**MCB 525, Fall 2016**

**1.5: Adaptor Ligation**

**Objective:** In this section, we will ligate partially double-stranded adaptors to the 36-bp DNA fragment from section 1.4. Both the T4 ligase enzyme and the ligation products are very fragile and temperature sensitive. Keep reactions on ice prior to and following ligation, and ***DO NOT vortex the ligase.***

**Protocol:**

1. Prepare an appropriate volume of partially double-stranded 2μM Adaptor 1 and 2 (in separate tubes) from 100 μM stock solutions of 5ILL-NN, 3ILL-NN, and Anti-ILL primers. Adaptor 1 will bind to one side of the 36 bp dsDNA fragment from Section 1.3. Adaptor 2 will bind to the other.

Adaptor 1: 5ILL-NN + Anti-ILL

Adaptor 2: 3ILL-NN + Anti-ILL

1. Anneal the adaptors by holding at room temperature for 10 minutes.
2. Prepare a master mix for 2.1 ligation reactions based on the following recipe for a single reaction:

Nuclease-free H2O 24.0 μL

10 mM ATP (not dATP) 1.0 μL

10X T4 ligase buffer 4.0 μL

2 μM Adaptor 1 5.0 μL

2 μM Adaptor 2 5.0 μL

T4 DNA ligase 1.0 μL (NEB #M0202)

1. Combine 40 μL master mix with remaining 10 μL of digested DNA. Incubate at 16°C for at least one hour or overnight. Store on ice after ligation.

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*The Meyer lab protocol gives the following sequences for the primers used to make the adaptors, where N represents a mix of A,G,C, and T, and InvdT is an inverted T that prevents extension during PCR by DNA polymerase:*

*5ILL-NN: 5’-* CTACACGACG CTCTTCCGAT CTNN *-3’*

*3ILL-NN: 5’-* CAGACGTGTG CTCTTCCGAT CTNN *-3’*

*Anti-ILL: 5’-* AGATCGGAAG AGC(InvdT)-*3’*

*The protocol also includes the following primers for “reduced tag representation”:*

*5ILL-NC: 5’-* CTACACGACG CTCTTCCGAT CTNC -*3’*

*3ILL-NC: 5’-* CAGACGTGTG CTCTTCCGAT CTNC *-3’*

1. *What would be the effect of using the 5ILL-NC/3ILL-NC primers rather than the 5ILL-NN/3ILL-NN primers and why might a researcher want to do this?*