Supplemental Figure S3. MPRA library cloning strategy. GFP, green fluorescent protein, minP, minimal promoter; *Sbf*1, *Eco*R1 restriction sites. The lenti vector pLS-mP is cut with *Sbf*I and *Eco*RI to remove the minimal promoter and GFP. The enhancer/barcode agilent array is amplified with adaptor primers, and these PCR products are cloned into the pLS-mP backbone using NEBuilder HiFi Assembly mix. Cloning disrupts the original *Sbf*I and *Eco*RI sites. This initial library can be sequenced to validate its accuracy and complexity. The library is then digested with *Sbf*I and *Eco*RI to re-insert the minimal promoter and GFP between the enhancer and the barcode with a sticky-end ligation.