Efficient DNA Extractions for CRISPR Toolkits

A Simplified CRISPR Genotyping Workflow

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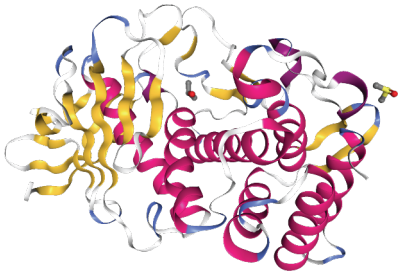


eXymes's *prep*GEM structure model

Introduction

For CRISPR genotyping to be successful, adequate DNA must be available for the downstream analysis. Extraction methods with poor DNA recovery, such as magnetic bead and silica-column based approaches, lose DNA during the extraction process. Therefore, large numbers of cells are required as a starting sample to extract enough DNA. As a result, labs must grow cells for longer. It is not uncommon for proliferation times upwards of 12 days.

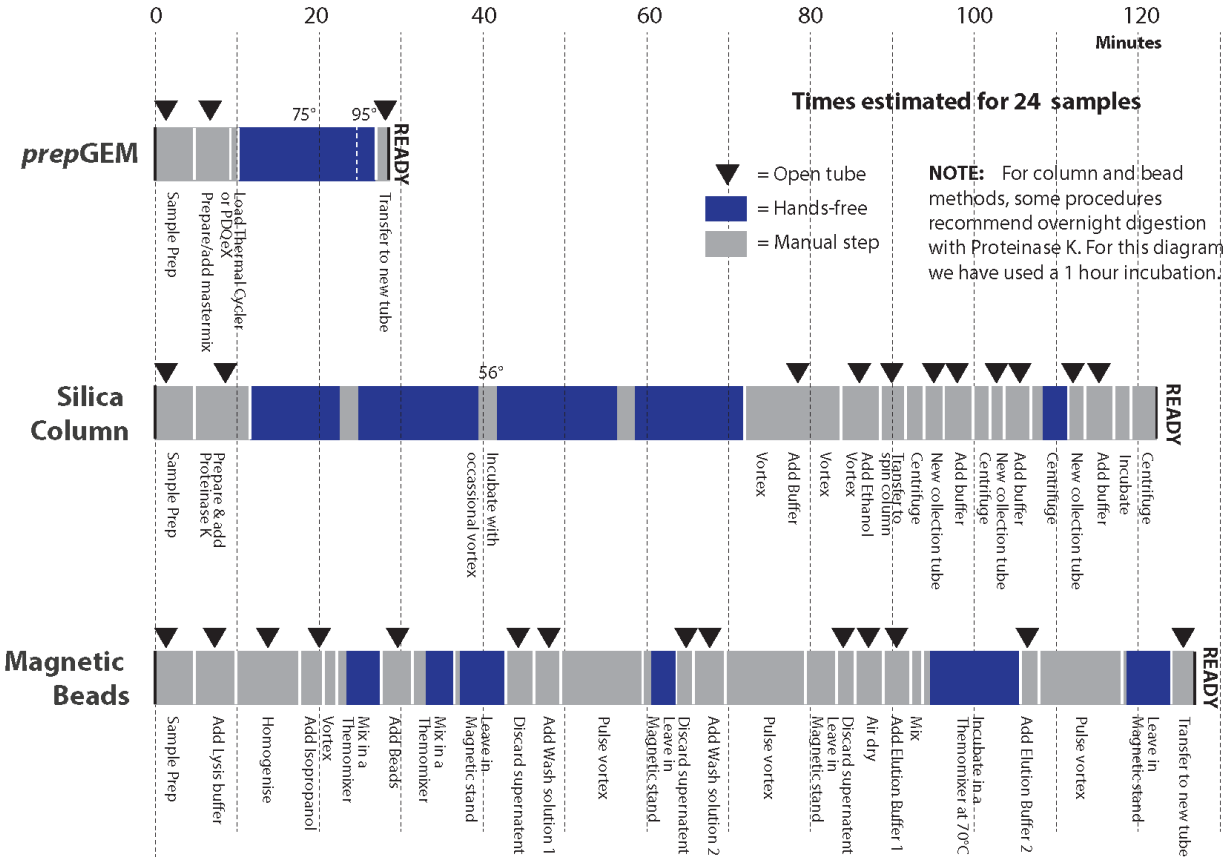
There is an easier, more efficient way. Enzymatic DNA extractions, driven by temperature changes in a single tube, effectively lyse nucleic acids from a very small number of cells, even single cells, without the need for harsh chemicals, multiple washes, or further purification. eXymes's *prep*GEM reagents solve the multi-step challenge, producing highquality extracts in minutes, not hours. They are ideal for extracting DNA from blood, saliva, cellculture, tissue, and bacteria, and produce single-stranded DNA suitable for STR, PCR,qPCR, whole genome amplification, amplicon sequencing, and gene editing.

*prep*GEM offers a simple, rapid DNA extraction solution that leverages a thermostable proteinase and single-tube protocol. Activated at 75°C and inactivated at 95°C, this powerful, broad-specificity enzyme protects precious samples from nucleases and so ensures all DNA is preserved. The method is automatable, closed-tube, and does not require furtherpurification for reliable downstream analysis. The reagents efficiently lyse the cells, hydrolyse nucleases, and clear protein from strands of nucleic acids, preparing them for analysis. Greater sensitivity is achieved, especially with low abundance transcripts and small sample volumes as nucleic acids are preserved in the process.

The prepGEM Advantage?

- Stream-lined approach extracts DNA in under 15 minutes
- Inhibitor-free reagents mean no harsh chemical washes or multiple steps
- Single-tube extractions protect the integrity of the sample
- Simplified workflow frees up preparation time, reduces lab costs, and significantly reduces environmental impact from hazardous and plastic waste.

The workflow is significantly faster than conventional column and bead methods and is much simpler:



A standard eXymes DNA Extraction Protocol

- Prepare sample
- Mix sample and reagents (*prep*GEM and buffers)
- Place in a standard thermocycler for 2-10 minutes at 75°C
- Denature *prep*GEM for 2 minutes at 95°C

The eXymes method has an exceptionally high DNA recovery with no loss of DNA during the extraction process. Researchers can work with fewer cells and still get enough DNA for downstream analysis. The time to grow cells is significantly reduced. Results are much faster.

Theapproachiseasilyscaledtohigh-throughputlevelsbysimplyusinga96-wellplateandPCRmachine. This allows researchers to genotype 96 cell lines simultaneously. With protocols as short as 4 minutes start to finish, a laborious process now becomes quick and very efficient.

Select Examples of DNA Extraction Using eXymes for CRISPR Protocols

The Wang group (2020) used the CRISPR-Cas spacer acquisition process to study horizontal gene transfer (HGT) between bacteria, in this case an E.coli recording strain and a microbial sample. Spacers were acquired from transferred plasmids and stored in genomic CRISPR arrays. DNA was extracted using *prep*GEM Bacteria as part of the group's established sequencing pipeline. The study found that HGT into the recording strain in human clinical fecal samples was driven by different plasmid types.[1]

Using a generalized platform for screening and selection of functional bacterial CRISPR-Cas transcription activators, the Wang group (2020) identified a novel CRISPR activator, dCas9AsiA, to activate gene expression by up to 200fold across genomic and plasmid targets, expanding capabilities to perform gene regulation in bacteria. To quantify abundance, plasmid DNA from each sample was extracted using *prep*GEM Bacteria to generate a DNA amplicon sequencing library.[2]

Zhang Y *et al* (2016) successfully used CRISPRCas9 mediated integration of a site-specific homozygous mutation in CHMP2B to generate a human-induced pluripotent stem cell line for the study of familial frontotemporal dementia. *prep*GEM was used for DNA extraction for downstream amplification.[3] The Church group (2014) described a protocol for Cas9-mediated human genome engineering to deliver Cas9 and guide RNA (gRNA) into human-induced pluripotent stem cells (hiPSCs) and HEK293 cells. It also described methods to assess genome editing efficiency using nextgeneration sequencing and isolate monoclonal hiPSCs with the desired modifications for downstream applications. *prep*GEM was used in basic protocol 3, *Genotyping Transfected Cells using Next-Generation Sequencing*, to harvest cells to create the sequencing library.[4]

In a study investigating single nucleotide variations impacting the specificity of Cas9, the Church group (2014) combined whole genome sequencing and deep-targeted sequencing to characterizetheoff-targeteffectsofCas9editing. Extracted genomic DNA using *prep*GEM was used for site-specific deep sequencing to predict off-target sites in a population of Cas9-treated cells.[5]

Conclusion

The CRISPR process can often be laborious and, with the inherent loss of DNA from conventional DNA extraction methods, require long proliferation times to ensure adequate quantities of cells and adequate quantities of DNA. The eXymes rapid protocol allows researchers to shorten proliferation time, limit extraction time, and process a large number of samples simultaneously to streamline the CRISPR genotyping workflow to a few simple, efficient steps.

References

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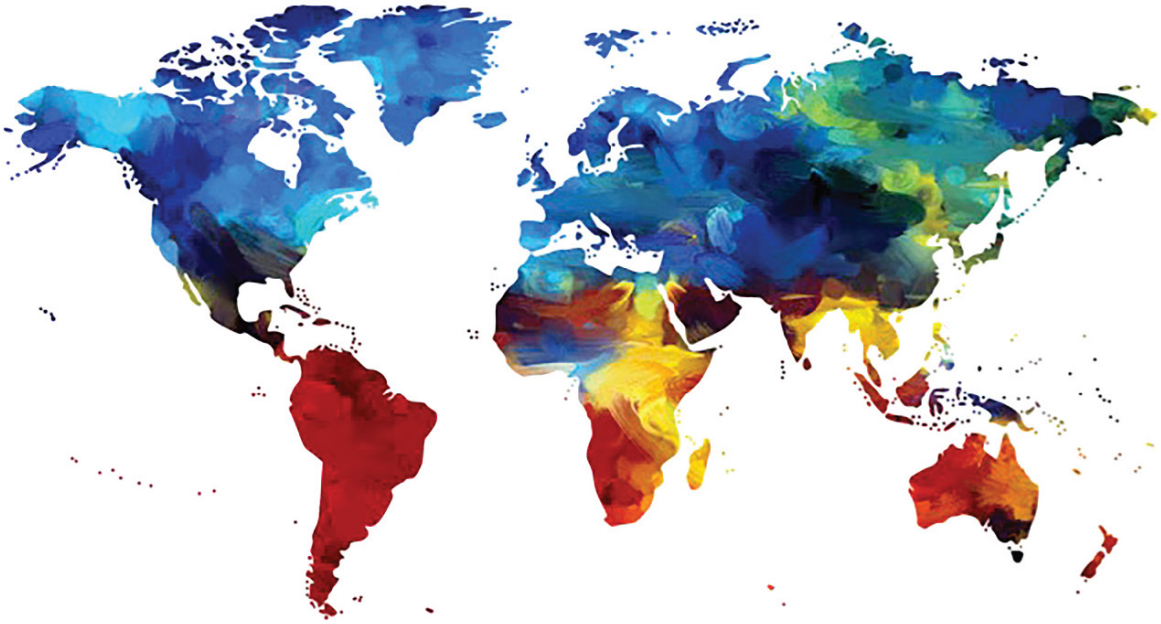

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At Exymes, our goal is to provide ultra fast DNA extraction enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

eXymes

Committed to minimal packaging, reduced plastic use, and a vibrant, sustainable world