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Molecular biology tools enable the custom generation of proteins with complete control of sequence, purification tags, secretion signals, and other performance characteristics. While the breadth of tools allows researchers to create their desired protein, this process often involves a low-throughput and time-consuming, multiday workflow using live cells. To overcome these limitations, we have demonstrated a completely in vitro workflow that combines Golden Gate DNA Assembly, rolling circle amplification (RCA), and cell-free protein expression (CFPE) to rapidly screen the impact of multiple protein designs simultaneously (Figure 1). This workflow enables researchers to generate an array of protein variants in as little as a single day using a basic set of custom DNA vectors or insertion fragments. It also provides a means to assess the engineering constraints that are unique to each protein of interest and allows for the rapid identification of soluble protein.

APPLICATION NOTES:

Accelerating DNA Construction to Protein Expression A Rapid 1-Day Workflow Using NEBridge Golden Gate Assembly

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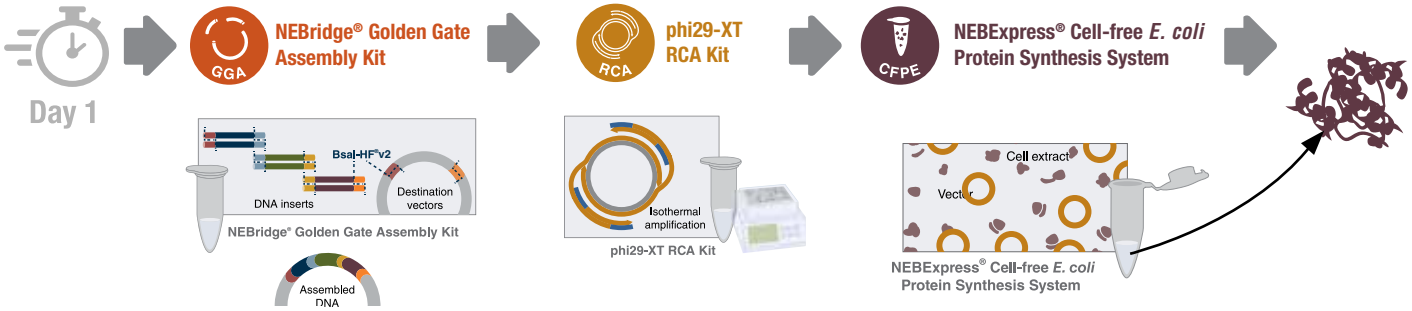
Figure 1: **Rapid expression of proteins using NEBridge® Golden Gate Assembly and NEBExpress Cell-free E. coli Protein Synthesis System**



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GGA Learn more about NEBridge Golden Gate Assembly at international.neb.com/goldengate. Try our NEBridge Ligase Fidelity Tools for Golden Gate Assembly at ligasefidelity.neb.com

RCA For more information about the phi29-XT RCA Kit, please visit www.neb.ca/E1603

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