Supplemental Table 1. Constructs delivered by Fast-TrACC.

| Construct  | MODULE A             | MODULE B             | MODULE C       | MODULE D             | VECTOR                  |
|-----------|----------------------|----------------------|----------------|----------------------|-------------------------|
| pRN62     | 35S:Cas9             | 35S:Non-Targeting gRNA | 35S:GFP       | --                   | BeYDV (pCambia T-DNA)  |
| pMM117    | 35S:Luciferase       | AtU6-PDS1 gRNA       | --             | --                   | BeYDV (pCambia T-DNA)  |
| pMKV60    | --                   | --                   | AtUb10:Luciferase | --                   | BeYDV (pCambia T-DNA)  |
| pMZ94     | --                   | AtU6:AtWUS gRNA      | CmILCV:Luciferase | --                   | BeYDV (pCambia T-DNA)  |
| pRN569    | --                   | --                   | 35S:Renilla & CmILCV:Firefly | -- | pCambia T-DNA |
| pRN570    | --                   | --                   | 35S:Renilla & AtHbcs:Firefly | -- | pCambia T-DNA |
| pRN591    | --                   | --                   | 35S:Renilla & SoStzS:Firefly | -- | pCambia T-DNA |
| pRN221    | 35S:Cas9             | AtU6-PDS1 gRNA       | --             | --                   | BeYDV (pCambia T-DNA)  |
| pRN417    | 35S:Cas9             | AtU6:PAPl gRNA1      | 35S:Luciferase | --                   | BeYDV (pCambia T-DNA)  |
| pRN418    | 35S:Cas9             | AtU6:PAPl gRNA2      | 35S:Luciferase | --                   | BeYDV (pCambia T-DNA)  |
| pRN419    | 35S:Cas9             | AtU6:PAPl gRNA3      | 35S:Luciferase | --                   | BeYDV (pCambia T-DNA)  |
| 35S:Cas9  | 35S:Cas9             | AtU6:ANTI Promoter gRNA | CmILCV:Luciferase | --       | BeYDV (pCambia T-DNA)  |
| pMM181    | 35S:Cas9             | AtU6:ANTI Promoter gRNA | CmILCV:Luciferase | -- | BeYDV (pCambia T-DNA)  |
| pMM182    | 35S:Cas9             | AtU6:ANTI Promoter gRNA | CmILCV:Luciferase | -- | ToLCV (pCambia T-DNA)  |
| pMM183    | 35S:Cas9             | AtU6:ANTI Promoter gRNA | CmILCV:Luciferase | -- | ToLCV (pCambia T-DNA)  |
| pMKV81    | 35S:Cas9             | AtUb10:ALS gRNA1 & 2 | AtUb10:Luciferase | -- | BeYDV (pCambia T-DNA)  |
### Supplemental Table 2. Sequencing Primers.

| Primer Name | Description | Sequence       |
|-------------|-------------|----------------|
| oRN652      | NbPAPI Rev Primer | GCCACTGTCAGCAGTCTGGCAGAGGTTG |
| oRN653      | NbPAPI Fwd Primer | ATGGGATTCAAGTAGGGATCA |
| oMKV108     | SIALS Fwd Amplification Primer | AGAAGGTTGAAGTACCACTGAGTTT |
| oMKV110     | SIALS Rev Amplification Primer | ATTAAGGAGAAATGTCGACCC |
| oCS1065     | SIALS Fwd Sequencing Primer | TGATTAGGACATGCGATTG |
| oCS1243     | SIALS Rev Sequencing Primer | GCCAACAGCCAGCGCAAATCA |

### NGS Amplicon Primers

| Primer Name | Description | Sequence |
|-------------|-------------|----------|
| oRN468      | NbPDS NGS Fwd Primer (Barcode 1) | ACACCTCTTCCCTACACGACGCTTCCGCATCTCAGGTTAAATTGAGTCGC |
| oRN469      | NbPDS NGS Fwd Primer (Barcode 2) | ACACCTCTTCCCTACACGACGCTTCCGCATCTCGTTGCGGTAATTGAGTCGC |
| oRN470      | NbPDS NGS Fwd Primer (Barcode 3) | ACACCTCTTCCCTACACGACGCTTCCGCATCTCCGAGTTAATTGAGTCGC |
| oRN471      | NbPDS NGS Fwd Primer (Barcode 4) | ACACCTCTTCCCTACACGACGCTTCCGCATCTCTCGAGTTAATTGAGTCGC |
| oRN472      | NbPDS NGS Fwd Primer (Barcode 5) | ACACCTCTTCCCTACACGACGCTTCCGCATCTGTCTGCGGTAATTGAGTCGC |
| oRN473      | NbPDS NGS Rev Primer (Barcode 1) | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN474      | NbPDS NGS Rev Primer (Barcode 2) | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN475      | NbPDS NGS Rev Primer (Barcode 3) | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN476      | NbPDS NGS Rev Primer (Barcode 4) | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN477      | NbPDS NGS Rev Primer (Barcode 5) | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN479      | Barcode Free NbPDS NGS Fwd | ACACCTCTTCCCTACACGACGCTTCCGCATCTCCGAGTTAATTGAGTCGC |
| oRN480      | Barcode Free NbPDS NGS Rev | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
| oRN501      | SIA1NT NGS Fwd Primer | ACACCTCTTCCCTACACGACGCTTCCGCATCTGGAGTTAATTGAGTCGC |
| oRN612      | SIA1NT NGS Rev Primer | GACTGAGAGTTCAAGGCTGCTTCCACCTGCAATAGTAGCTGAGCTGACATCAAATTC |
Supplemental Figure 1. Fast-TrACC Delivery of Transgenes. (a) For transgene delivery by Fast-TrACC, Agrobacterium cultures are grown and treated with AB:MES200 salt media to increase vir gene expression. (b) T-DNAs are designed to contain geminiviral replicons to increase DNA copy number as well as either a reporter (top) or gene editing reagents and a reporter (bottom). The treated bacterial culture is added to seedlings two days after germination in liquid media. Delivery of reagents, such as GFP (c-e) or luciferase (f-h) to whole seedlings can be performed in multiple species like Arabidopsis thaliana (c), Nicotiana benthamiana (d-e), habanero pepper (Capsicum chinense) (f), eggplant (Solanum melongena) (g) or canola (Brassica napus) (h).
Supplemental Figure 2. Determining the transient nature of Fast-TrACC delivery. Fast-TrACC was used to deliver a firefly luciferase reporter driven by either the 35S or AtUbi10 promoter to tomato seedlings. Luminescence serves as a proxy for gene expression from the delivered T-DNA, and the duration of luminescence was recorded. As seen with both 35S and AtUbi10, the luminescence signal is strongest 24 hours after co-culture. This signal drops substantially by 48 hours, and only small sectors of luminescence are observed 72 hours post co-culture. The sectors that remain after 72 hours are likely sites of transgene integration.
Supplemental Figure 3. Fast-TrACC delivery to various species to determine promoter efficacy. To determine how broadly Fast-TrACC can be applied across species, a firefly luciferase reporter, driven by one of three promoters (35S, AtUbi10, CmYLCV), was delivered to canola (Brassica napus), eggplant (Solanum melongena), and habanero pepper (Capsicum chinense) seedlings. Across the species, 35S:luciferase manifested strong luminescence, whereas AtUbi10:luciferase showed more modest expression. The CmYLCV promoter was more species-specific: the strongest expression was observed in eggplant seedlings, but essentially no expression was observed in either the canola or pepper seedlings.
Supplemental Figure 4. Normalized luminescence values from a dual luciferase assay. In order to directly compare activity of the CmYLCV, AtRbcs, and StSTLS promoters, a dual luciferase was implemented with firefly and Renilla luciferases. Two independent experiments (a-b, experiment one; c-d, experiment 2) were performed in N. benthamiana. Raw luminescence values were normalized to the background levels for the no luciferase controls to determine the ratio between the Renilla and firefly enzymes. Variability in the Renilla luciferase luminescence correspond to differential amounts of delivery across the seedlings, whereas the differences in the firefly luciferase values correspond to differing expression levels from the tested promoters. The values from the normalized firefly luciferase measurements are divided by the normalized Renilla luciferase measurements to give a relative luminescence unit (RLU) for comparison.

| Promoter Treatment | Normalized Firefly | Normalized Renilla |
|--------------------|--------------------|--------------------|
| RN569 (CmYLCV)     | 234.0363993        | 646.3191768        |
| RN570 (AtRbcs)     | 102.1347399        | 1086.951415        |
| RN571 (StSTLS)     | 29.3358311         | 1700.73103         |

| Promoter Treatment | Normalized Firefly | Normalized Renilla |
|--------------------|--------------------|--------------------|
| RN569 (CmYLCV)     | 944.1493645        | 1037.547211        |
| RN570 (AtRbcs)     | 21.5758153         | 1041.326504        |
| RN571 (StSTLS)     | 11.72038915        | 1788.561881        |