

<https://doi.org/10.7124/bc.000B48>

**R.M. Vietrov, T.B. Papurina, N.O. Hrubian,
S.O. Novosolov, R.I. Sydor, O.M. Hubar**

Yuria-Pharm LLC

19, Sviatoslava Khorobroho Str., Kyiv, Ukraine, 03151

ruslan.vietrov@uf.ua

DEVELOPMENT AND FUNCTIONAL VALIDATION OF T7 RNAP-BASED ORTHOGONAL TRANSCRIPTION SYSTEM IN MAMMALIAN CELLS

Background. RNA therapeutics are becoming an important therapeutic modality. However, screening large mRNA libraries remains challenging due to yield and quality variability from screening-scale *in vitro* transcriptions (IVT). To accelerate this process, we propose an *in-cell* RNA synthesis system enabling direct, phage single-subunit polymerase-based transcription of candidates within cells. This allows robust screening of RNA candidates without IVT-introduced variability, facilitating ribozymes and aptamers evaluation in relevant cellular environments. **Aim.** Development of T7 RNAP-based orthogonal transcription system in mammalian cells. **Methods.** T7 RNAP expression constructs (mCMV promoter, chimeric Intron A, codon-optimized Myc-tagged T7 RNAP ORF, and either SV40 pA or a reporter element (EMCV IRES LP and CyOFP1)) were assembled by two-stage Golden Gate assembly using PaqCI (NEB) with intermediate PCR for PaqCI sites reintroduction. Reporter constructs (T7 promoter, optional DiLCR(WT), EMCV IRES, hGluc-Dura CDS, pA120, T7 terminator) were assembled by Golden Gate using PaqCI (NEB). Endotoxin-free plasmids were delivered to CHO-DG44 cells by electroporation (Neon NxT, 1600 V, 3 * 10 ms), cells were transferred to Cellovento 4CHO SFM with 8mM glutamine and after 4, 26, 52 and 72h at 37 °C media aliquots were collected and GLuc quantified (Pierce Gaussia Luciferase Glow Assay Kit). **Results.** Expression of T7 RNAP in CHO cells was confirmed by flow cytometry (anti-MycTag), with CyOFP1 co-transcriptional reporter having no effect on the expression. Co-transfecting T7 RNAP and pT7-driven reporter plasmids produced luciferase signal starting from 4h post-transfection, reaching 64% of ARCA-capped mRNA reporter signal, transfected at 2.86x molar load, at 26h. Transcription from pT7-driven reporter doesn't influence transcription from T7 RNAP-expressing construct, measured as CyOFP1 reporter expression in absence of pT7 template. Also, upon co-transfection of IRES-deficient pT7 construct, no luciferase activity was detected, confirming orthogonality to nuclear RNA polymerase II-driven transcription. Titration of pT7-containing plasmid confirms linear dose-dependent response in range 1–16 nM at all timepoints tested from 6h to 50h, supporting hypothesis of cytoplasmic transcription. **Conclusions.** We demonstrated an orthogonal T7 RNAP-based expression system in mammalian cells, that allows expression of transiently transfected pT7-containing plasmids in cellulo with template dose-dependent product accumulation. Future work will focus on validating transient system and developing a CHO-based stable cell line as a high-throughput screening host for mRNA constructs.

Keywords: IVT, mRNA production, IRES, T7 RNAP.

Received: 07.07.2026; Accepted: 08.09.2026; Published: 29.09.2026

Citation: Vietrov R.M., Papurina T.B., Hrubian N.O., Novosolov S.O., Sydor R.I., Hubar O.M. (2026) Development and functional validation of T7 RNAP-based orthogonal transcription system in mammalian cells. *Biopolymers & Cell*, Special issue(42), 15. <https://doi.org/10.7124/bc.000B48>

© Publisher PH "Akademperiodyka" of the NAS of Ukraine, 2026. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited