

INTRODUCTION

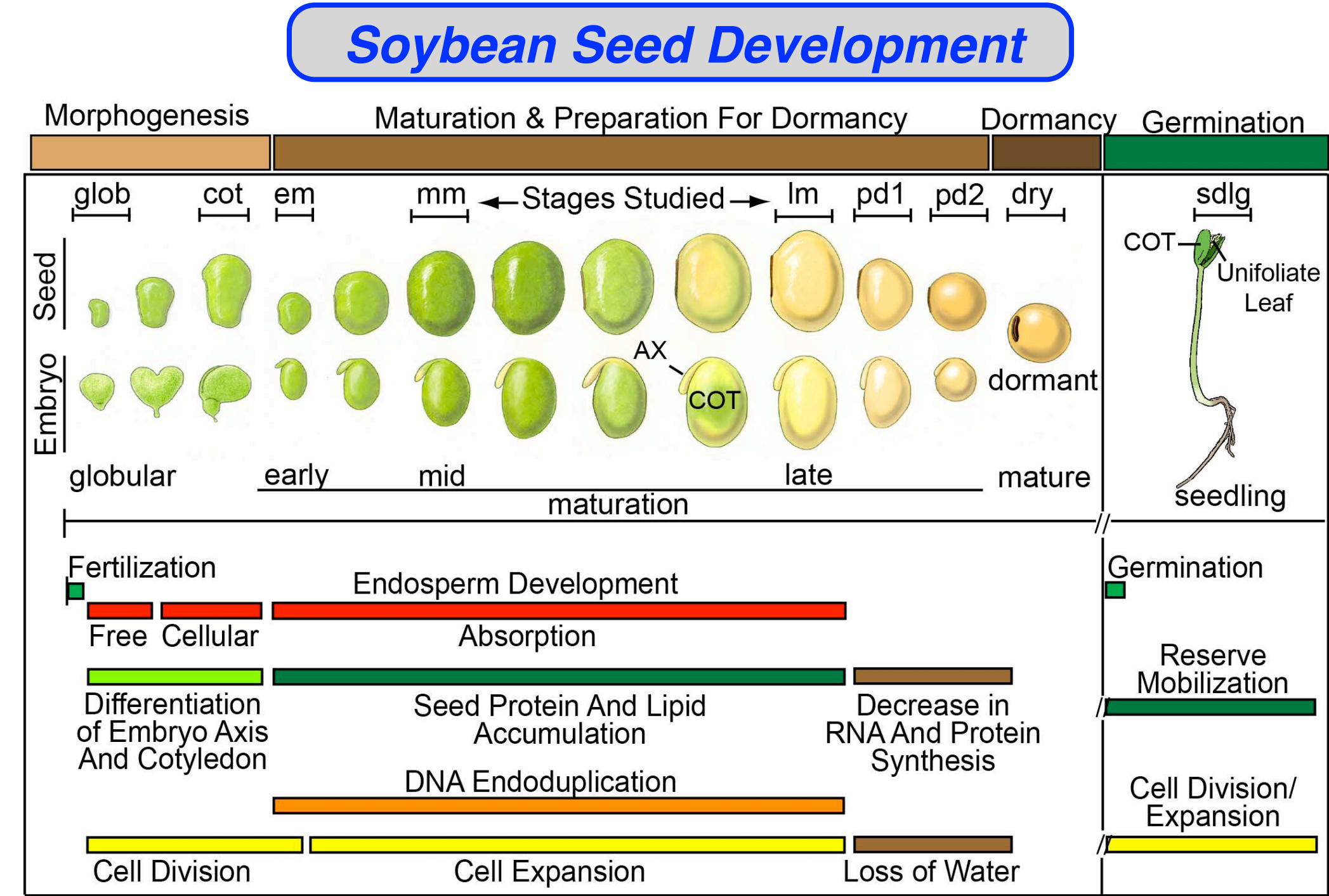


Figure 1. Major Events in Soybean Seed Development. glob, hrt, cot, em, mm, lm, pd, and dry refer to globular, heart, cotyledon, early maturation, late maturation, pre-dormancy, and dormant seed stages, respectively. COT and sdlg refer to cotyledon and seedling, respectively.

Major Questions Being Addressed In Soybean Seed Development

- What are the targets of seed region- & subregion-specific transcription factors?
- What processes do seed region- and subregion-specific transcription factors regulate?
- What cis-regulatory motifs do seed region- and subregion-specific transcription factors interact with?
- Are seed region- and subregion-specific transcription factor targets and interacting cis-regulatory modules conserved between different plant species?
- What are the gene networks required to program seed differentiation and maturation?

RESEARCH PLAN & STRATEGY

- Identification of Soybean Seed Subregion-Specific Transcription Factor Targets and Control DNA Motifs.** ChIP-Seq experiments will be used to identify targets of transcription factors that are specific for all major seed regions and subregions throughout development.
- Characterization of Soybean Seed Subregion Regulatory Genome.** ATAC-Seq experiments will be used to identify transcription factor-binding domains in the chromatin of seed subregions throughout development.
- Determining the DNA Binding Motifs for Soybean Seed Subregion-Specific Transcription Factors in vitro.** SELEX experiments will be carried out to define DNA sequences bound by subregion-specific transcription factors.
- Functional Validation of Target Genes Regulated by Subregion-Specific Transcription Factors.** The activity of endogenous transcription factors will be down-regulated in seed protoplasts to identify genes regulated directly by the subregion-specific transcription factors.

PROGRESS & RESOURCES

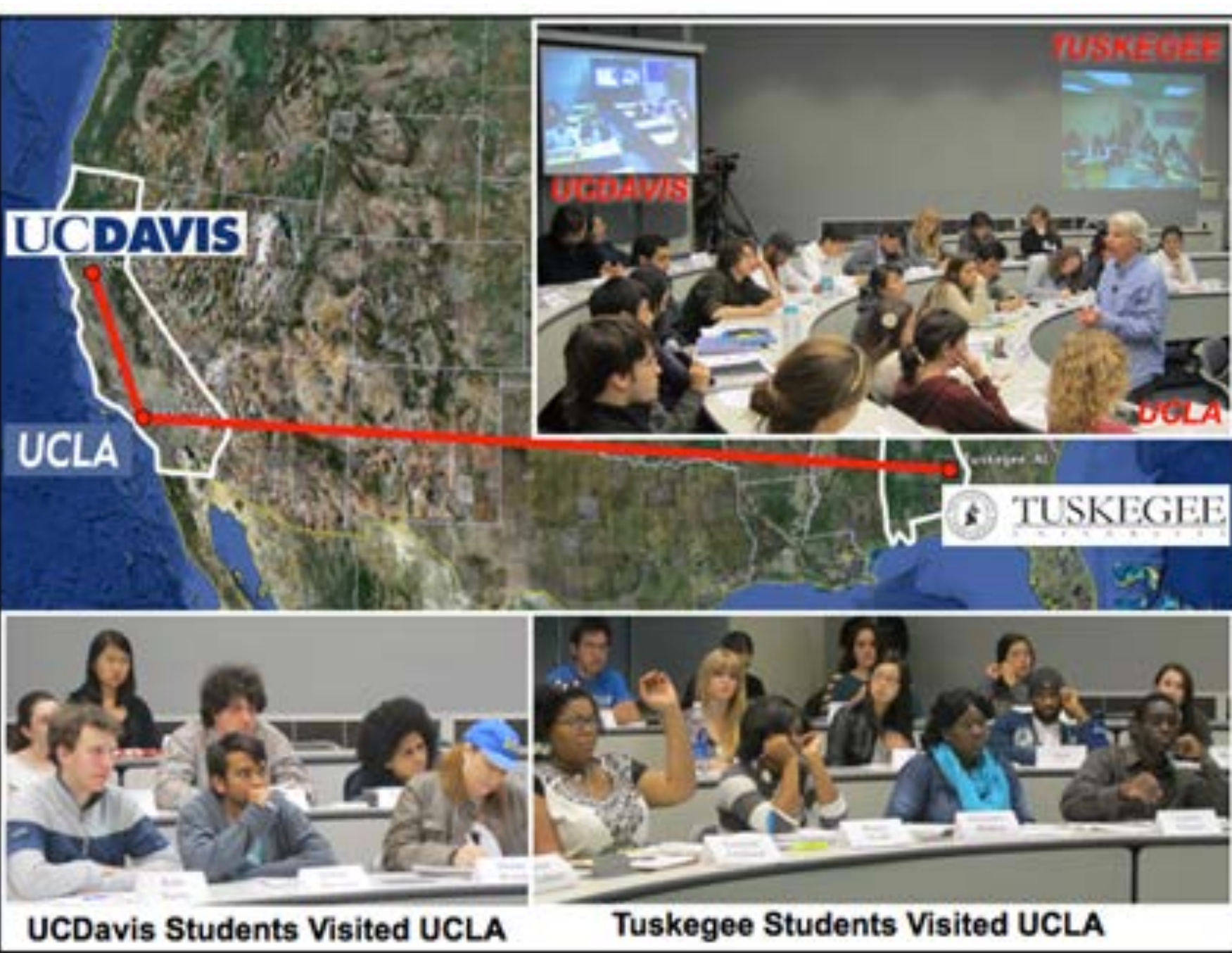
Summary of Soybean Seed ChIP-Seq Experiments

Title	GEO Accession	No. Datasets	No. Reads	No. Bases
Identification of LEC1 binding sites in soybean embryos at cotyledon, early-maturation and mid-maturation stages	GSE99882	16	429 M	21.7 Gb
Identification of AREB3 and bZIP67 binding sites in soybean early-maturation embryos	GSE101672	6	265 M	13.5 Gb
Identification of bZIP67 binding sites in soybean mid-maturation embryos	GSE101663	4	89 M	4.5 Gb
Identification of ABI3 binding sites in soybean mid-maturation embryos	GSE101648	8	171 M	8.8 Gb
Identification of ABI3 binding sites in soybean early-maturation embryos	GSE101649	4	112 M	5.7 Gb
Identification of LEC1 binding sites in Arabidopsis bent cotyledon stage seeds	GSE99587	4	41 M	2.1 Gb
Total		42	1,107 M	56.3 Gb

GEO, Gene Expression Omnibus; M, million; Gb, Gigabyte

EDUCATION AND OUTREACH

Broader Impacts - Using Long-Distance Learning To Teach Students Simultaneously At UCLA, UC Davis, And Tuskegee University



- "Genetic Engineering in Medicine, Agriculture, and the Law" was taught using a novel long-distance teaching approach that linked ~183 UCLA (HC70A), UC Davis (SAS70A) and Tuskegee (PLS550) students over the high speed internet-based video conferencing system into one "inter-campus" classroom. During the quarter UC Davis and Tuskegee students visited UCLA allowing in-person interactions with their UCLA counterparts.
- A novel online class, "Genetic Engineering and Society" (MCDB 70) was created in partnership with the UCLA School of Theater, Film, and Television. This online class provided a unique online educational experience for non-science students across the "globe" to learn first-hand about the importance of genetic engineering, genomics, and science to society.
- A lab class, *Gene Discovery Laboratory (HC70AL)* was taught to entering life science and non-science students to teach them about the "excitement of discovery."

CONCLUSIONS

- Genomic regions devoid of methylation (DMVs) are highly enriched in transcription factor genes in seeds suggesting that transcriptional processes regulate seed transcription factor genes without involvement of DNA methylation, and that understanding seed development requires identifying the networks that integrate transcription factor genes in seed genomes.
- LEC1, which acts at the highest level in the regulatory hierarchy controlling the maturation phase, regulates gene sets involved in distinct developmental processes at different development stages.
- LEC1 partners with different transcription factors leading to functionally distinct roles to regulate distinct gene sets at different stages of seed development. Consistent with this hypothesis, the upstream regions of each target gene set are overrepresented for different DNA motifs.
- A transient assay system has been developed to evaluate the functional consequences of down-regulating the expression of transcription factors.

RESULTS

Characterizing Regulatory Network That Operated During the Maturation Stage of Seed Development

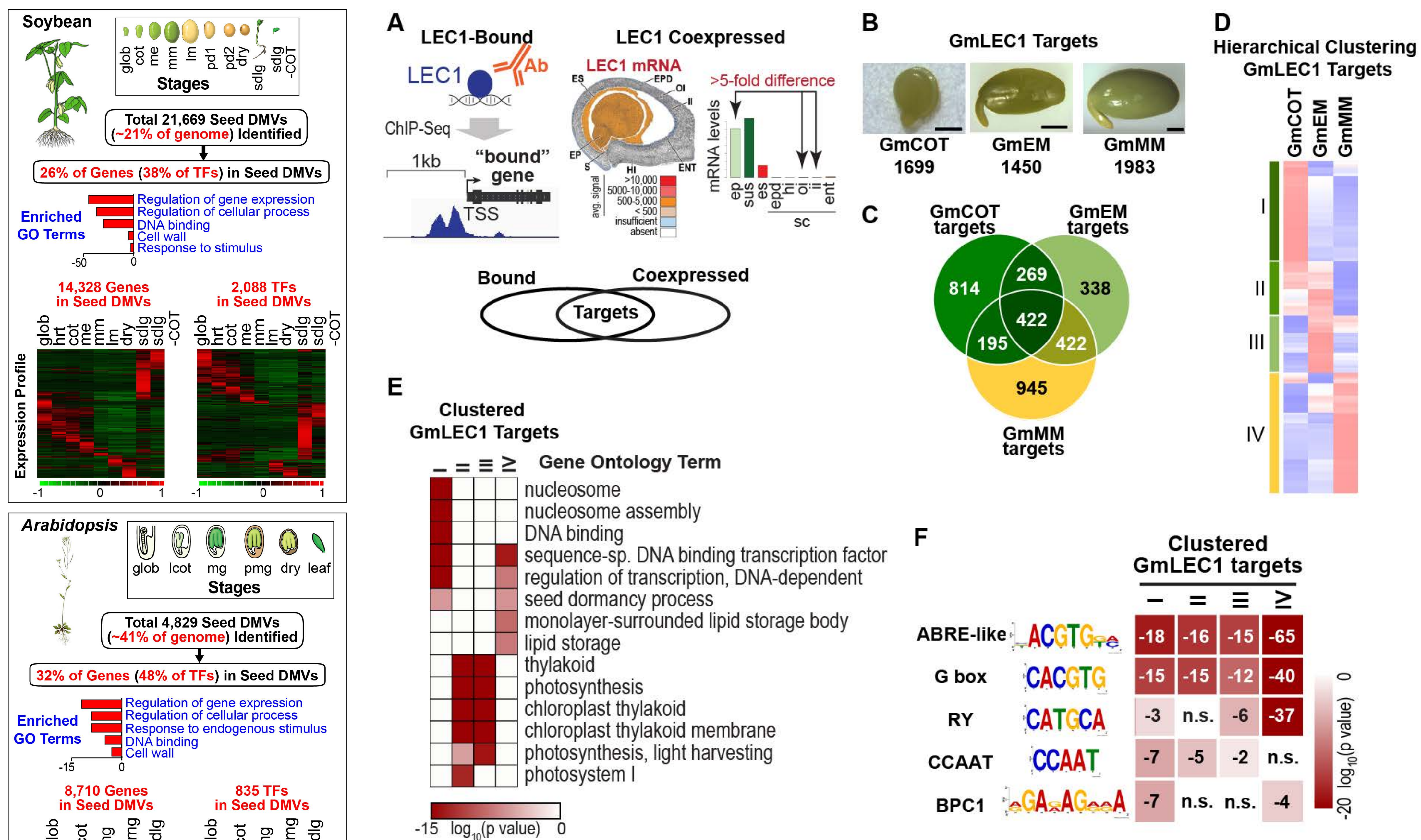


Figure 2. Genes Targeted by the LEC1 Transcription Factor During Soybean Seed Development. (A) Strategy used to identify LEC1 target genes. (B and C) The number of LEC1 target genes identified at three stages. (D) Clustering of mRNAs encoded by LEC1 target genes. Enriched GO terms (E) and DNA motifs (F) of LEC1 target genes from clusters in (D).

Figure 1. DNA Methylation Valleys (DMVs) in Soybean and Arabidopsis Seeds. Seed DMVs were identified by scanning soybean and Arabidopsis methylomes throughout seed development from fertilization through dormancy and germination with < 5% methylation levels. TFs, transcription factors.

Figure 3. Gene Regulatory Networks Within Early Maturation (EM) Stage Soybean Seeds. (A) The number of ABI3, bZIP67 and AREB3 target genes. (B) Overlap between LEC1, ABI3, bZIP67 and AREB3 target genes: (i) L Genes targeted exclusively by LEC1, (ii) LAR Genes targeted by LEC1 and AREB3, (iii) LARZ Genes targeted by LEC1, AREB3 and bZIP67, and (iv) LARZab genes targeted by all four transcription factors, including ABI3. Enriched GO terms (C) and DNA motifs (D) of gene sets in (B).

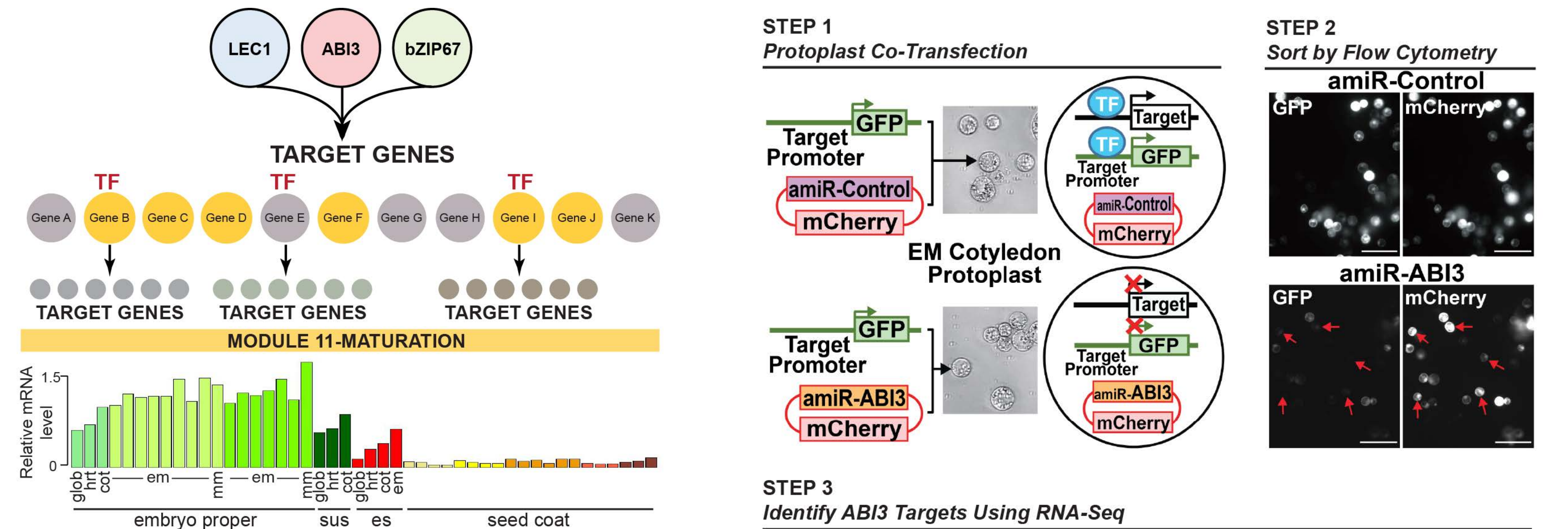
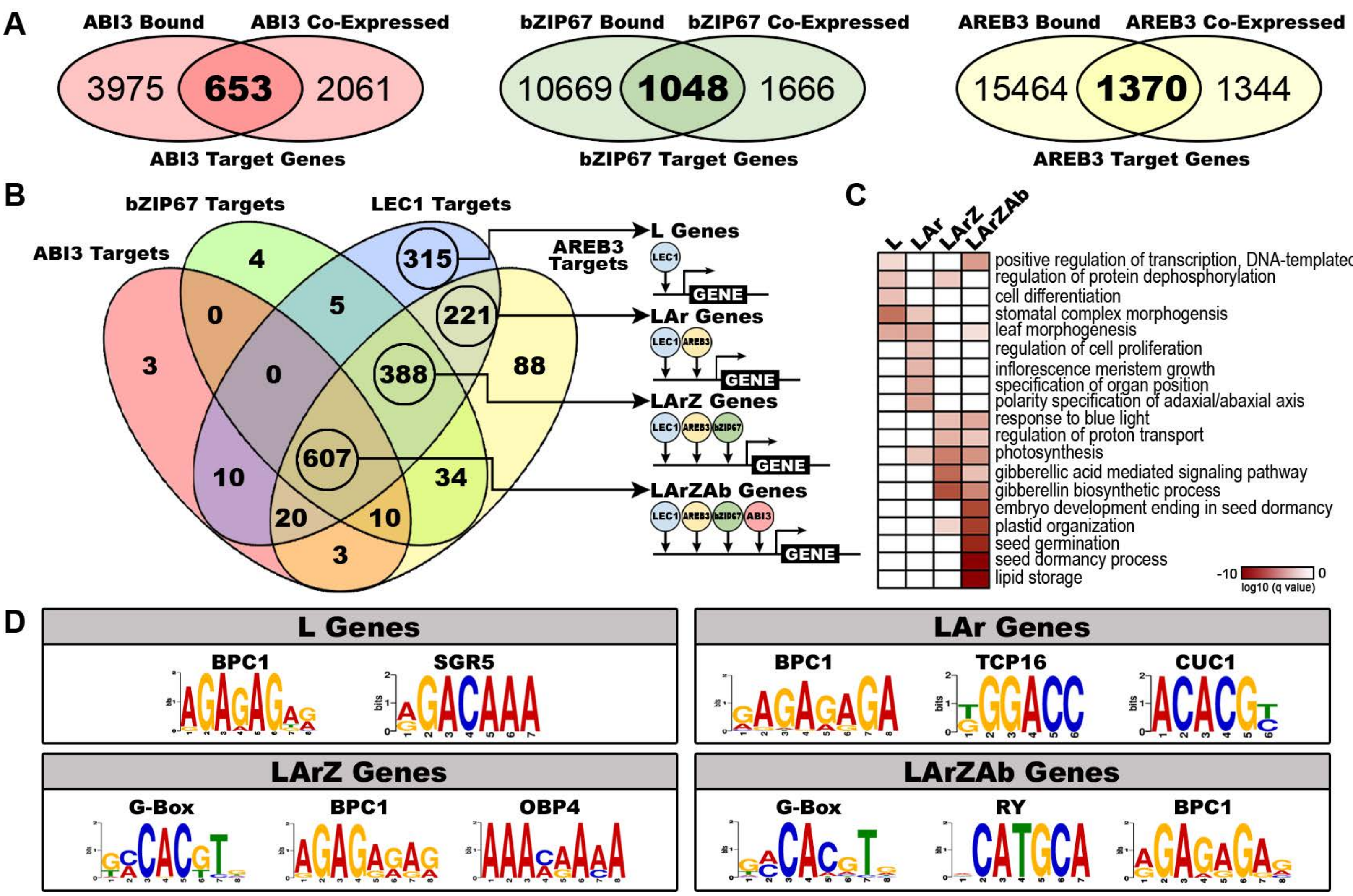


Figure 4. Using LEC1, ABI3, and bZIP67 As An Example To Demonstrate A Strategy For Identifying Gene Networks Controlling Soybean Seed Maturation. LEC1, ABI3, and BZIP67 target genes with expression patterns correlating with the maturation gene module 11.

Figure 5. Using Soybean Embryo Protoplasts to Identify ABI3 Targets. (Step 1) Embryo protoplasts were co-transfected with a fluorescent marker (mCherry), and either an artificial micro-RNA directed against ABI3 mRNA (amiR-ABI3) or a control micro-RNA (amiR-Control). (Step 2) Transfected protoplasts were collected using flow cytometry. (Step 3) RNA-Seq experiments identified three gene sets: ABI3-Bound and ABI3-Activated (A), ABI3-Bound, ABI3-Activated, and ABI3-Expressed (B), and ABI3-Bound and ABI3-Expressed (C). Enriched GO terms of three gene sets.

