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Automated DNA template preparation and quantitation methods

Jane Romantseva[1], David Ross[1], Nina Alperovich[2], Elizabeth Strychalski[2]

[1]National Institute of Standard and Technology; [2]National Institute of Standards and Technology

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DNA template preparation for CFE

Jane Romantseva
National Institute of Standard and Technology

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Concentrated, intact, and biologically-active DNA templates are essential for protein production using cell-free expression. We present automated methods for DNA template preparation and quantitation towards improving reproducibility in cell-free expression.

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document

Romantseva E.F., Tack D.S., Alperovich N., Ross D., Strychalski E.A. (2022) Best Practices for DNA Template Preparation Toward Improved Reproducibility in Cell-Free Protein Production. In: Karim A.S., Jewett M.C. (eds) Cell-Free Gene Expression. Methods in Molecular Biology, vol 2433. Humana, New York, NY. https://doi.org/10.1007 /978-1-0716-1998-8_1

cell-free expression, automated DNA extraction, automated DNA purification, DNA template preparation, automated DNA quantitation

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Concentrated, intact, and biologically-active DNA templates are essential for protein production using cell-free expression. We present automated methods for DNA template preparation and quantitation towards improving reproducibility in cell-free expression.

The following automated methods for DNA template preparation and quantitation were developed for improving reproducibility in cell-free expression and are described in detail elsewhere.

Romantseva EF, Tack DS, Alperovich N, Ross D, Strychalski EA (2022). Best Practices for DNA Template Preparation Toward Improved Reproducibility in Cell-Free Protein Production.. Methods in molecular biology (Clifton, N.J.). https://doi.org/10.1007/978-1-0716-1998-8_1

These methods were developed using the Hamilton STAR automated liquid handler with the Monitored Multi-Flow Positive Pressure Evaporative Extraction ([MPE]^2) module. For questions about methods or implementation, contact Jane Romantseva (eugenia.romantseva@nist.gov).

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1. Load deck with tips for DNA template preparation methods

0_LoadDeckwithTips.hsl

0_LoadDeckwithTips.med

0_LoadDeckwithTips.stp

0_LoadDeckwithTips.sub

2. Aliquot cell culture into tubes and wells

Aliquot pEFR40019 culture.hsl

Aliquot pEFR40019 culture.med

Aliquot pEFR40019 culture.stp

Aliquot pEFR40019 culture.sub

3. Resuspend cell culture for DNA preparation with two kits

Resuspend pEFR40019 culture.prepare for 2 kit DNA extraction.hsl

Resuspend pEFR40019 culture.prepare for 2 kit DNA extraction.med

Resuspend pEFR40019 culture.prepare for 2 kit DNA extraction.stp

Resuspend pEFR40019 culture.prepare for 2 kit DNA extraction.sub

4. DNA extraction using QIAGEN QIAprep 96Turbo kit with MPE2

DNA Extraction.QIAGEN QIAprep 96 Turbo.with MPE2.hsl

DNA Extraction.QIAGEN QIAprep 96 Turbo.with MPE2.med

DNA Extraction.QIAGEN QIAprep 96 Turbo.with MPE2.stp

DNA Extraction.QIAGEN QIAprep 96 Turbo.with MPE2.sub

5. DNA extraction using Omega Biotek Mag-Bind Ultra-Pure kit with MPE2

DNA Extraction.OmegaBiotek Mag-Bind Ultra-Pure.with MPE2.Version2med.hsl

DNA Extraction.OmegaBiotek Mag-Bind Ultra-Pure.with MPE2.Version2med.med

DNA Extraction.OmegaBiotek Mag-Bind Ultra-Pure.with MPE2.Version2med.stp

DNA Extraction.OmegaBiotek Mag-Bind Ultra-Pure.with MPE2.Version2med.sub

6. DNA Purification using Invitrogen PURELink Pro96 kit with MPE2

DNA Purification.InvitrogenPURELink Pro96.with MPE2.hsl

DNA Purification.InvitrogenPURELink Pro96.with MPE2.med

DNA Purification.InvitrogenPURELink Pro96.with MPE2.stp

DNA Purification.InvitrogenPURELink Pro96.with MPE2.sub

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7. Load deck with tips for DNA quantitation methods

00_LoadQuantDeckwithTips.hsl

00_LoadQuantDeckwithTips.med

00_LoadQuantDeckwithTips.stp

00_LoadQuantDeckwithTips.sub

8. Aliquot QuantIT Master Mix for DNA quantitation using fluorometry and QuantIT BroadRange kit

DNA Quantitation.Aliquot QuantIT Master Mix.dsDNA Broad Range Assay.hsl

DNA Quantitation.Aliquot QuantIT Master Mix.dsDNA Broad Range Assay.med

DNA Quantitation.Aliquot QuantIT Master Mix.dsDNA Broad Range Assay.stp

DNA Quantitation.Aliquot QuantIT Master Mix.dsDNA Broad Range Assay.sub

9. Prepare DNA samples for DNA quantitation using fluorometry and QuantIT BroadRange kit

DNA Quantitation.Prep Quant Plates.hsl

DNA Quantitation.Prep Quant Plates.med

DNA Quantitation.Prep Quant Plates.stp

DNA Quantitation.Prep Quant Plates.sub

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