5' single-fragment DNA pooled together, all 3' single-fragment DNA pooled together, and all dual-fragment DNA pooled together. At this stage all the PCR runs use the same primers, but each DNA group should still be run separately to promote even amplification.

The following primers are necessary:

Primer	**Sequence**
C1	*CAAGCAGAAGACGGCATACGAGAT*
C2	*AATGATACGGCGACCACCGAGATCTACAC*AGCGATATACACTCTTTCCCTACACGA

Prepare each PCR mixture according to the following table:

(If only single-fragment data is desired, the dual-fragment DNA PCR mixture can be omitted)

Component	**50 µl Reaction**	**Final Concentration**
Nuclease-free water	to 50 µl	
5X HF PCR Buffer	10 µl	1X
10 mM dNTPs	1.25 µl	250 µM
Primer C1 5 µM	6 µl	0.6 µM
Primer C2 5 µM	6 µl	0.6 µM
Template DNA 15 nM	1 µL	0.3 nM
HF Polymerase	0.5 µl	1.0 units

Run each PCR according to the following program:

STEP	TEMP	TIME
Initial Denaturation	98°C	60 seconds
20 Cycles	98°C 62°C 72°C	30 seconds 60 seconds 30 seconds
Final Extension	72°C	10 minutes
Hold	4°C	

7) Agarose Gel Electrophoresis

Next the amplified sequencing-ready constructs must be separated with another agarose gel electrophoresis run.

A 2.0% agarose gel should be used with either TAE or TBE buffer. Use a gel that can accommodate all 50 µL of the PCR amplification product. Use a DNA ladder covering a 100 to 500 bp range to track the product bands.

Expected product sizes are:

- 3' single-fragment DNA – 185 nt
- 5' single-fragment DNA – 187 nt
- Ligated dual-fragment DNA – 250 nt

8) Agarose Gel Purification

Finally, the DNA constructs must be extracted from the agarose gel. As in step 5, follow the directions of your preferred agarose gel purification kit.

Tip: After dissolving the agarose gel in your kit's dissolving buffer, mix the dissolved sample with isopropanol 1:1 for better DNA yield.

Afterward, quantify each sample via Nanodrop or equivalent spectrophotometer. You may need to dilute the sample if it is outside the linear range.

Decide on the final pooling of samples based on the read depth that is desired (review section 3). Submit the DNA for Illumina sequencing. The resulting sequencing data can be loaded on our portal for analysis to determine hits. See the DEL NGS Analysis Site Use Guide for instructions on using the portal.

Appendix A: MIDs

These 15, 12-base pair MIDs have been designed with an equal representation of the 4 bases at each position. These can be used for the MIDs in primers B3, B4, and B5 in section 3.

TCGAACCTAGAC
TAGCACGAACTC
TCGCTGAGACTC
TATGATCTACTG
ATTGAGAGCGAG
AGAGGCGATGCG
ATACGCCCTTCT
AGTCCAAAGTAG
CGATCATATCGA
CGCGTATATCGC
CACACATCGAGC
GCGTCATAGTAT
GATCAGAGCATA
GAGTTTCTCGAT
GCTCGCGCTGAT

Appendix B: Primers

Here is a list of all of the primers required for the protocol. More details are offered in the protocol text, but here is a summary. Primers A1-A4 are used to quantify the DNA. Primers B1 and B2 are used to introduce one of the sequences for the flow cell binding (italics). Primers B3, B4, and B5 introduce the multiplexing Read 1 Illumina sequence (underlined) and the MIDs (bold). A final PCR protocol is now performed to introduce the other Illumina sequence for flow cell binding (italicized) with primer C2. The underlined sequence in C2 overlaps with the Read 1 sequence in primers B3, B4, and B5. Primer C1 contains the other sequence for flow cell binding (italics) that was introduced with primers B1 and B2. When ordering primers, using unformatted sequences may be preferable for some vendors. Appendix C contains the unformatted primers.

Primer	Sequence
A1	GGAGGTGTAGACGACAGAGT
A2	CCGACCGAATCCAGAAGG
A3	CAGATCGAGCAACTCCAC
A4	TGGTCTCAGCCGCCCTAT
B1	*CAAGCAGAAGACGGCATACGAGAT*GGAGGTGTAGACGACAGAGTATTTGAC
B2	*CAAGCAGAAGACGGCATACGAGAT*TGGTCTCAGCCGCCCTAT
B3 with MID1	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TCGAACCTAGAC**CCGACCGAATCCAGAAGG
B4 with MID2	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TAGCACGAACTC**GGAGGTTGGGATCGCAG
B5 with MID3	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TCGCTGAGACTC**CCGACCGAATCCAGAAGG
B3 with MID4	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TATGATCTACTG**CCGACCGAATCCAGAAGG
B4 with MID5	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ATTGAGAGCGAG**GGAGGTTGGGATCGCAG
B5 with MID6	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**AGAGGCGATGCG**CCGACCGAATCCAGAAGG
B3 with MID7	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ATACGCCCTTCT**CCGACCGAATCCAGAAGG
B4 with MID8	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**AGTCCAAAGTAG**GGAGGTTGGGATCGCAG
B5 with MID9	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CGATCATATCGA**CCGACCGAATCCAGAAGG
B3 with MID10	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CGCGTATATCGC**CCGACCGAATCCAGAAGG

B4 with MID11	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CACACATCGAGC**GGAGGTTGGGATCGCAG
B5 with MID12	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GCGTCATAGTAT**CCGACCGAATCCAGAAGG
B3 with MID13	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GATCAGAGCATA**CCGACCGAATCCAGAAGG
B4 with MID14	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GAGTTTCTCGAT**GGAGGTTGGGATCGCAG
B5 with MID15	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GCTCGCGCTGAT**CCGACCGAATCCAGAAGG
C1	*CAAGCAGAAGACGGCATACGAGAT*
C2	*AATGATACGGCGACCACCGAGATCTACAC*AGCGATATACACTCTTTCCCTACACGA

Appendix C: Primers Without Formatting

Here is a list of all of the primers required for the protocol. Formatting has been removed, which may be preferable when ordering.

Primer	Sequence
A1	GGAGGTGTAGACGACAGAGT
A2	CCGACCGAATCCAGAAGG
A3	CAGATCGAGCAACTCCAC
A4	TGGTCTCAGCCGCCCTAT
B1	CAAGCAGAAGACGGCATACGAGATGGAGGTGTAGACGACAGAGTATTTGAC
B2	CAAGCAGAAGACGGCATACGAGATTGGTCTCAGCCGCCCTAT
B3 with MID1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAACCTAGACCCGACCGAATCCAGAAGG
B4 with MID2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCACGAACTCGGAGGTTGGGATCGCAG
B5 with MID3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGCTGAGACTCCCGACCGAATCCAGAAGG
B3 with MID4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATGATCTACTGCCGACCGAATCCAGAAGG
B4 with MID5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGAGAGCGAGGGAGGTTGGGATCGCAG
B5 with MID6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAGGCGATGCGCCGACCGAATCCAGAAGG
B3 with MID7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATACGCCCTTCTCCGACCGAATCCAGAAGG
B4 with MID8	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTCCAAAGTAGGGAGGTTGGGATCGCAG
B5 with MID9	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATCATATCGACCGACCGAATCCAGAAGG
B3 with MID10	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCGTATATCGCCCGACCGAATCCAGAAGG
B4 with MID11	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACACATCGAGCGGAGGTTGGGATCGCAG
B5 with MID12	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGTCATAGTATCCGACCGAATCCAGAAGG
B3 with MID13	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCAGAGCATACCGACCGAATCCAGAAGG

B4 with MID14	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGTTTCTCGATGGAGGTTGGGATCGCAG
B5 with MID15	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCGCGCTGATCCGACCGAATCCAGAAGG
C1	CAAGCAGAAGACGGCATACGAGAT
C2	AATGATACGGCGACCACCGAGATCTACACAGCGATATACACTCTTTCCCTACACGA

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