

High-throughput, transfection-grade plasmid purification without centrifugation using paramagnetic particles.



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Abstract #B120

1. Abstract

Antibody-based therapeutics is a promising field for the development of new treatments for many diseases. The increasing interest in screening engineered antibodies raises new challenges in High-Throughput (HT) antibody production. Plasmids encoding engineered antibodies transfected to cultured cells can be used for *in vitro* antibody production. Automation of steps such as plasmid purification can greatly enhance lab throughput capabilities.

In order to automate plasmid purification, 3 main challenges inherent to standard protocols must be overcome: 1. remove centrifugation steps, 2. purify enough plasmid for screening, transfection and library storage purposes, and 3. obtain transfection-grade quality plasmids with low to no inhibitors after purification.

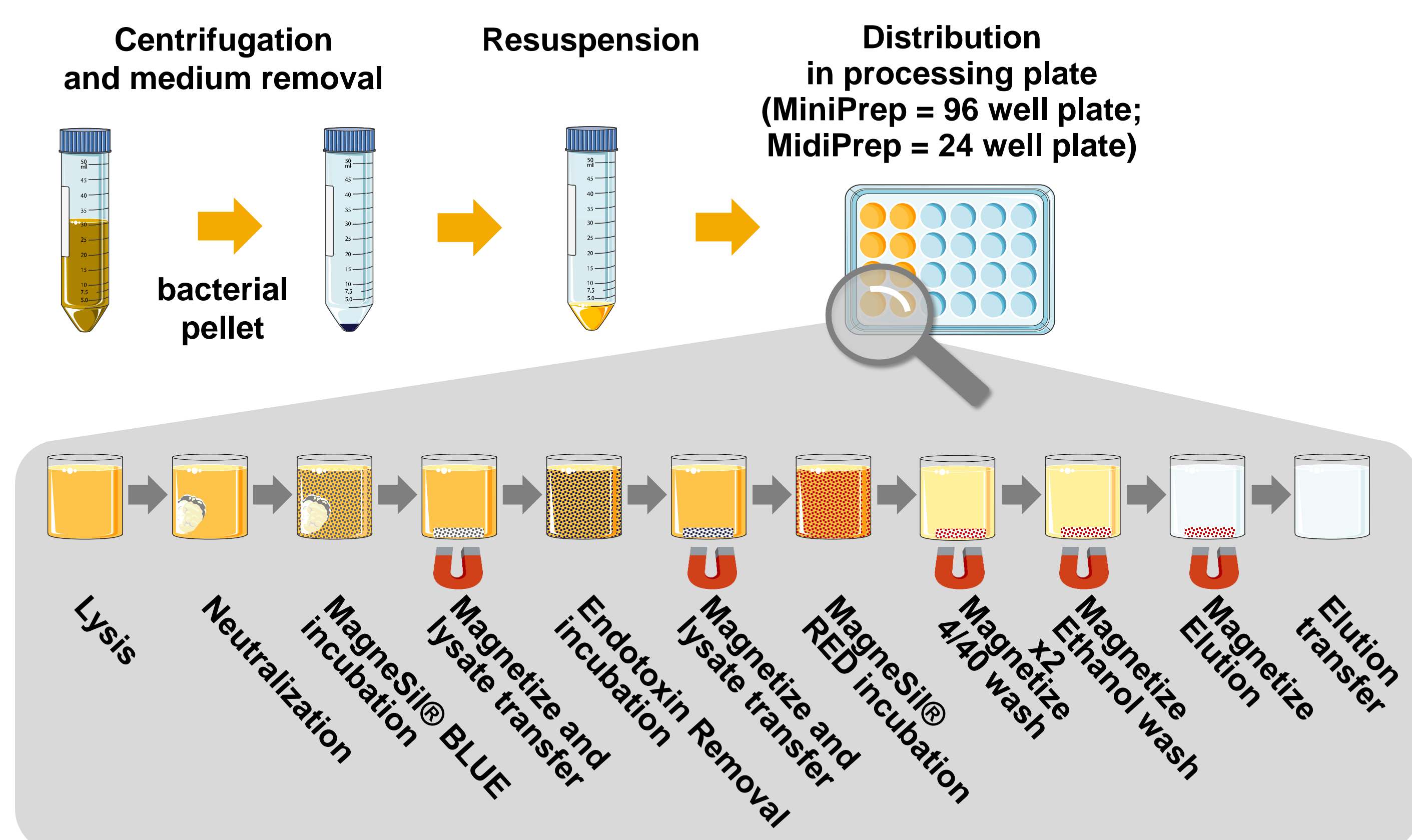
In this poster, we present protocols for High Throughput plasmid purifications using 96- or 24-deep well plate formats on liquid handler platforms that overcome these challenges. These protocols are fully automated with no hands-on intervention required during the purification process. In addition to standard miniprep scale (1.5ml input culture) we also demonstrate the efficiency of our purification protocols from large volume bacterial inputs (up to 30ml bacterial culture input) to maximize plasmid yields. Finally, our protocols contain a specific endotoxin-removal step to obtain high quality plasmids suitable for cell transfection.

These plasmid purification protocols were developed to simplify the engineered antibody screening workflow.

2. Workflow

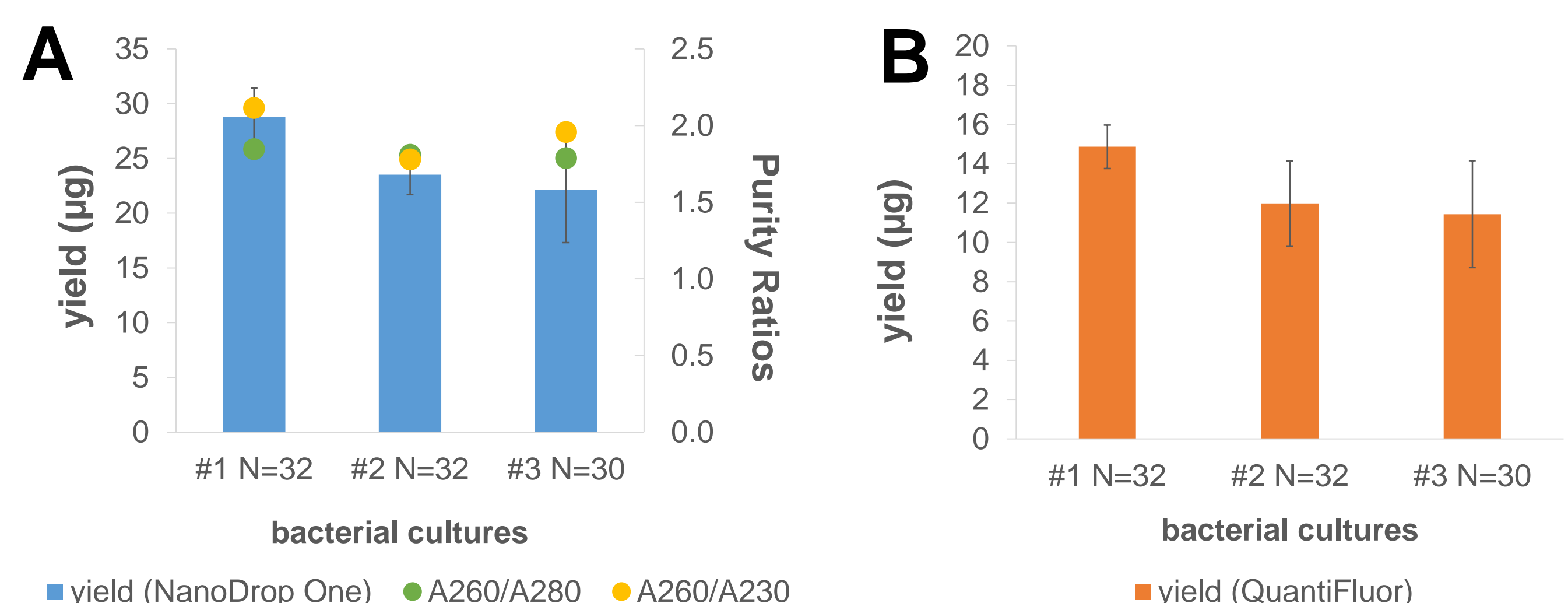
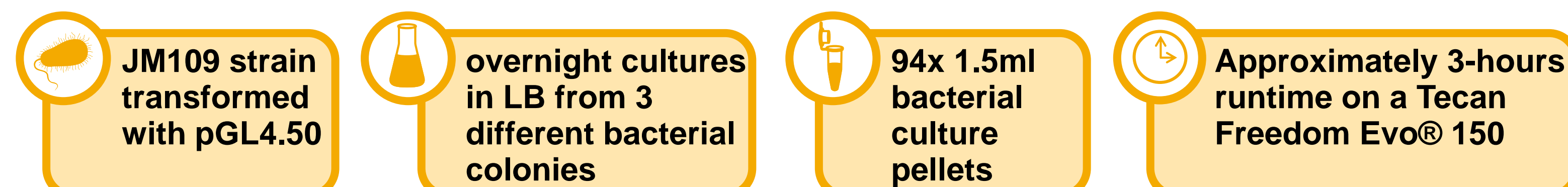


3. Protocol



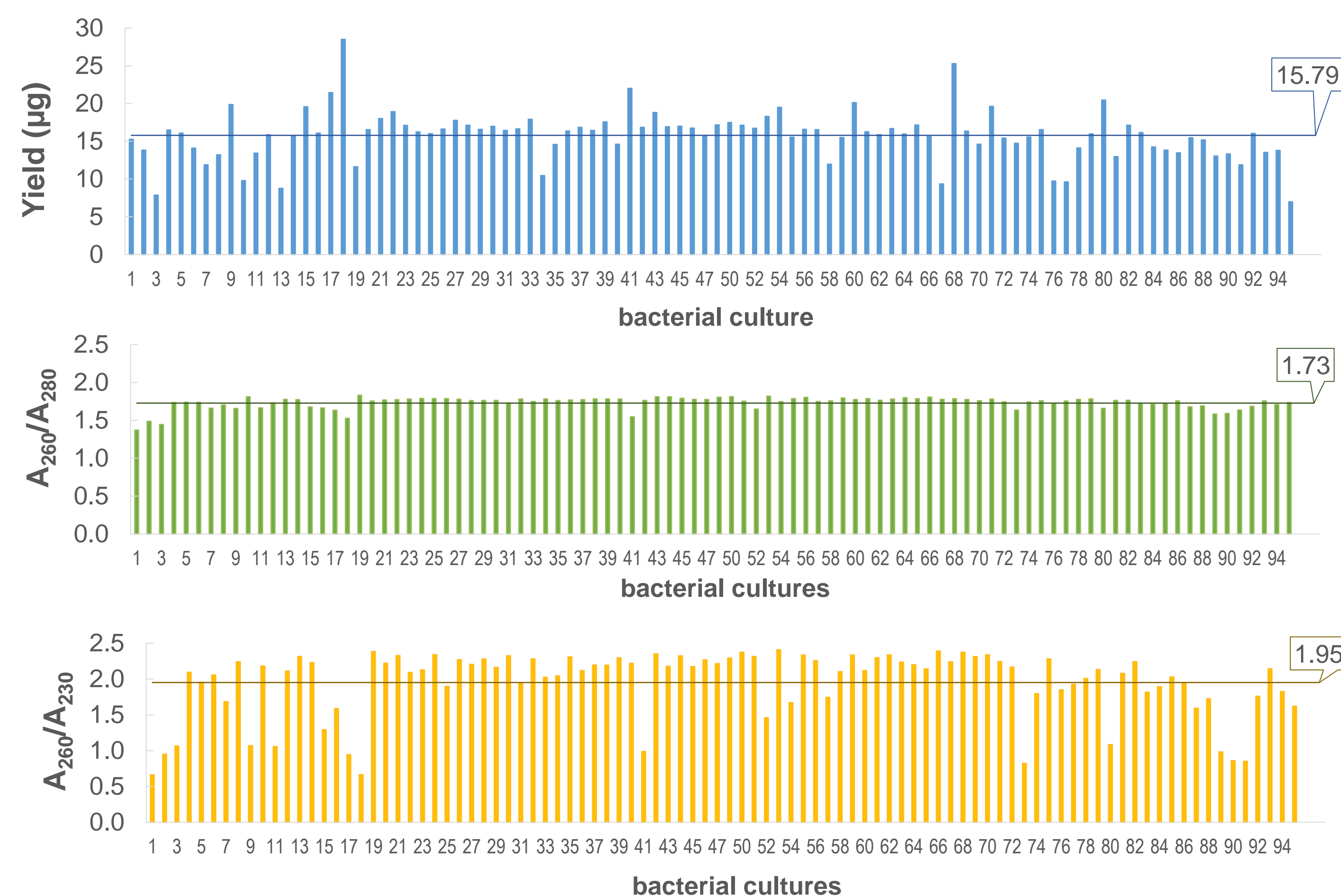
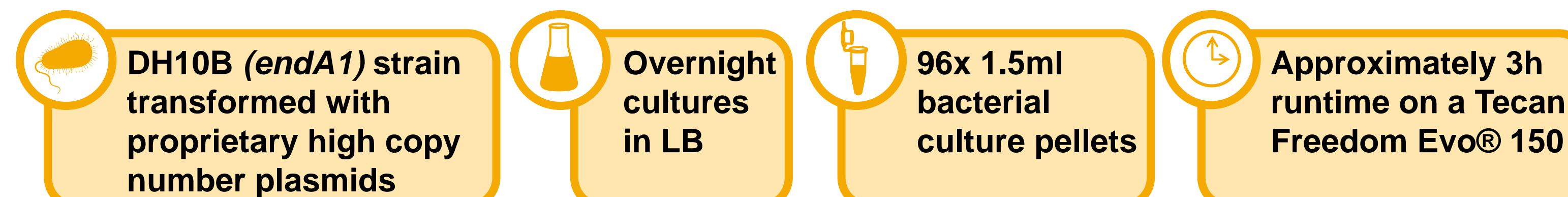
Process fully automated (no hands-on) from cell pellet resuspension to elution using the Promega Wizard® Magnesil® Tfx System which includes proprietary Magnesil® BLUE beads for cell debris removal & Magnesil® Endotoxin Removal beads.

4. Plasmid Purification from 1.5ml Bacterial Cultures: "MiniPrep" Format



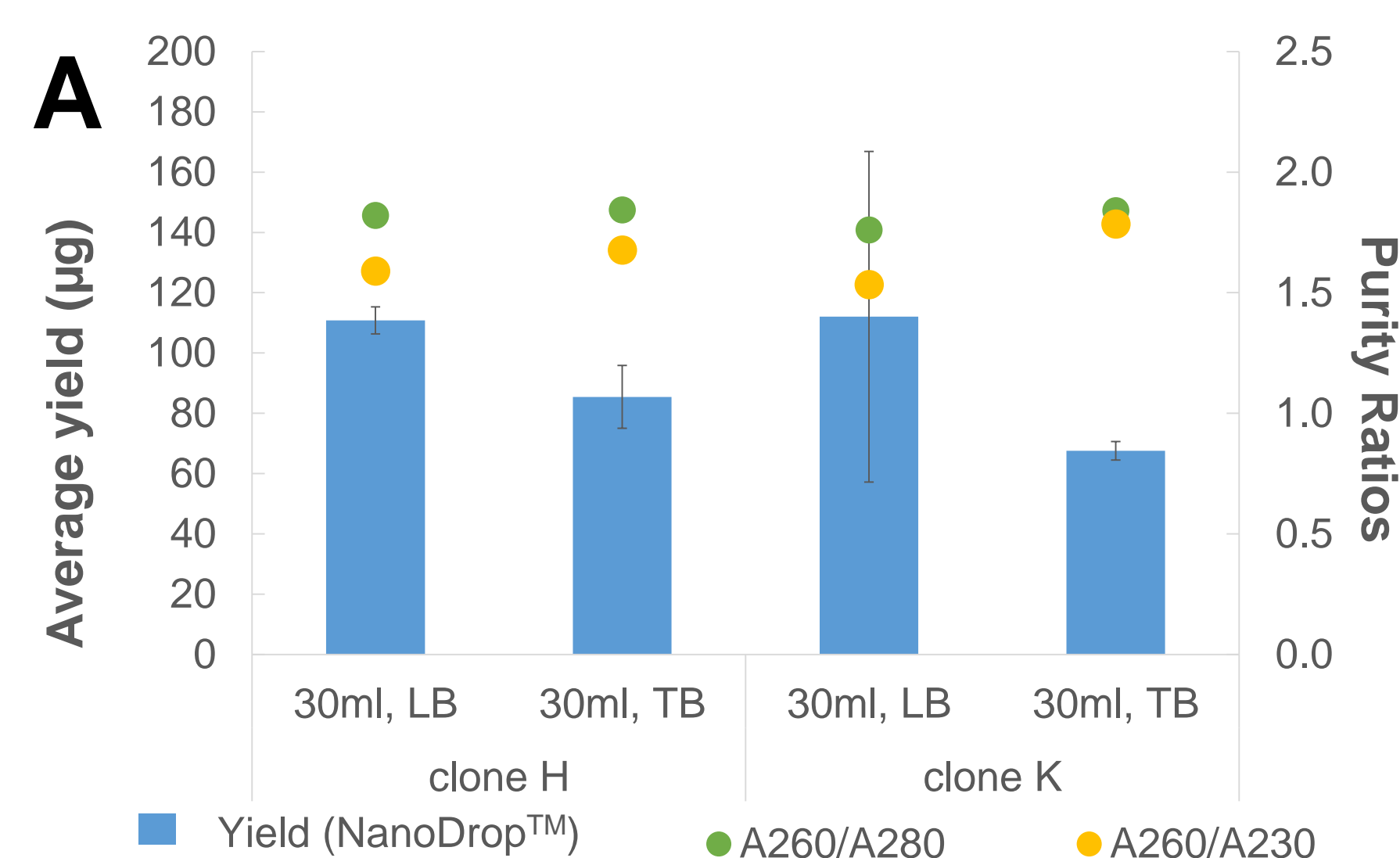
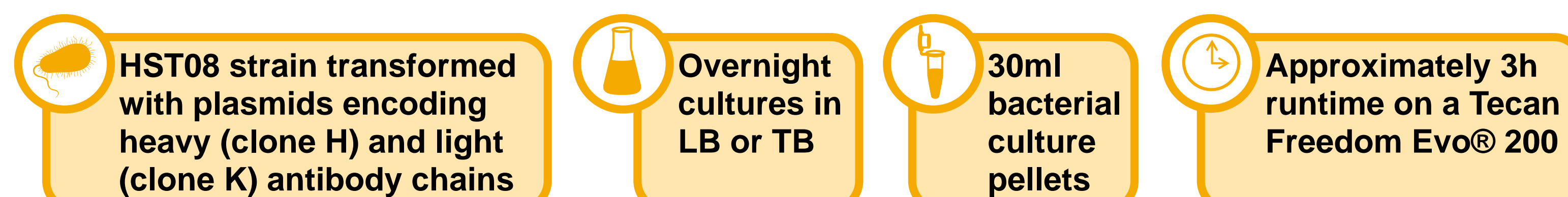
Average yield and purity of plasmid DNA from 1.5ml bacterial culture on a Tecan Freedom Evo® 150. A. Yields and purity of isolated plasmid DNA were measured by absorbance on a NanoDrop™ One Spectrophotometer. B. Yields were measured by fluorescence with QuantiFluor® ONE dsDNA System on a Promega GloMax® Discover instrument.

5. Plasmid purification from 1.5ml bacterial cultures

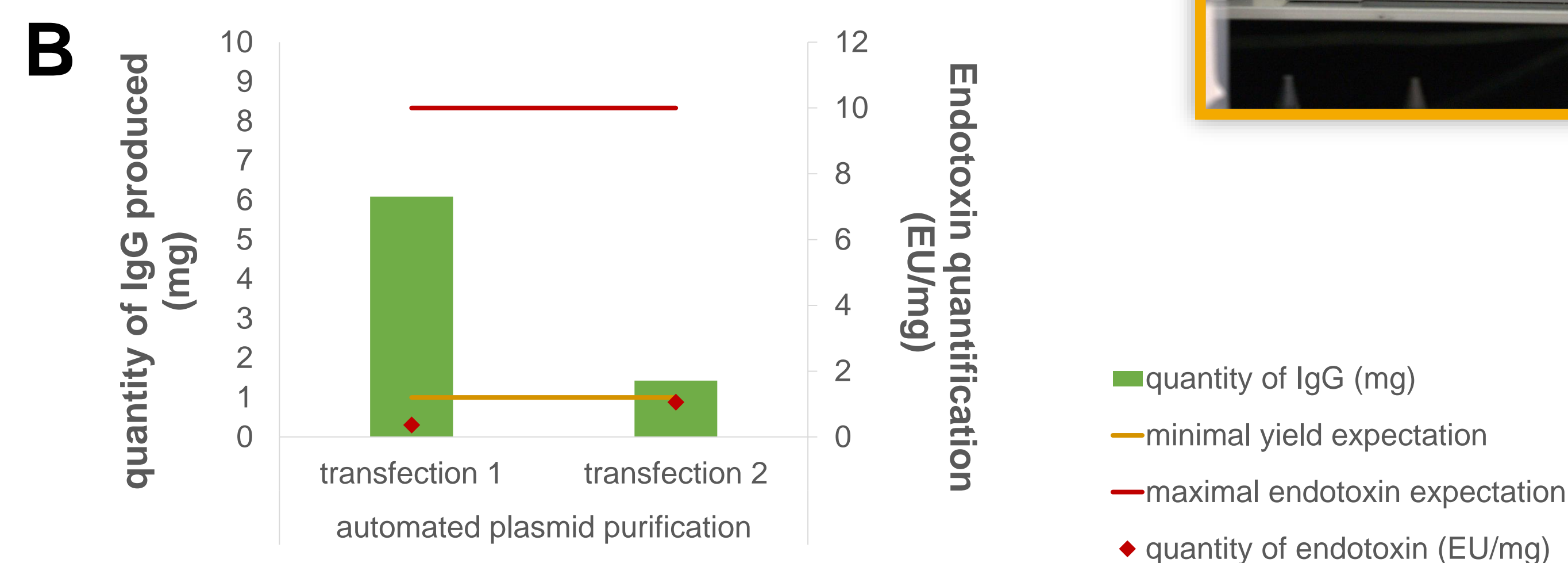


Average yield and purity of plasmid DNA from 1.5ml bacterial culture on a Tecan Freedom Evo® 150. Yield and purity of isolated plasmid DNA were measured by absorbance on a NanoDrop™ One Spectrophotometer.

6. Large Volume Plasmid Purification from 30ml Bacterial Cultures: "MidiPrep" format (Innate Pharma Strain)



Average yield and purity of DNA from 30ml bacterial culture on a Tecan Freedom Evo® 200. Yield and purity of isolated plasmid DNA were measured by absorbance on a NanoDrop™ Spectrophotometer. Shown is the average ± standard deviation for N=3 replicates.



Antibody production and Endotoxin levels quantification. Customer's antibody production results from cell transfection using plasmids purified with the automated Wizard® Magnesil® Tfx System.

7. Conclusions

Getting sufficient plasmids using standard commercial kits is a challenge for screening and many other applications.

High-throughput automated plasmid purification using the Wizard® Magnesil® Tfx System provides high quality transfection-grade plasmids for antibody production. The protocol can be adapted for standard ("MiniPrep") or large volume ("MidiPrep") bacterial inputs in 96 or 24-deep well plate formats. The developed protocol is fully automated in 3 hours runtime on Tecan Freedom Evo® platforms. Runtime will vary depending on the instrument and configuration. A dedicated team of automation specialists can help you to adapt the protocol to your liquid handler platform.