Table 2. Each part(s) and plasmid listed in the first column must be individually digested by the corresponding enzyme(s) listed in the second column (i.e. Plasmid 1 is digested with SmaI). The digested products can then be placed in the same reaction tube with the Gibson reagents added for the plasmid in column three to form. These plasmids are classified as plasmids made in a sequential Gibson reaction as the initial digested parts include a digested plasmid made from a Gibson reaction in Table 1.

Parts/Previously made plasmids to add	**Digest with**[1]	**Resulting plasmid (composition)**
Only CBH-containing Gene Cassettes		
1. Plasmid 1[1] 2. BBa_K3629012 (TrCBHII construct)	1. SmaI 2. SmaI and BamHI	Plasmid 14: Modified PfCBHI + TrCBHII + Nourseothricin[2]
1. Plasmid 2 2. BBa_K3629012 (TrCBHII construct)	1. SmaI 2. SmaI and BamHI	Plasmid 15: NcCBHI + TrCBHII + Nourseothricin
1. Plasmid 8 2. BBa_K3629014 (NcCBHI construct)	1. SmaI 2. NcoI and BamHI	Plasmid 16: Modified PfCBHI + NcCBHI + TrCBHII + Nourseothricin
1. Plasmid 15 2. BBa_K3629013 (Modified PfCBHI construct)	1. EcoRV 2. XhoI and EcoRV	Plasmid 17: Modified PfCBHI + NcCBHI + TrCBHII + Nourseothricin
1. Plasmid 9 2. BBa_K3629013 (Modified PfCBHI construct)	1. EcoRV 2. XhoI and EcoRV	Plasmid 18: Modified PfCBHI + NcCBHI + TrCBHII +

		Nourseothricin
Only EG-containing Gene Cassettes		
1. Plasmid 4 2. BBa_K3629017 (TrEGII construct)	1. SmaI 2. XhoI and BamHI	Plasmid 19: Modified TrEGI + TrEGII + Nourseothricin
CBH + EG Gene Cassettes		
1. Plasmid 16 2. BBa_K3629016 (Modified TrEGI construct) 3. BBa_K3629017 (TrEGII construct)	1. EcoRV 2. XhoI and SmaI 3. XhoI and EcoRV	Plasmid 20: Modified PfCBHI + NcCBHI + TrCBHII + Modified TrEGI +TrEGII + Nourseothricin
1. Plasmid 7 2. BBa_K3629016 (Modified TrEGI construct) 3. BBa_K3629017 (TrEGII construct)	1. EcoRV 2. XhoI and SmaI 3. XhoI and EcoRV	Plasmid 22: Modified PfCBHI + NcCBHI + TrCBHII + Modified TrEGI +TrEGII + Nourseothricin

[1]Digest with these enzymes to expose the appropriate Gibson homology sequence to create the desired plasmid in column three.

[2]Nourseothricin= Nourseothricin resistance gene