**MCB 525, Fall 2016**

**1.3: Digestion with type IIb restriction enzyme**

**Objective:** For preparation of genomic libraries, we follow the 2bRAD library preparation protocol developed by the Meyer lab at OSU (http://people.oregonstate.edu/~meyere/docs/2bRAD\_11Aug2015.pdf)[[1]](#footnote-1). The first step of the 2bRAD library preparation is to cut small 36-bp fragments from genomic DNA. We will use the type IIb restriction enzyme BcgI, which recognizes the following site:

**Protocol:**

1. Prepare a BcgI digestion master mix for 2.1 reactions based on the recipe below for a single reaction. Add reagents in the order listed. For template DNA you will use the DNA you concentrated earlier, as well as an additional sample that your TA will provide. **Hold everything on ice unless otherwise noted.**

Nuclease-free water 1.5 μL

10X NEBuffer 3.1 1.2 μL

150 μM S-adenosylmethionine (SAM) 0.8 μL

BcgI (2 Units μL-1) 0.5 μL (NEB #R0545S)

1. Combine 4 μL master mix in a PCR tube containing 8 μL of DNA. Mix by vortexing *very briefly* (touch tube to vortexer very briefly 2-3 times) or by flicking the tube.
2. Incubate at 37°C for at least 1 hr, or as long as O/N.
3. Heat-inactivate the BcgI by incubating the sample at 65°C for 20 minutes.
4. Hold samples on ice after heat-inactivation.

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1. *The fragment we have cut out has “sticky ends”. What would the ends of a fragment with “blunt ends” look like?*
2. *Different type IIb restrictions enzymes could be used instead of BcgI (e.g. AlfI, BsaXI). Why might one want to choose a different enzyme in a 2bRAD experiment?*
3. *What other modifications might need to be made to the protocol if one were to choose a different restriction enzyme?*

1. Wang, S., Meyer, E. & M. Matz (2012). 2bRAD: A simple and flexible method for genome-wide genotyping. *Nature Methods* 9: 808-810. [↑](#footnote-ref-1)