Double DAP-seq – A New Technical Platform for Studying Synergistic DNA binding of Interacting Transcription Factors

Background

- Sequence-specific transcription factors (TF) regulate the expression of their target genes by recognizing short DNA sequences known as transcription factor binding sites (TFBS). Most TFs work within a complex or interact with other proteins to regulate gene expression *in vivo*; and homo- and heterodimerization are important features of DNA recognition and regulatory function for many TFs.
- Dimerization of BASIC LEUCINE ZIPPER (bZIP) TFs are critical for their functions, but the molecular mechanism underlying the DNA binding and functional specificity of homo- or heterodimers is elusive.

Approach

- This study extends the previously established high-throughout TF-DNA binding assay, DAP-seq (DNA Affinity Purification and sequencing), to develop a new technical platform (double DAP-seq, dDAP-seq) that enables to study synergistic DNA binding of interacting TFs – specifically the role of functional TF heterodimers.
- Twenty pairs of C/S1 bZIP heterodimers and S1 homodimers in Arabidopsis were profiled and compared using dDAP-seq.

Results

- Heterodimerization significantly expands the DNA binding preferences of these TFs.
- Analysis of dDAP-seq binding sites reveals the function of bZIP9 in abscisic acid response and the role of bZIP53 heterodimer-specific binding in seed maturation.
- The C/S1 heterodimers show distinct preferences for the ACGT elements recognized by plant bZIPs and motifs resembling the yeast GCN4 cis-elements.

Significance

- This study establishes a new technical platform enabling determination of functional heterodimer targets based on hetero-TF-TF DNA binding specificities, which is essential for defining combinatorial gene regulation by interacting TFs.

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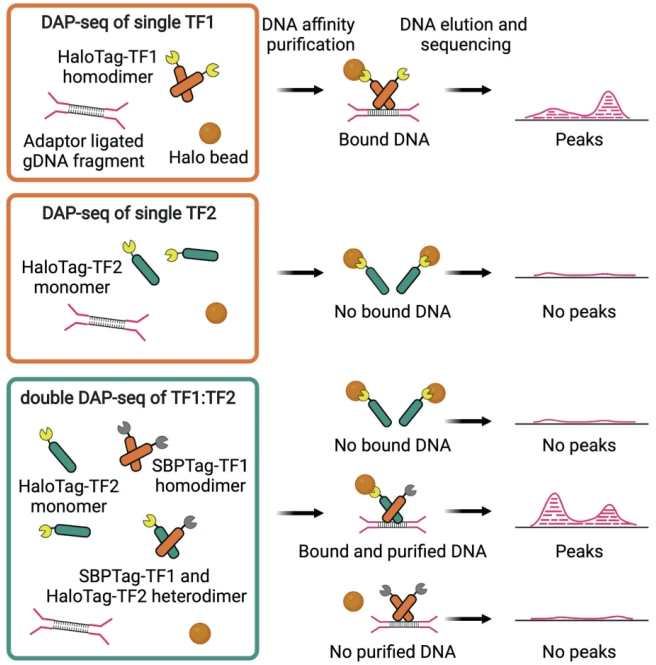

Schematic of DAP- and dDAP-seq.

Top panel: in DAP-seq, in vitro expressed HaloTag-fused TF (HaloTag-TF1) forms homodimers that bind to genomic DNA (gDNA) fragments ligated to Illumina-compatible sequencing adapters. The TF-DNA complex is purified by HaloTag ligand-coupled magnetic beads, from which bound gDNA is eluted and sequenced. Mapping the sequenced reads to the reference genome allows identification of TF binding location as peak regions of significant read enrichment.

Middle panel: Performing DAP-seq for a TF that cannot bind DNA by itself does not produce any peaks.

Bottom panel: in dDAP-seq, two TFs are fused to SBPTag and HaloTag separately, SBPTag-TF1 and HaloTag-TF2, and co-expressed in vitro and allowed to form heterodimers. HaloTag ligand-coupled magnetic beads purify the complex of SBPTag-TF1:HaloTag-TF2 with the bound DNA. Although the beads can pull down the HaloTag-TF2 monomer, no peaks will be detected for them without the bound DNA.