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# Automated DNA template preparation and quantitation methods

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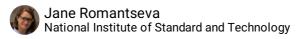
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#### DNA template preparation for CFE



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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

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These methods were developed using the Hamilton STAR automated liquid handler with the Monitored Multi-Flow Positive Pressure Evaporative Extraction ([MPE]<sup>2</sup>) 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

- $\\ \textbf{0} \\ \textbf{0} \\ \textbf{LoadDeckwithTips.hsl} \\$
- 0 0\_LoadDeckwithTips.med
- 0 0\_LoadDeckwithTips.stp
- (i) O\_LoadDeckwithTips.sub

# 2. Aliquot cell culture into tubes and wells

- (l) Aliquot pEFR40019 culture.hsl
- (i) 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**
- $\ensuremath{\emptyset}$  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
- $@ \ DNA \ Extraction. OmegaBiotek \ Mag-Bind \ Ultra-Pure. with \ MPE2. Version 2 med. med \\$
- DNA Extraction.OmegaBiotek Mag-Bind Ultra-Pure.with MPE2.Version2med.stp
- UDNA 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**

- 7. Load deck with tips for DNA quantitation methods
- @ 00\_LoadQuantDeckwithTips.hsl
- (i) 00\_LoadQuantDeckwithTips.med
- 0 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**
- **NA Quantitation. Prep Quant Plates. med**
- **DNA Quantitation.Prep Quant Plates.stp**
- **DNA Quantitation.Prep Quant Plates.sub**