# CrY2H-seq: a massively-multiplexed assay for deep coverage interactome mapping

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## Editorial Summary

CrY2H-seq, a Cre recombinase reporter-mediated yeast two-hybrid method coupled with next-generation sequencing, enables ultra-high-throughput screening of transcription factor interactions in *Arabidopsis thaliana*.


## ABSTRACT

Broad-scale protein-protein interaction mapping is a major challenge given the cost, time, and sensitivity constraints of existing technologies. Here, we present a massively-multiplexed yeast two-hybrid method, CrY2H-seq, that uses a Cre recombinase interaction reporter to intracellularly fuse the coding sequences of two interacting proteins, and next-generation DNA sequencing to identify these interactions *en masse*. We applied CrY2H-seq to investigate sparsely annotated combinatorial interactions among *Arabidopsis thaliana* transcription factors. By performing ten independent CrY2H-seq screens each testing 3.6 million interaction combinations, we report a deep coverage network of 8,577 interactions among 1,453 transcription factors, demonstrating CrY2H-seq's high capacity, efficiency, and sensitivity. In addition to recapitulating one third of previously reported interactions derived from diverse methods, we expand the number of known plant transcription factor interactions by three-fold in a resource we call AtTFIN-1, revealing previously unknown family-specific interaction module associations with plant reproductive development, root architecture, and circadian coordination.