GINKGO BIOWORKS

High-throughput, Microscale Magnetic Bead Protein Purification on the Ginkgo Reconfigurable Automation Cart (RAC) Platform

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100%	96	10s-100s
Hands-free runtime	Member library purified in 1 hour	µg of protein purified per sample

High-throughput, Microscale Magnetic Bead Protein Purification on the Ginkgo Reconfigurable Automation Cart (RAC) Platform

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Here, we describe onboarding a simple, tip-based, generalized magnetic bead protein purification workflow onto Ginkgo's RAC platform. Configured through Ginkgo's Catalyst software, the same protocol supports diverse magnetic bead-based purifications with different bind, wash, and elute schemes. Using only a minimal device set centered around a compact 96-well liquid handling system, the system processes 96 cell lysate samples to yield 10s–100s µg purified protein in ~1 h and, unlike typical large-deck liquid handling system implementations, is straightforward to scale and requires no hands-on involvement during runtime.

INTRODUCTION

High-throughput protein engineering workflows require efficient processes for characterizing candidate proteins. Biologics drug discovery, protein engineering cycles and rapid prototyping necessitate a consistent automated protein purification protocol to reliably produce clean material for downstream assays from a variety of cell-based and cell-free expression platforms[1].

Most previous published work has focused on semi-automated protocols or requires specialized purpose-built equipment. Our work demonstrates a straightforward robust protocol utilizing general RAC-integrated hardware that can be used alone or together with other protocols as part of end-to-end protein engineering workflows[1,2,3,4].

FULLY AUTOMATED WORKFLOW

The fully automated biological workflow consisted of the following main steps (see **FIG. 1**), previously executed in a partially automated manner (using Hamilton Microlab STAR liquid handling workstation):

1. **Plate setup**: Cell lysate or cell-free expression material, washed magnetic

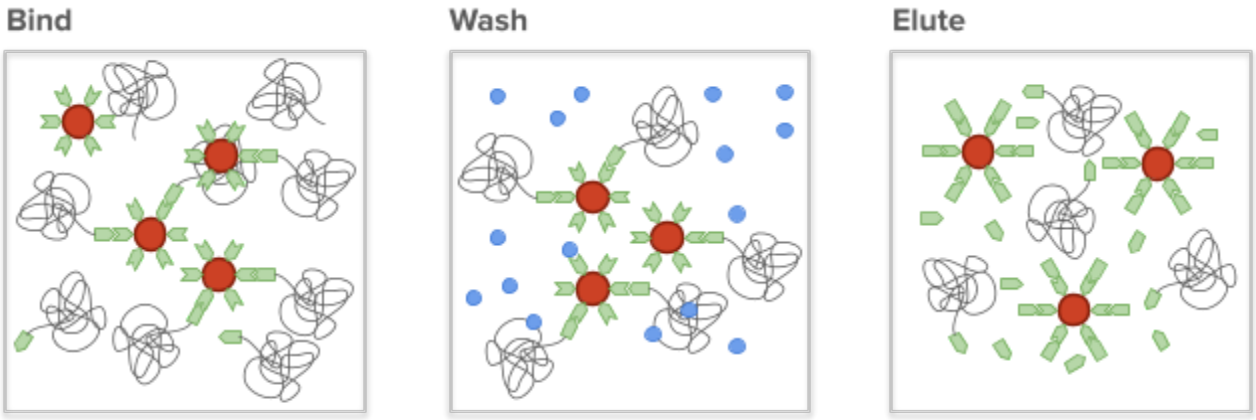


FIGURE 1: Fully Automated Magnetic Bead Protein Purification Workflow

beads, wash buffer, elution buffer, and a waste trough are prepared and positioned in storage.

2. **Bind tagged protein to magnetic beads**: Magnetic beads are incubated with the desired volume (50 µL - 1 mL) of crude protein input. Optional parameterized orbital shaking and/or pipette mixing can be applied to facilitate binding. Post-incubation, onboard magnetic separation permits removal of unbound material.

3. **Wash away unbound material**: Magnetic beads are washed one or more times. Each iteration executes the parameterized number of mix cycles with the provided wash buffer. Washes are removed with onboard magnetic separation and transferred to the provided waste trough.

4. **Elute bound protein**: Protein is eluted from magnetic beads with the parameterized volume of provided elution buffer. Optional parameterized orbital shaking and/or pipette mixing can be applied to facilitate elution. Eluted material is collected.

FIG. 2 shows Ginkgo's Catalyst Orchestrator scheduling results. The run reflects a single re-usable parameterizable protocol capable of executing magnetic bead purification with user-specified parameters, wash buffer and elution buffer. **The protocol run completed in less than 52 minutes, processing 96 samples in parallel without any in-person monitoring and runtime issues. Standard preventative remote monitoring was performed by Ginkgo's Apex Support Team**.

As expected, most of the protocol runtime involved the Agilent Bravo 96 liquid handler, where optimized stamp and mix operations occur once plates have been automatically positioned on the liquid handling deck with the integrated Meca500 (Mecademic Robotics) robotic arm. Plate moves to and from the

FIGURE 2: Magnetic Bead Protein Purification Catalyst Protocol Run completed in under one hour to process one 96-well plate of input protein. Liquid handling steps performed on Bravo 96. Optional orbital shaking included for both bind and elute steps.

magnet pad were flexibly executed by the liquid handler's incorporated gripper through operations orchestrated by Catalyst. Orbital shaking steps were executed on one of the integrated BioShake (QInstruments) orbital shaking RAC accessories. Plates were peeled before arriving to and sealed upon leaving from the Bravo liquid handler. **Overall, full end-to-end automation reduced the runtime by ~50% (compared to the existing semi-automated solution). The generalized nature of the protocol permits users to execute protocol runs with their choice of magnetic beads and buffers based on the intent of the protocol, making it extensible to other purifications, e.g. of nucleic acids. Multiple executions of the protocol can be queued using different protocol inputs and allowed to run hands-free.**

LIQUID HANDLING VALIDATIONS

FIG. 3 shows the plates after completion of a magnetic bead transfer validation. Magnetic beads remained in the magnetic bead plate

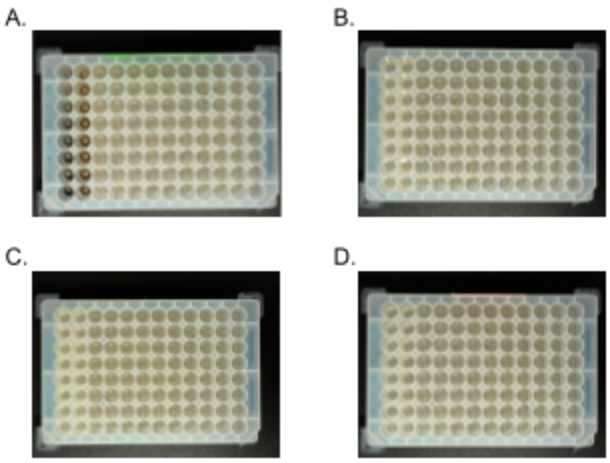


Figure 3: Bead Transfer Validation Beads remained in the bead plate at the end of the protocol, the bead plate (A) with Ni-NTA beads from two different suppliers, load waste (B), wash waste (C), and output (D) were checked for the presence of bead.

and did not migrate to load waste, wash waste, or output plates. One minute of magnetic separation was sufficient to sequester beads

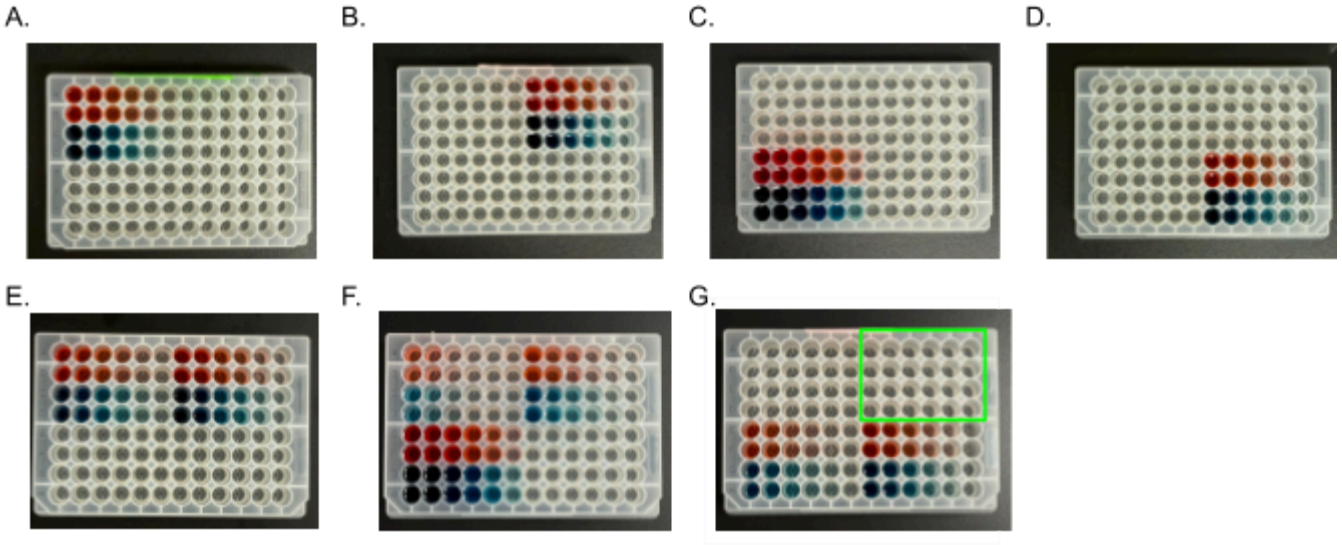


Figure 4: Carryover Test inputs: Bead buffer (A), sample (B), wash buffer (C), elution buffer (D). outputs: Load waste (E), wash waste (F), and output (G). Green box: no carryover from input to output.

during each liquid removal step. **FIG. 4** shows a summary of carryover testing to confirm that washes were sufficient to prevent magnetic bead buffer and input sample from remaining in

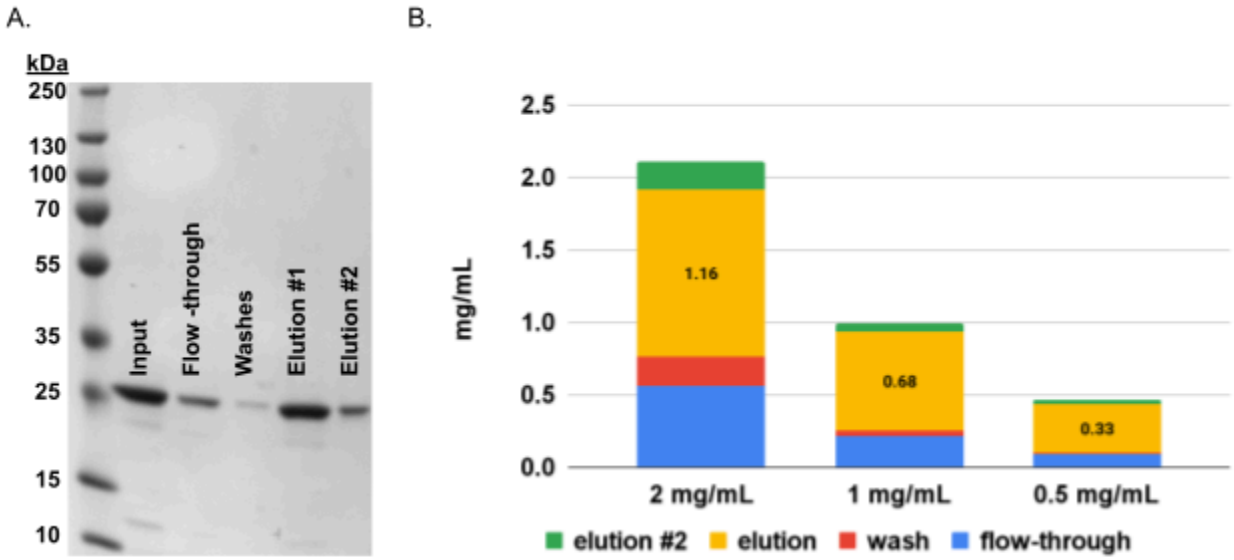


Figure 5: Purified GFP Recovery Representative protein gel showing input, flow-through, washes, automatic elution, and a second manual elution performed off-system (A). Protein recovery saturation test based on fluorescent intensity (B).

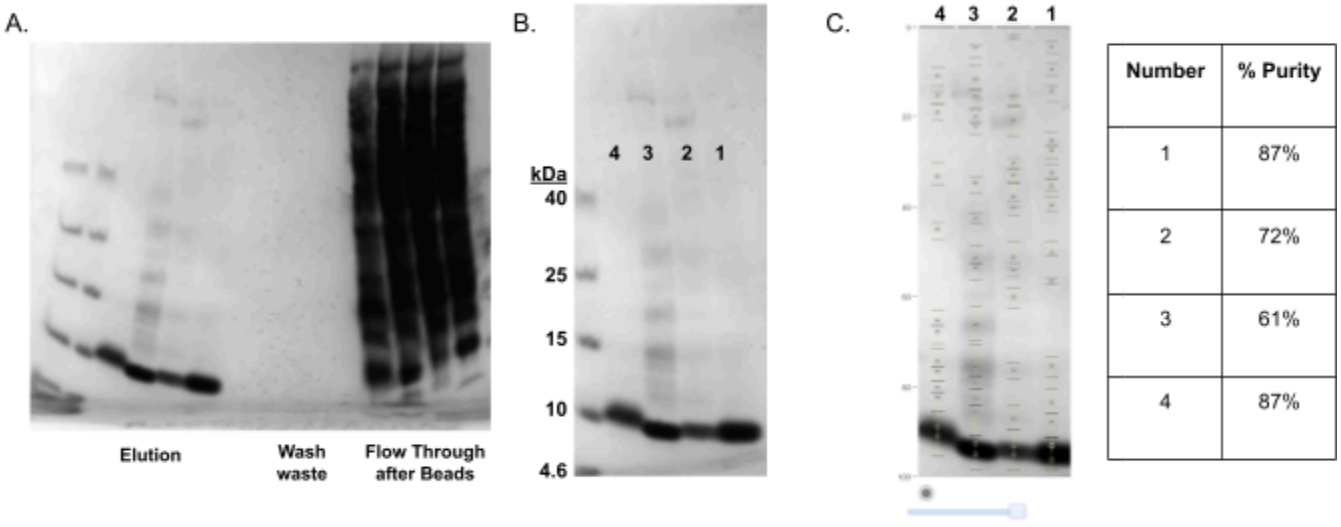
the output samples. Each of four inputs (magnetic beads, input samples, wash buffer, and elution buffer) were labeled with duplicate serial dilutions of red and blue food coloring in separate quadrants. Load waste is expected to show color in magnetic bead and input sample quadrants. Wash waste is expected to show the strongest color in the wash waste quadrant, less in the input sample quadrant, and least in the magnetic bead buffer quadrant. Output is expected to show strongest color in the elution buffer quadrant, less in the wash buffer quadrant, and none in the magnetic bead buffer or input sample quadrants. The lack of color in the input sample quadrant of the output plate confirms that **the wash procedure is effective at minimizing carryover from input sample to output**.

GFP VALIDATION

Purified GFP was subjected to the protein purification protocol to test recovery. A representative protein gel (**FIG. 5A**) shows that the majority of the protein is being recovered in the first elution, with less being lost to flow-through and washes. A second elution was performed and showed that most of the protein is released during the first elution. **FIG. 5B** shows the results of varying protein concentration on recovery fraction. 1 mg/mL represents the manufacturer's suggested binding capacity and yielded a ~68% recovery. Optimization of magnetic bead binding, and eluate mixing and shaking may further improve these results.

E. COLI CELL LYSATE

E. coli cell lysate from strains expressing four different glucose mini-binders (~10 kDa) were next processed using the protocol. Eluates



Number	% Purity
1	87%
2	72%
3	61%
4	87%

Figure 6: Purification of mini-binders from E. coli Protein gel showing elution, flow-through, and wash waste (A). Protein gel showing elution (B) with densitometry analysis (C).

showed 61-87% purity across the set of four samples purified (**FIG. 6C**). In **FIG. 6A**, unbound protein was primarily observed in the flow through material. Wash waste represented the collected volume from two sequential washes and showed far lower concentrations of protein. Eluate showed a strong band at the anticipated molecular weight and corresponding depletion in the flow through.

CONCLUSIONS

Robust small-scale protein purification is a vital step in many biological screening workflows. The automated protein purification workflow outlined herein demonstrates how Ginkgo's RAC platform, equipped with state-of-the-art lab automation equipment, can be leveraged to reliably reduce hands-on time for this critical process.

This protein purification protocol can be chained together with upstream and downstream protocols to enable end-to-end screening workflows. For example, combining protein purification with strain growth, protein production, and cell lysis protocols upstream creates a workflow capable of generating a high-throughput glycerol stock to purified protein pipeline capable of producing material for myriad downstream characterization assays. Scientists can schedule such a pipeline to occur during off-hours allowing their time to be focused on assay development, data analysis, and understanding.

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MATERIALS

- One (1) 96-well half-deep plate filled with input samples.
- One (1) 96-well half-deep plate filled with a wash buffer.
- One (1) 96-well half-deep plate filled with an elution buffer.
- One (1) 96-well half-deep plate to collect waste
- One (1) rack of 96-well 250 uL tips