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Identification of Bread Wheat Seed-specific bZIP Transcription Factors Binding Sites by Genome-wide *In Vitro* Binding Analysis

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Basic leucine zipper (bZIPs) are dimeric sequence-specific transcription factors (TFs) that are unique to eukaryotes and play an important role in various biological processes including seed development and maturation, plant immunity and defense, and biotic and abiotic stress. In humans and other vertebrates bZIP TFs DNA binding sites gene promoters are well characterized but not much is known in plants. Problem is more pronounced in economically important crop like wheat. Recently an ordered draft sequence of hexaploid wheat (Triticum aestivum) genome and transcriptome analysis has opened new opportunities to study the roles of TFs proteins in gene regulation in wheat. Particularly, we are interested in analyzing the structure and functions of bZIP TFs of wheat. In last few years there has been a spurt in breakthrough technologies that identify TF-binding sites in whole genome (e.g. PBM, ChIP-seq, Bind-n-seq/DAP-seq). The new protocols are helping in identify hitherto unknown binding sites of TFs both *in vivo* and in vitro. We have cloned seed- specific bZIPs and adapted high-throughput bind-n-seq methodology to identify their genome-wide binding sites. Bind-n-Seq is a simple and robust method in which bZIPs are incubated with bar coded random oligonucleotides libraries (70 mer) with random binding sequences. Pure bZIP bound oligonucleotides are isolated and are sequenced using illumina platform. Based on abundance binding sites are scored. One of significant advantage of bind-n-seq over other method is that large numbers of binding sites are captured for each bZIP that is used in constructing transcription factor binding landscape.