

High-Throughput, Transfection-Grade Plasmid Purification without Centrifugation using Paramagnetic Particles

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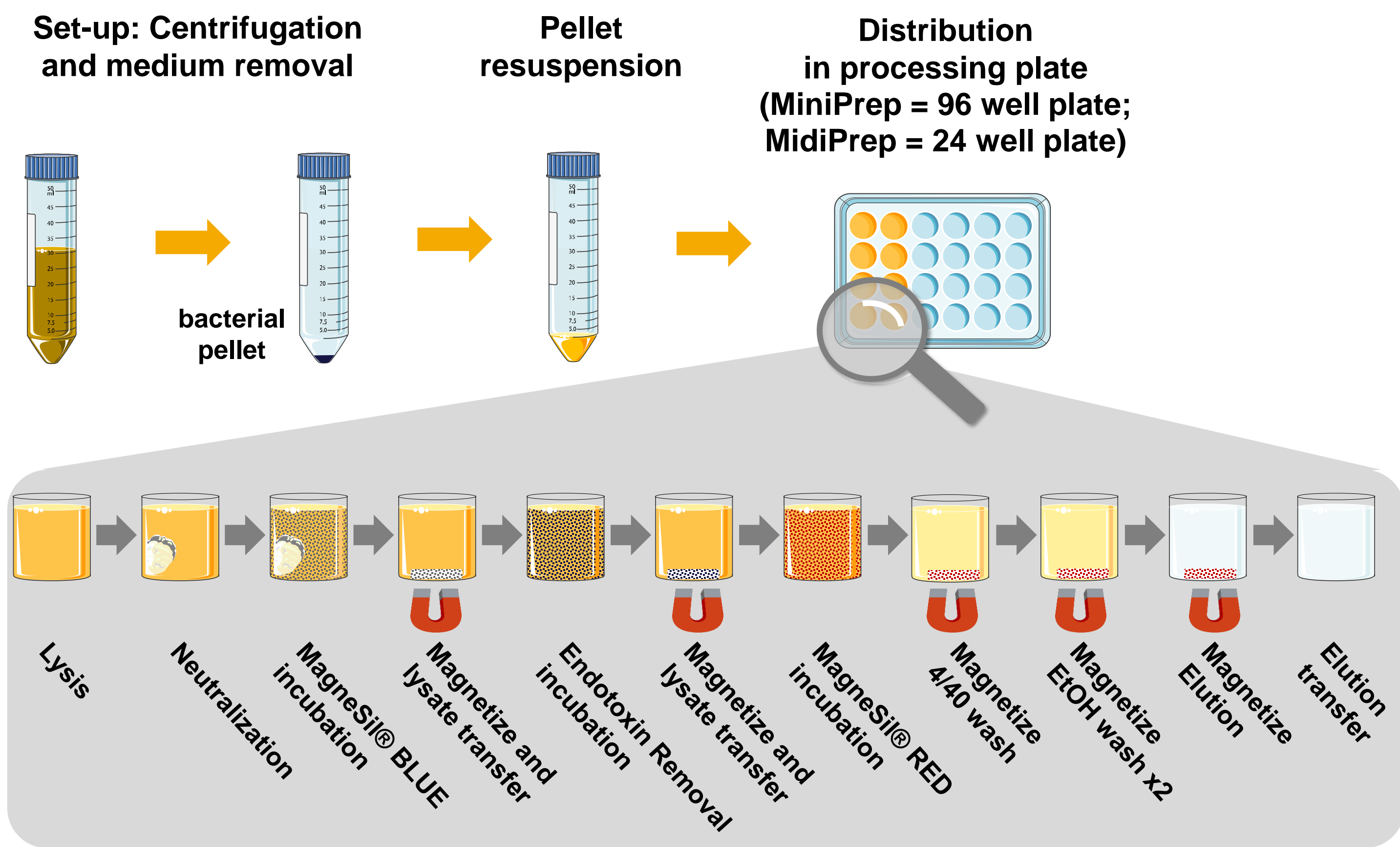
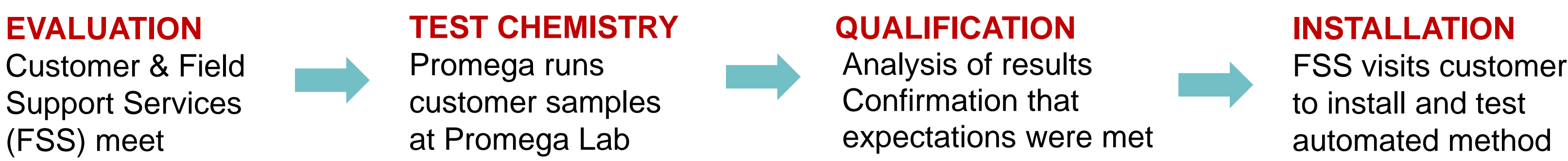


1. Introduction

Antibody-based therapeutics is a promising field for the development of new treatments for many diseases. Increasing interest in screening engineered antibodies raises new challenges in High-Throughput (HT) antibody production. In this workflow, plasmids encoding engineered antibodies are transfected to cultured cells for production in vitro. Promega developed protocols for HT plasmid purification on liquid handler platforms to overcome challenges in standard protocols. The purification protocols presented here are fully automated once the cell pellet is obtained with no hands-on intervention required.

Getting sufficient plasmids using standard commercial kits is a challenge for screening, transfection and library storage and typically involves centrifugation steps. Here we demonstrate the efficiency of the first fully automated plasmid purification from large volume bacterial inputs to maximize plasmid yields. A final challenge is to obtain transfection-grade quality plasmids with low-to-no inhibitors after purification. Our plasmid purification protocols contain a specific endotoxin-removal step to obtain high quality plasmids suitable for cell transfection. Promega protocols for HT plasmid purification will help simplify antibody production. Our team of automation experts (FSS) is dedicated to supporting customers during the installation process.

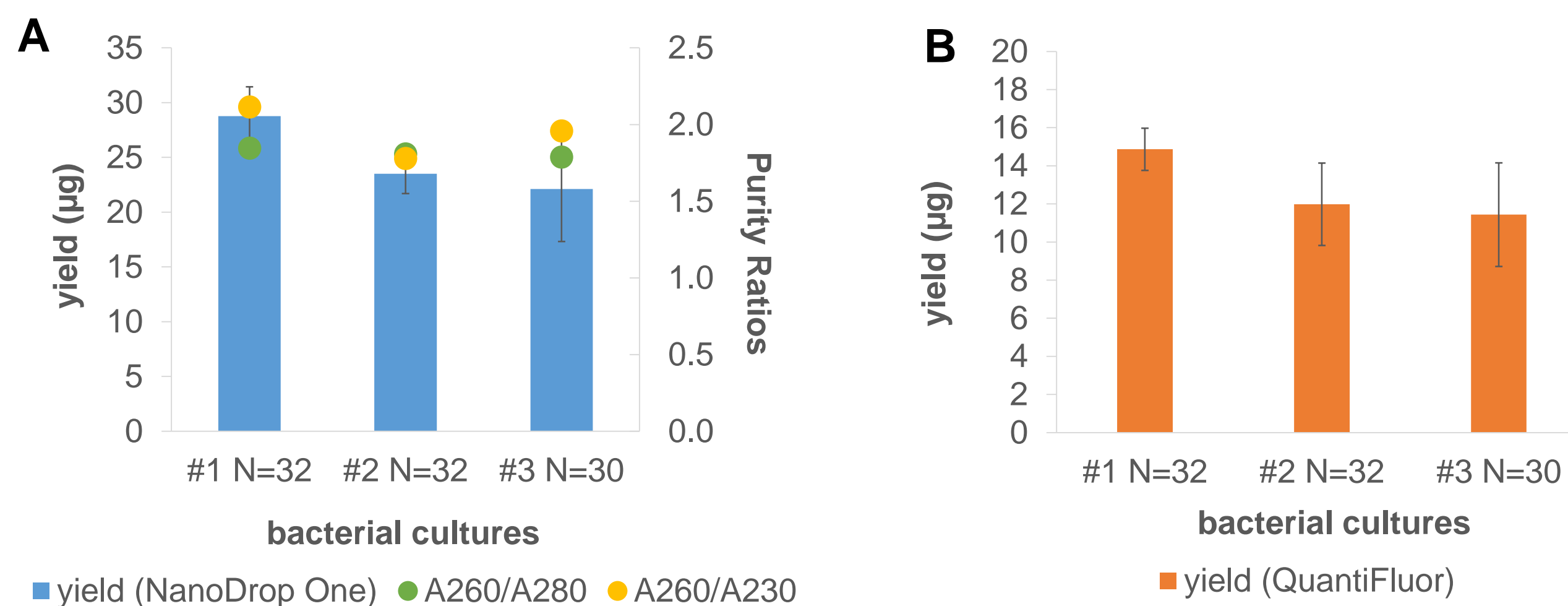
2. Workflow and Protocol



Process is fully automated (no hands-on) beginning with cell pellet resuspension and elution using the Promega Wizard MagneSil Tfx System. The system includes proprietary MagneSil BLUE beads for cell debris removal & MagneSil Endotoxin Removal beads.

3. Plasmid Purification from 1.5ml JM109 Bacterial Cultures: “MiniPrep” Format

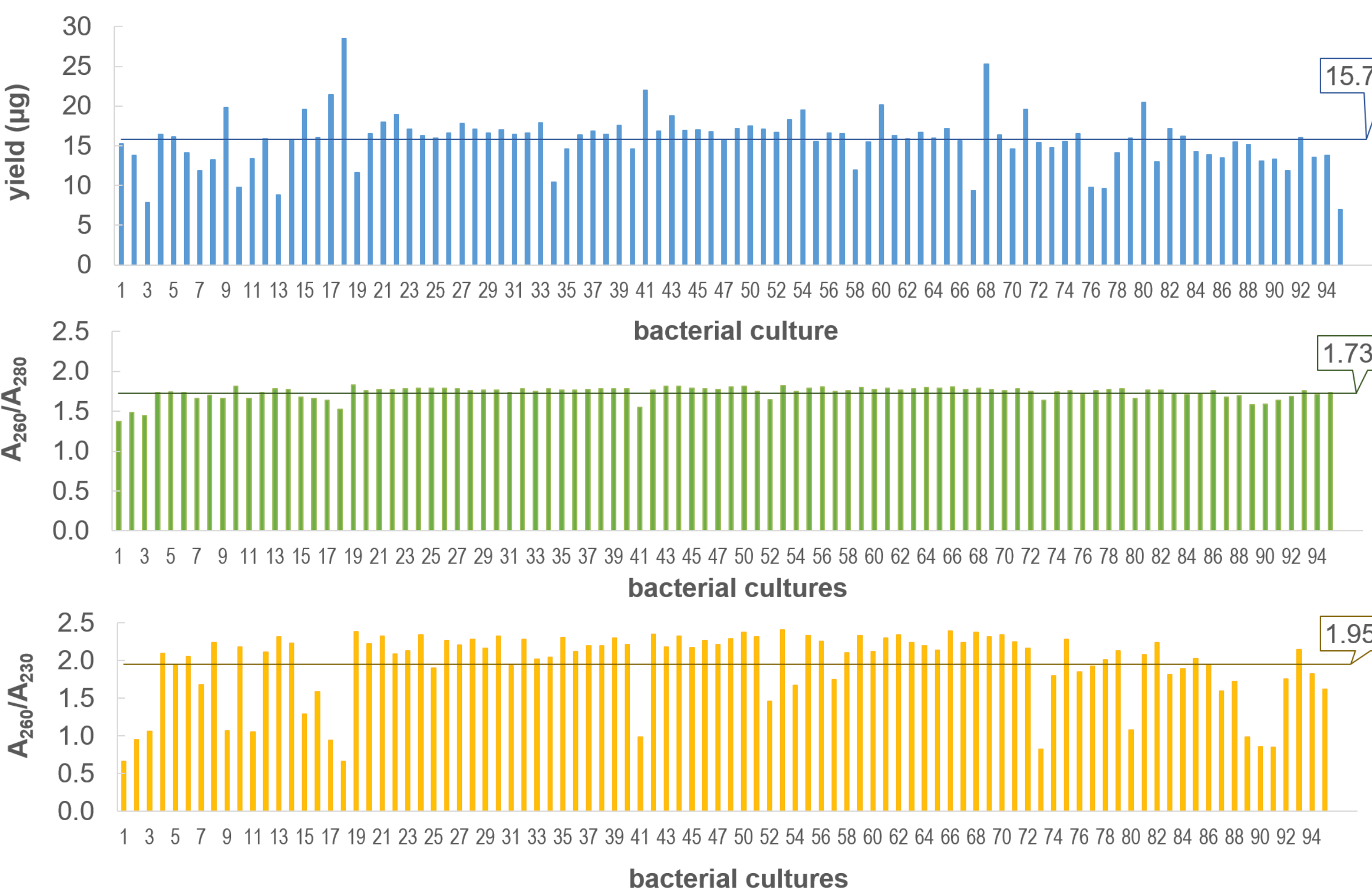
Transform JM109 strain with pGL4.50 → Overnight cultures in LB from 3 different bacterial colonies → 94 x 1.5ml bacterial culture pellets → Apprx 3hr runtime on Tecan Freedom Evo® 150



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.

4. Plasmid Purification from 1.5ml Bacterial Cultures (Innate Pharma Strain)

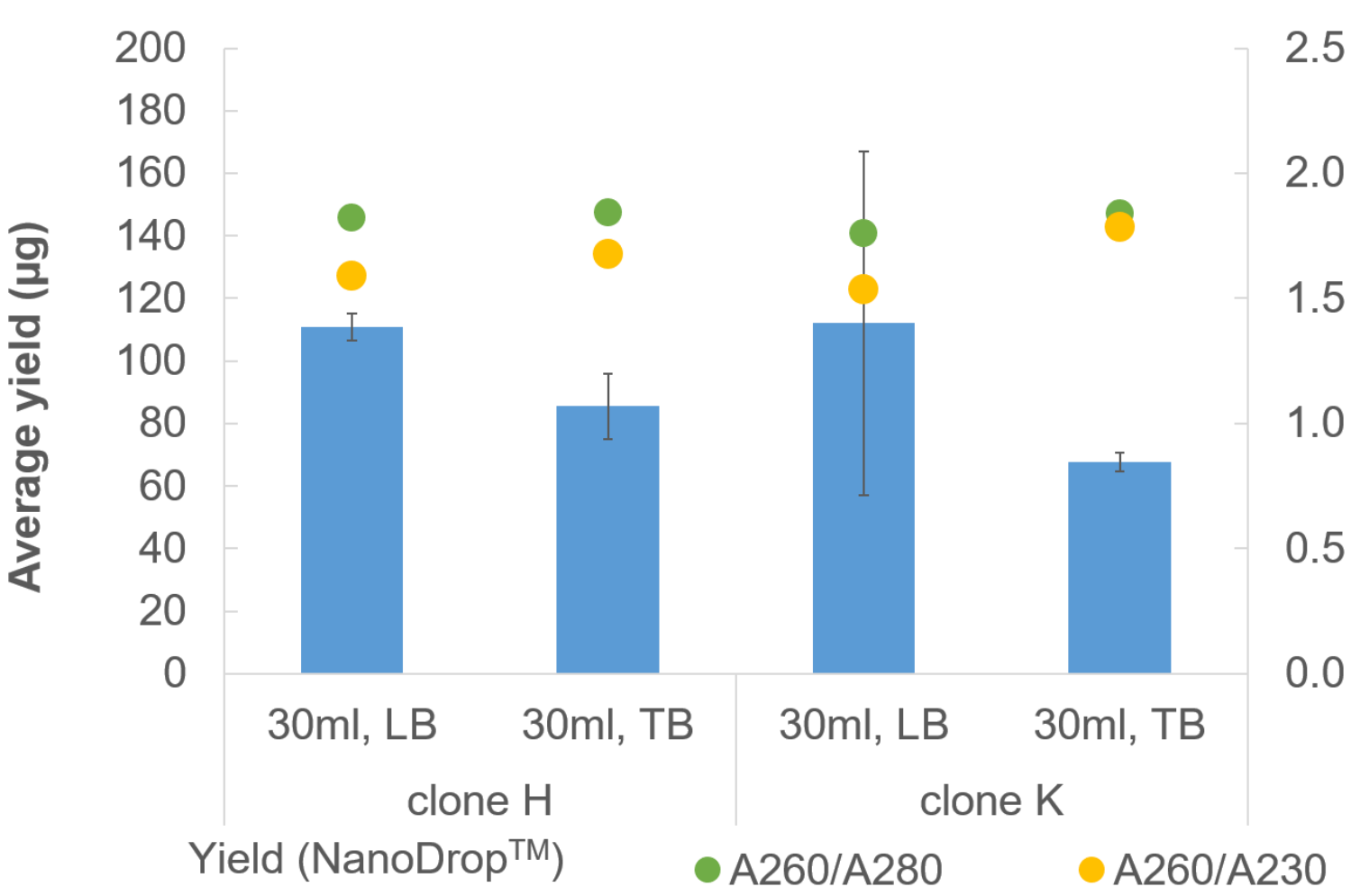
Transform DH10B (endA1) strain with proprietary high copy number plasmids → Overnight cultures in LB → 96 x 1.5ml bacterial culture pellets → Apprx 3hr runtime on Tecan Freedom Evo® 150



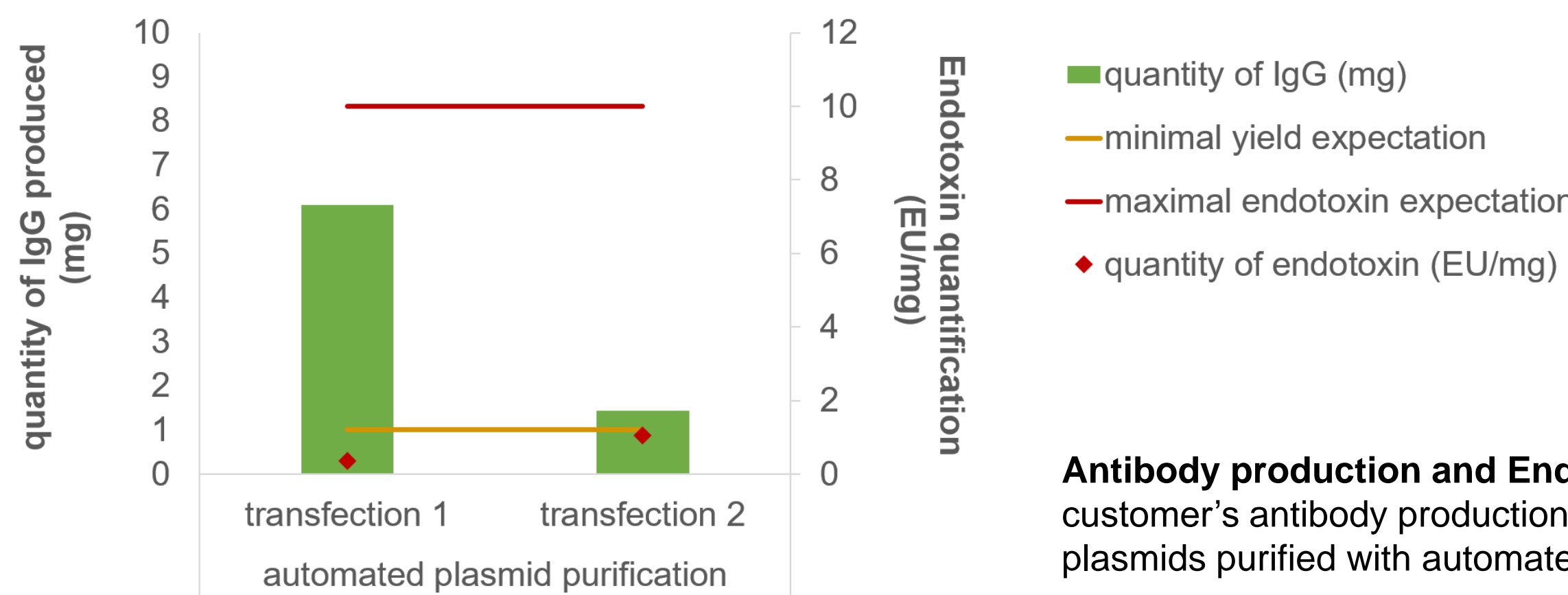
Average yield and purity of plasmid DNA from 1.5ml bacterial culture on a Tecan Freedom Evo® 150. Yield and purity of isolated plasmid DNA were measured by absorbance on a NanoDrop™ One Spectrophotometer.

5. Large Volume Plasmid Purification from 30ml Bacterial Cultures: “MidiPrep” format (Innate Pharma Strain)

Transform HST08 strain with plasmids encoding heavy (clone H) and light (clone K) Ab chains → Overnight cultures in LB or TB → 30ml bacterial culture pellets → Apprx 3hr runtime on Tecan Freedom Evo® 200



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.



6. 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 (“MidiPrep”) volume 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 configuration and materials set-up (i.e., initial centrifugation to generate cell pellet). A dedicated team of automation specialists (FSS) can help you to adapt the protocol to your liquid handler platform.