

CRISPR-Cas9 has emerged as a highly efficient and programmable tool for genome engineering. Beyond simple gene knockouts, Cas9 enables precise gene editing and transgene insertion through homology-directed repair (HDR), wherein an exogenous donor template guides repair of the double-strand break. HDR is critical for applications in tool development, disease modeling, and gene therapy. However, achieving efficient HDR remains challenging and often requires extensive field expertise, limiting broader adoption. Compounding this issue, existing computational design tools are trained using wild-type Cas9 primarily on immortalized cell line data, which poorly reflects editing outcomes in therapeutically relevant primary cells. To address these gaps, we developed HDR-GPT, an agentic AI platform that guides users through HDR-optimized sgRNA selection and donor template design tailored for primary cell editing with p.R691A high-fidelity Cas9. Additionally, HDR-GPT incorporates a retrieval-augmented generation (RAG) system trained on curated CRISPR and HDR literature, enabling users to query the agent for evidence-based guidance on experimental design. ou