

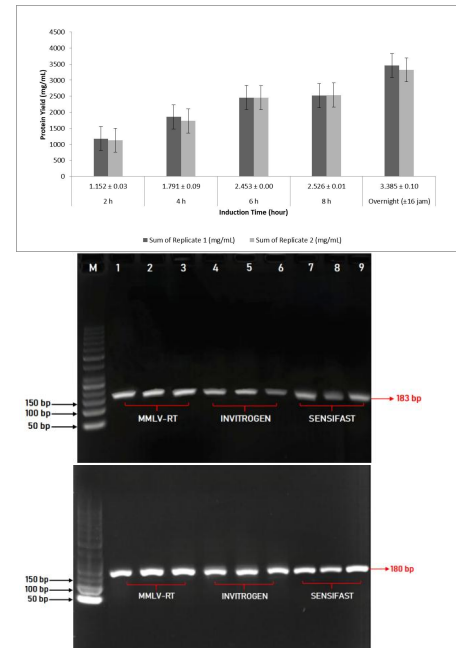
Recombinant MMLV-RT as an Alternative to Commercial Reverse Transcriptase

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Introduction. Reverse transcriptase (RT) is a critical enzyme in RNA-based molecular diagnostics, including RT-PCR assays widely used for viral detection. Many laboratories in low- and middle-income countries remain dependent on imported RT enzymes, increasing diagnostic costs and vulnerability to global supply disruptions. Developing locally produced recombinant RT may enhance sustainability and diagnostic independence. **Aims.** This study aimed to construct and express a codon-optimized Moloney Murine Leukemia Virus reverse transcriptase (MMLV-RT) in *E. coli* BL21 (DE3) and evaluate its functional performance compared to commercial RT enzymes.

Methods. A synthetic codon-optimized MMLV-RT gene (CAI 0.73; GC 57.14%) was cloned into the pET-21a(+) vector and expressed in *E. coli* BL21 (DE3). Expression was induced with 1 mM IPTG and optimized across multiple induction periods. Recombinant His-tagged protein was purified using Ni-affinity chromatography. Functional activity was assessed via conventional RT-PCR using SARS-CoV-2 RNA, targeting ORF1ab and β -actin genes, and compared with commercial RT enzymes under equivalent conditions.

Results. The recombinant MMLV-RT was successfully expressed in *E. coli* BL21 (DE3) with a molecular weight of approximately 55.4 kDa and high purity following Ni-affinity chromatography. Protein expression increased



progressively with induction time, reaching a maximal yield of 3.385 ± 0.10 mg/mL after overnight induction. Functional validation demonstrated efficient cDNA synthesis from viral (SARS-CoV-2 N and ORF1ab) and housekeeping (β -actin) RNA templates. Amplification efficiency and band intensity were comparable to commercial RT enzymes under equivalent conditions, with no detectable non-specific amplification, indicating preserved enzymatic specificity and activity.

Discussion. The recombinant MMLV-RT demonstrated robust reverse transcription activity comparable to commercially available enzymes, supporting its functional reliability for molecular diagnostic applications. Importantly, the development of a locally produced RT enzyme may contribute to improving diagnostic sustainability, reducing dependence on imported reagents, and enhancing supply-chain resilience in resource-limited settings. Further quantitative and clinical validation studies are warranted to assess its scalability and implementation potential in routine diagnostic workflows.

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