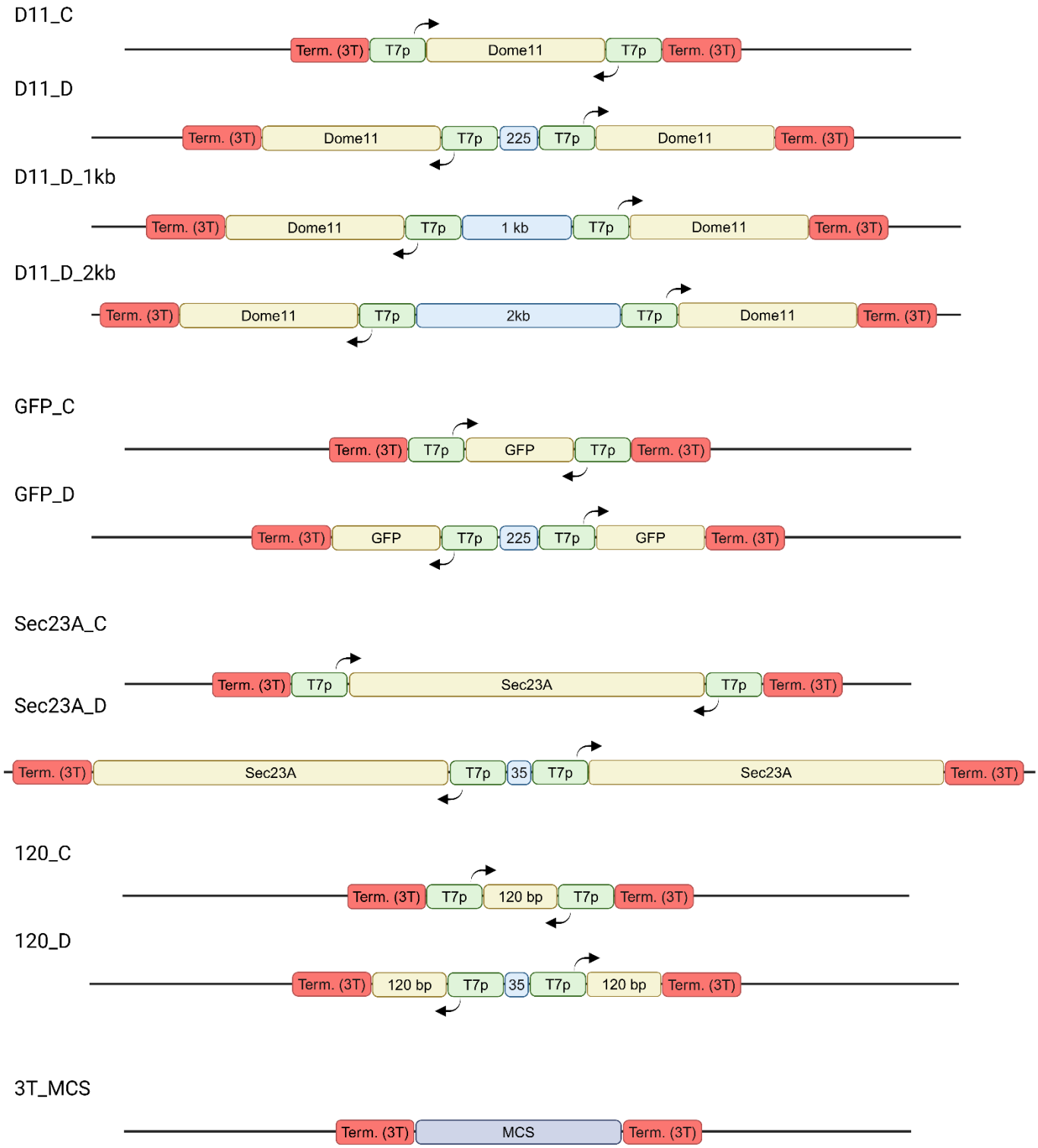
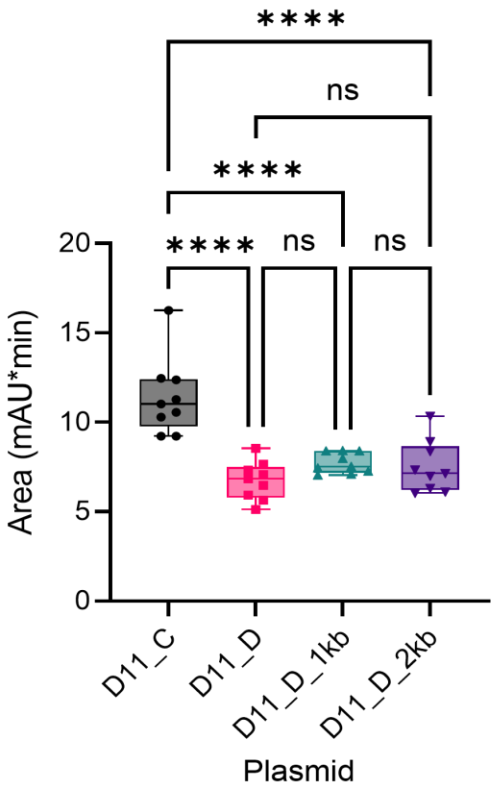



**Supplementary figure S1. Schematic diagram of all plasmid constructs used in this study.**

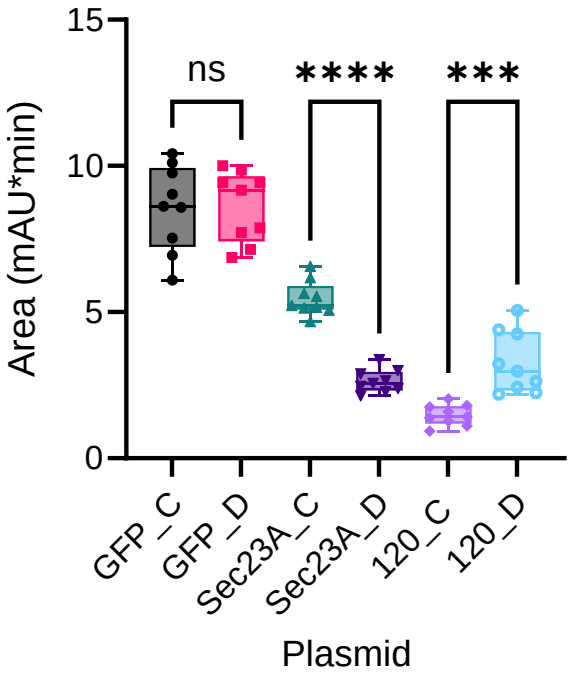
Visual representation of all 11 plasmid constructs used. The target dsRNA sequence name, orientation of T7 polymerases, transcriptional terminators and size of spacer region are annotated. Additionally, a multiple cloning site (MCS) is annotated for the cloning vector 3T_MCS. Created in BioRender. Dickman, M. (2025) https://BioRender.com/t9ofu4q

Supplementary figure 1



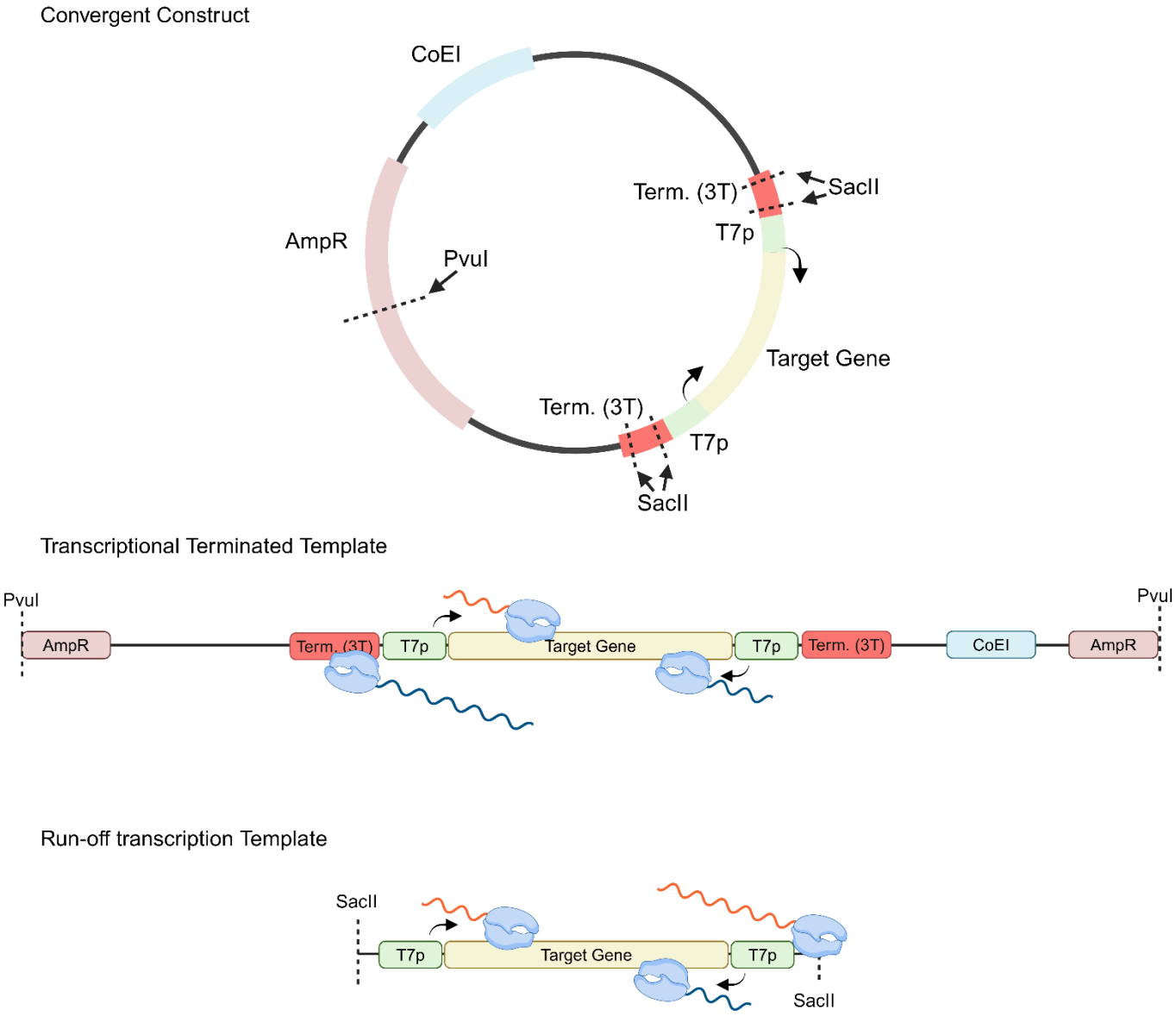

**Supplementary figure S2. Relative quantification of convergent and divergent synthesised Dome11 dsRNA *in vivo* within *E.coli*.** RNA extractions were performed in the absence of RNase T1. RNA samples were analysed using relative peak area (mAU*min). Significance was calculated using a one-way ANOVA with multiple comparisons. Convergent-produced Dome11 (pD11_C) demonstrates the highest relative yield of 11.41. Data is shown as box plots of triplicate technical replicates and are representative of three biological replicates; $p<0.05$ = (*), $p<0.01$ = (**), $p<0.001$ = (***), $p<0.0001$ (****).

Supplementary figure 2



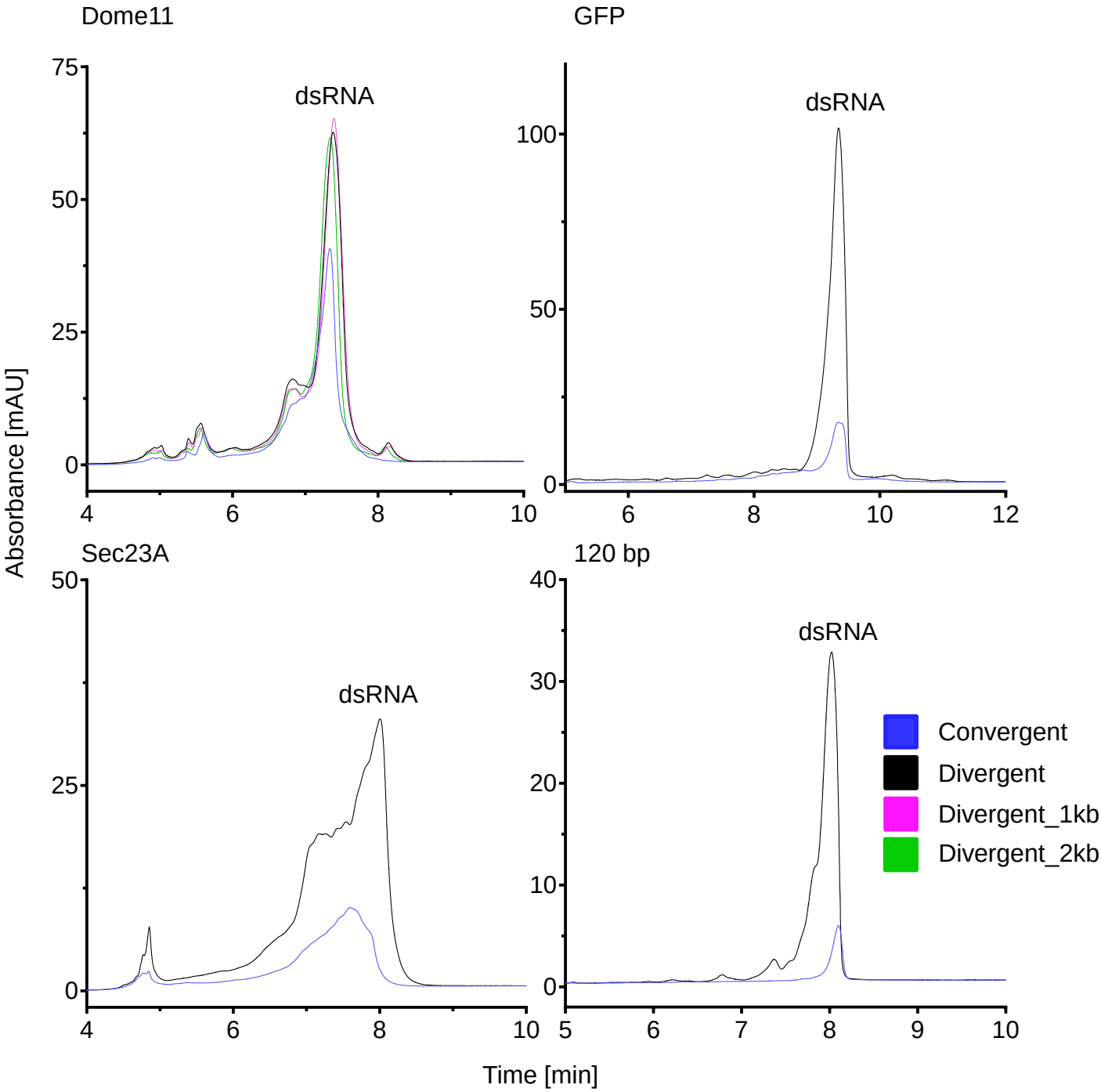

**Supplementary figure S3. Relative quantification of convergent and divergent synthesised GFP/Sec23A/120 bp dsRNA *in vivo* within *E.coli*.** RNA extractions were performed in the absence of RNase T1. RNA samples were analysed using relative peak area (mAU*min). Significance was calculated using unpaired T tests, for GFP, Sec23A and 120 bp RNA samples. No significant difference was noted between GFP constructs. Convergent-produced Sec23A (pSec23A_C) demonstrated the highest relative yield (5.46) between Sec23A constructs. Divergent-produced 120 bp (p120_D) demonstrated the highest relative yield (3.27) between 120 bp constructs. Data is shown as box plots of triplicate technical replicates and are representative of three biological replicates; $p<0.05$ = (*), $p<0.01$ = (**), $p<0.001$ = (***), $p<0.0001$ (****).

Supplementary figure 3



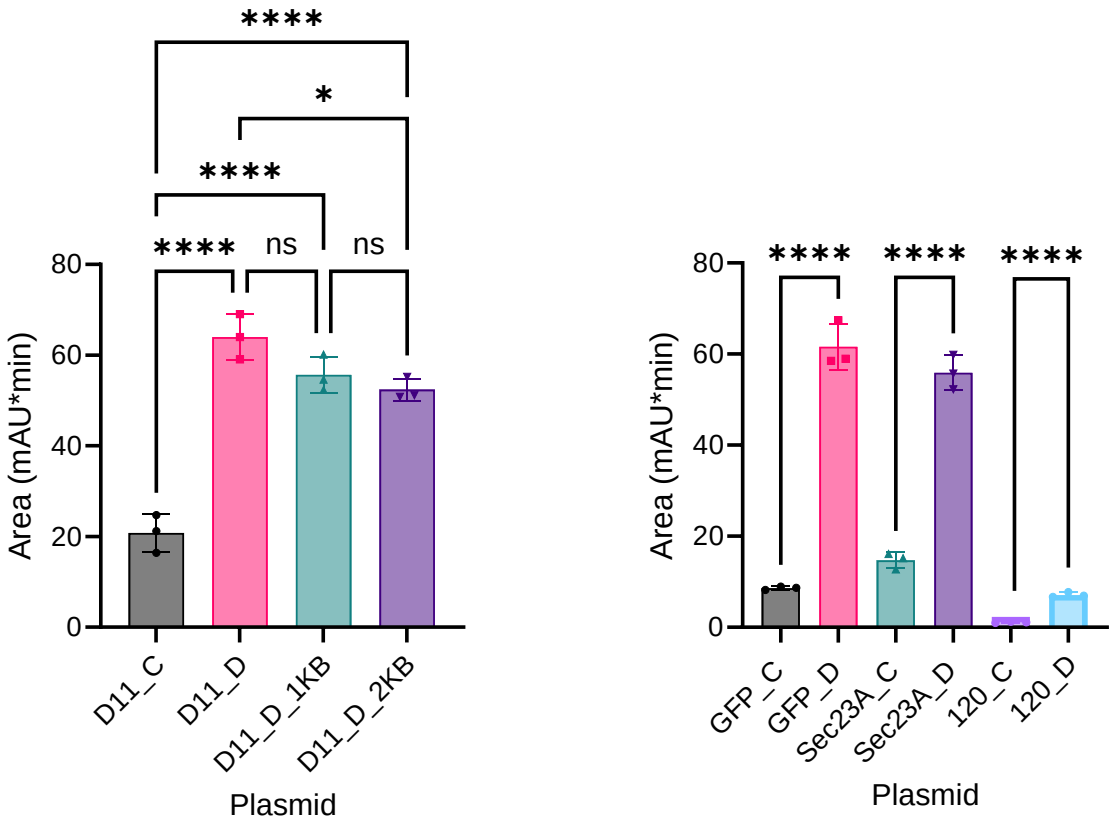

**Supplementary figure S4. Schematic diagram of *in vitro* transcription templates.** Visual representation of the generation of IVT templates used in this study. Transcriptional terminated templates were generated via linearization of plasmid templates within the Ampicillin resistance marker (AmpR) using restriction enzyme PvuI. Run-off transcription templates were generated by removing the triple transcriptional terminators (Term. (3T)), using restriction enzyme SacII. Created in BioRender. Dickman, M. (2025) https://BioRender.com/5qr6sa8

Supplementary figure 4

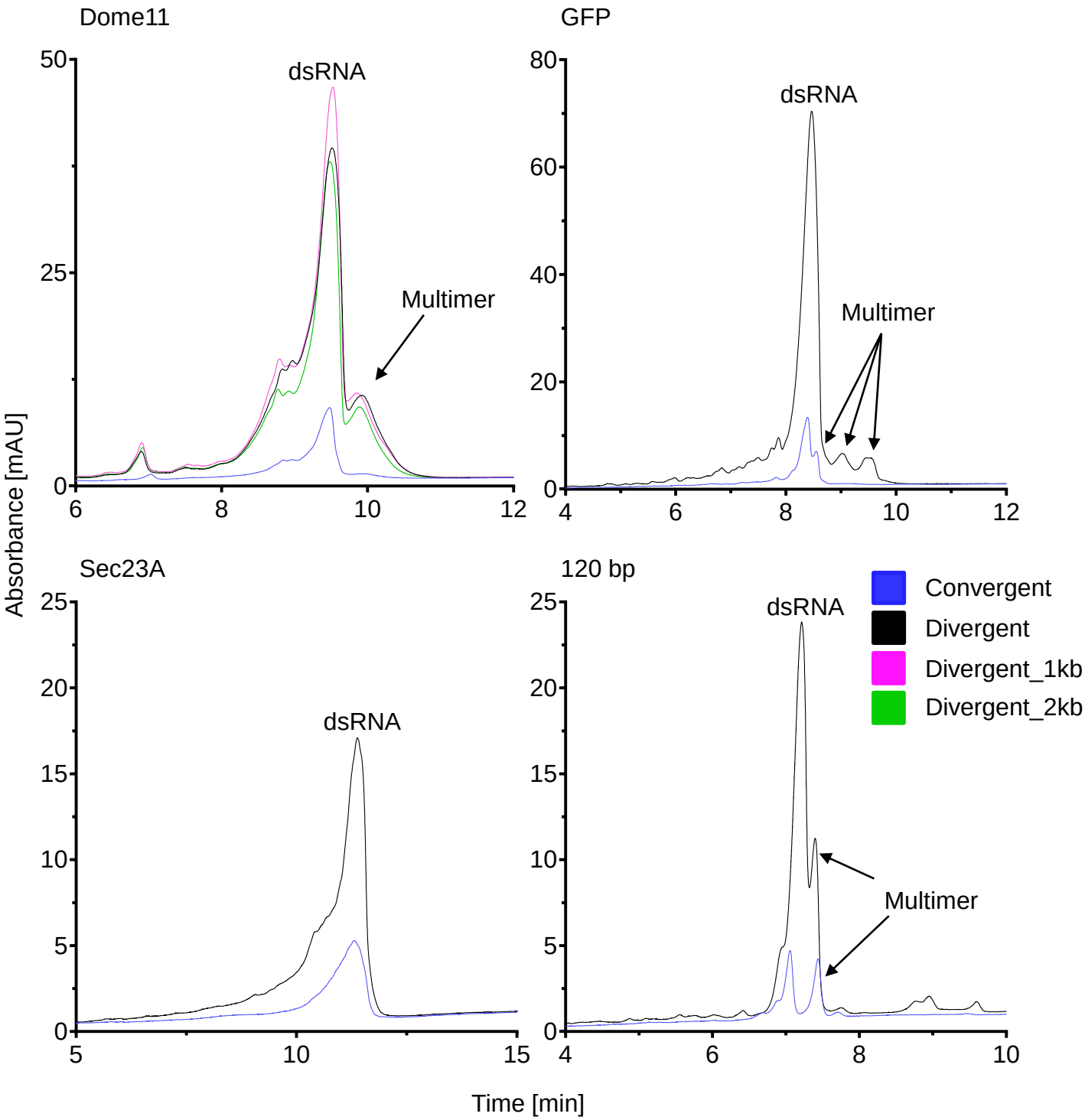



**Supplementary figure S5. IP-HPLC analysis of dsRNA synthesised from transcriptional terminated IVT reactions.** IP-HPLC analysis of IVT RNA samples for all Dome11, GFP, Sec23A and 120 bp plasmid constructs. dsRNA products are annotated. RNA samples are colour coded and indicated in figure. Analysis was performed at 20 °C; Mobile phase A, 15 mM dibutylamine (DBA) and 50 mM 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP). Mobile phase B, 15 mM dibutylamine (DBA), 50 mM 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP) and 50% acetonitrile (ACN).

# Supplementary Figure 5

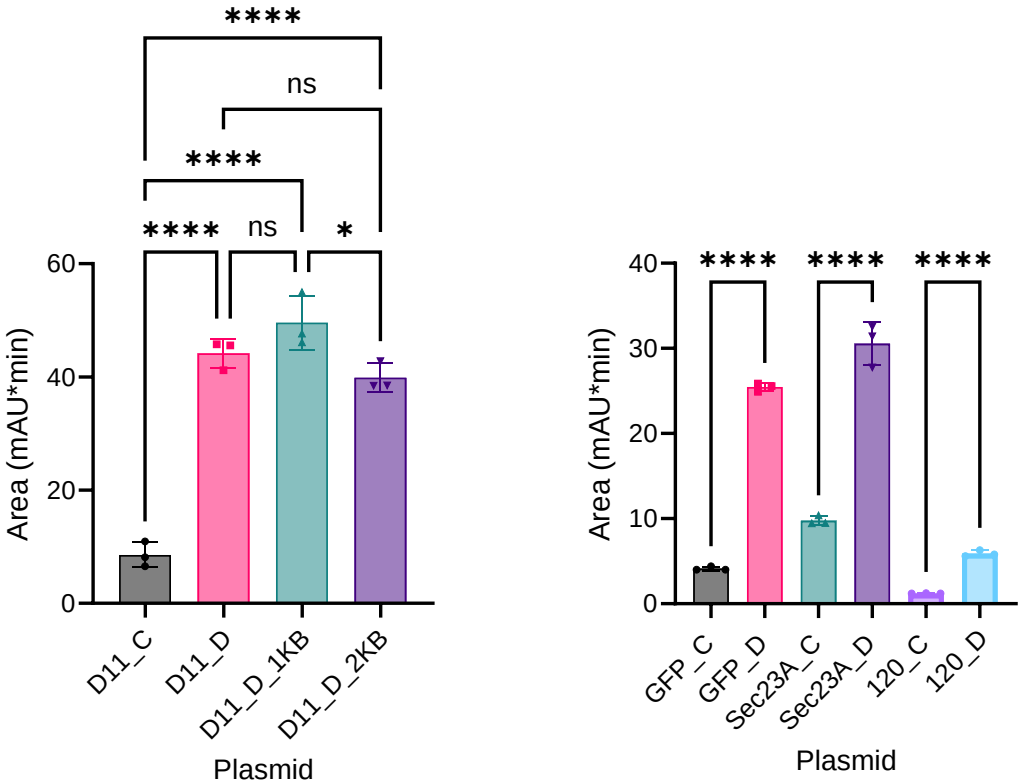



**Supplementary figure S6. Relative quantification of convergent and divergent synthesis of Dome11, GFP, Sec23A and 120 bp dsRNA in vitro utilising transcriptional terminators.** RNA samples were generated from 20 µl IVT reactions, followed by the addition of RNase T1 and DNase prior to purification. RNA samples were analysed using relative peak area (mAU*min), Significance was calculated using a one-way ANOVA test with multiple comparions, for Dome11 RNA samples. Divergent-produced Dome 11 (pD11_D) demonstrates the highest relative yield of 64.03. Significance was calculated using unpaired T tests, for GFP, Sec23A and 120 bp RNA samples. Divergent-produced, GFP (pGFP_D), Sec23A (pSec23A_D), and 120 bp (p120_D) demonstrate the highest relative yields of, 61.60, 55.93, and 7.11, respectively. Data is shown as bar charts of triplicate technical replicates; $p<0.05$ = (*), $p<0.01$ = (**), $p<0.001$ = (***), $p<0.0001$ (****).

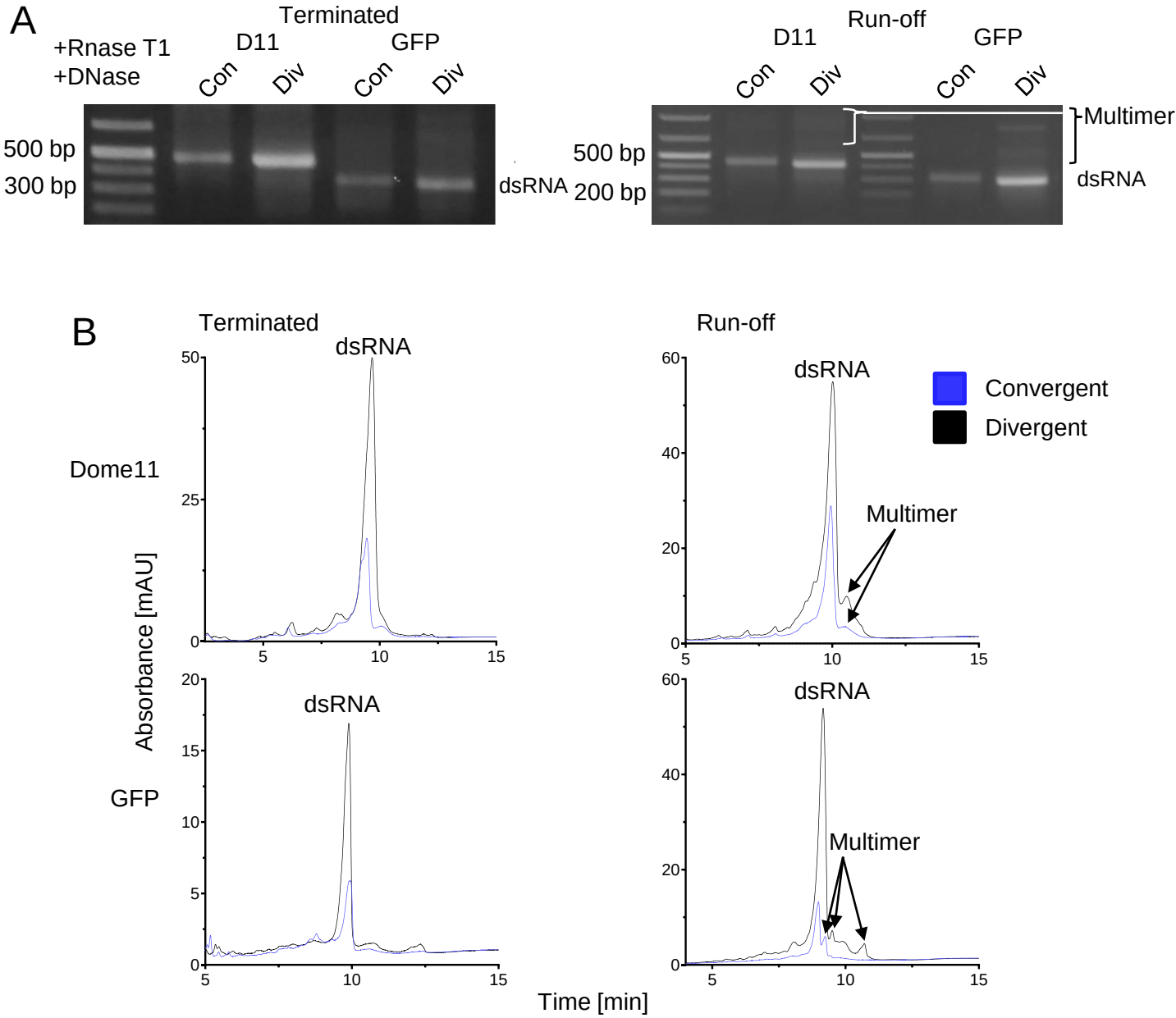



**Supplementary figure S7. IP-HPLC analysis of dsRNA synthesised from run-off transcription IVT reactions.** IP-HPLC analysis of IVT RNA samples for all Dome11, GFP, Sec23A and 120 bp plasmid constructs. dsRNA and multimer products are annotated. RNA samples are colour coded and indicated in figure. Analysis was performed at 20 °C; Mobile phase A, 15 mM dibutylamine (DBA) and 50 mM 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP). Mobile phase B, 15 mM dibutylamine (DBA), 50 mM 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP) and 50% acetonitrile (ACN).

# Supplementary Figure 7

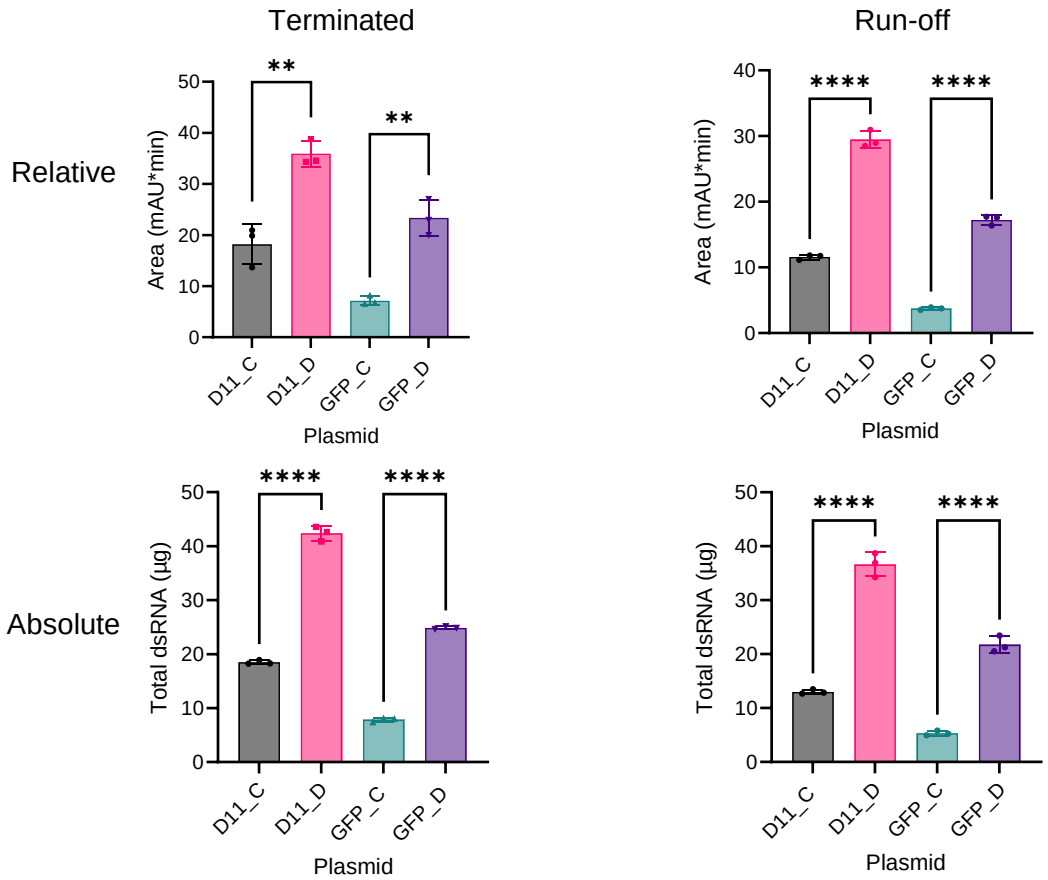



**Supplementary figure S8. Relative quantification of convergent and divergent synthesis of Dome11, GFP, Sec23A and 120 bp dsRNA in vitro utilising transcriptional terminators.** RNA samples were generated from 20 µl IVT reactions, followed by the addition of RNase T1 and DNase prior to purification. RNA samples were analysed using relative peak area (mAU*min). Significance was calculated using a one-way ANOVA test with multiple comparisons for Dome11 RNA samples. Divergent-produced Dome11 (pD11_D_1kb) demonstrates the highest relative yield of 49.59. Significance was calculated using unpaired T tests, for GFP, Sec23A and 120 bp RNA samples. Divergent-produced, GFP (pGFP_D), Sec23A (pSec23A_D), and 120 bp (p120_D) demonstrate the highest relative yields of, 24.45, 30.56, and 5.90, respectively. Data is shown as bar charts of triplicate technical replicates; $p<0.05$ = (*), $p<0.01$ = (**), $p<0.001$ = (***), $p<0.0001$ (****).




**Supplementary Figure S9. Investigations of convergent and divergent synthesis of N1-methylpseudouridine modified Dome11 and GFP, dsRNA *in vitro* utilising transcriptional terminators and run-off transcription.** Plasmids constructs pD11_C, pD11_D, pGFP_C and pGFP_D were investigated **A.** Agarose gel electrophoresis analysis of RNA extractions in the presence of RNase T1 and DNase. The corresponding Dome 11 (400 bp) and GFP (263 bp) dsRNA are highlighted. **B.** IP-RP HPLC chromatography analysis in the absence and presence of RNase T1. Left set of HPLC traces represent RNA produced utilising transcriptional terminators. Right graphs represent RNA produced via run-off transcription. Top row graphs represent Dome11 traces, bottom represent GFP traces. dsRNA and multimer products are annotated.

Supplementary Figure 9




**Supplementary Figure S10. Quantification of convergent and divergent synthesis of N1-methylpseudouridine modified Dome11 and GFP, dsRNA *in vitro* utilising transcriptional terminators and run-off transcription.** RNA samples were generated from 20 **UL** IVT reactions, followed by the addition of RNase T1 and DNase prior to purification. RNA samples were analysed using relative peak area (mAU*min), results are indicated on the top two graphs, the left graph represents transcriptional terminated dsRNA results, and the right represents run-off transcription dsRNA results. Significance was calculated using unpaired T-tests, for Dome11 and GFP RNA samples. pD11_D Dome11 and pGFP_D GFP demonstrate the highest relative yields of, 35.90 and 23.34 (Terminated), and for run-off transcription, 29.45 and 17.22, respectively. The bottom two graphs represent the comparison of absolute dsRNA yield of all RNA samples, the left represents transcriptionally terminated results, and the right run-off transcription results. RNA samples were analysed using UV spectrophotometry to determine RNA concentration using a mass concentration/A260 unit (46.52 µg/mL). Significance was calculated using unpaired T tests, for Dome11 and GFP RNA samples. pD11_D Dome11 and pGFP_D GFP demonstrate the highest absolute yields of, 42.35 and 24.87 (Terminated), and for run-off transcription, 36.61 and 21.75, respectively. Data is shown as bar charts of triplicate technical replicates; $p<0.05$ = (*), $p<0.01$ = (**), $p<0.001$ = (***), $p<0.0001$ (****).

Supplementary Figure 10