Automated Workflow for Antibody Production and Analysis on a Robotic Liquid Handling Platform

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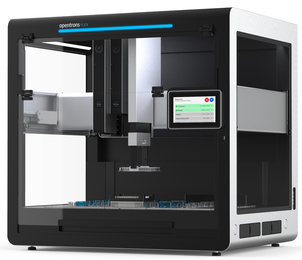
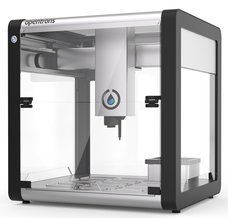
INTRODUCTION

Using a genetically engineered expression vector is a more efficient approach to reproduce a molecularly defined monoclonal antibody (mAb) with long-term stability, compared to hybridoma technology. Automation of the workflow to produce a collection of therapeutic mAb candidates (e.g., full-length IgGs with modified variable regions) for preclinical studies has been of great interest to those at the earlier stage of R&D. Opentrons robotic liquid handler OT-2 and Flex were tested for this purpose.



Opentrons Flex™



OT-2

METHOD

PROTOCOL PROTEIN EXPRESSION

Platform: Opentrons OT-2 with HEPA Module
Workflow:

- Day 1: Prepare HeLa cells in a 6-well plate (*Induction at 37°C and 5% CO_2 off deck*)
- Day 2: Prepare DNA/transfection reagent mixture (pRABBIT IgG IRES-EmGFP Positive Control Vector from Thermo Scientific, Waltham, MA, and FuGENE HD Transfection Reagent from Promega, Madison, WI) and add above mixture to HeLa cell culture (*Induction at 37°C and 5% CO_2 off deck*)
- Day 5: Harvest culture medium (subjected to protein purification)

PROTOCOL PROTEIN PURIFICATION

Platform: Opentrons Flex with Heater Shaker Module and Magnetic Module
Workflow:

1. Prepare Dynabeads Protein G (Thermo Scientific, Waltham, MA) with equilibration buffer
2. Incubate and agitate samples/beads mixture to capture target protein (agitation for 2 hours)
3. Wash x2
4. Elute target protein

Final products subjected to SDS-PAGE and Western Blot or protein quantification

PROTOCOL PROTEIN QUANTIFICATION

Platform: Opentrons Flex with Temperature Module
Workflow:

1. Prepare working reagent (BCA Protein Assay from Thermo Scientific, Waltham, MA)
2. Prepare working plate by adding working reagent, samples and standards into a 96-well plate
3. Incubation (37°C, 30 minutes)

Readouts obtained by measuring absorbances at 560 nm on a plate reader off deck, and protein levels estimated by referencing the standard curve.

RESULTS

HeLa cells transfected with the vector carrying IgG cDNA by automated Protein Expression protocol on OT-2 platform, and success of transfection confirmed by GFP expression visualized by fluorescence microscopy. (Figure 1)

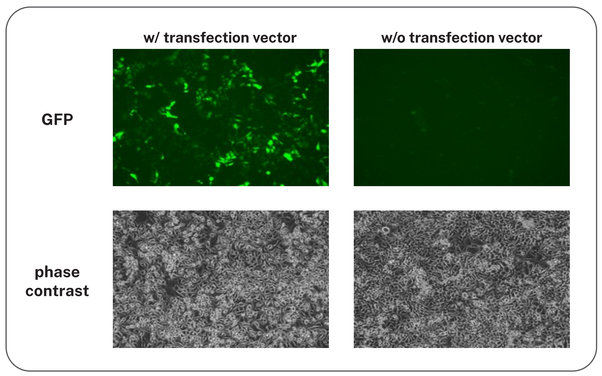


Figure 1. HeLa cells were seeded in a 6-well plate at 70% confluency and then cultured overnight. Transfection master mix per well was prepared by mixing DNA (2 µg) with FuGene Transfection Reagent (6 µL) in serum free (DMEM 100 µL). After 10-minute incubation at room temperature, Protein Expression protocol was run on OT-2 equipped with HEPA module. Transfected cells were cultured for 72 hours, and images captured by EVOS M7000 (Thermo Scientific, Waltham, MA) and culture medium harvested.

Supernatants harvested from transfected HeLa cell culture processed to collect secreted IgG by automated Protein Purification protocol on Flex platform, and purified IgG confirmed by SDS-PAGE and Western Blot (Figure 2).

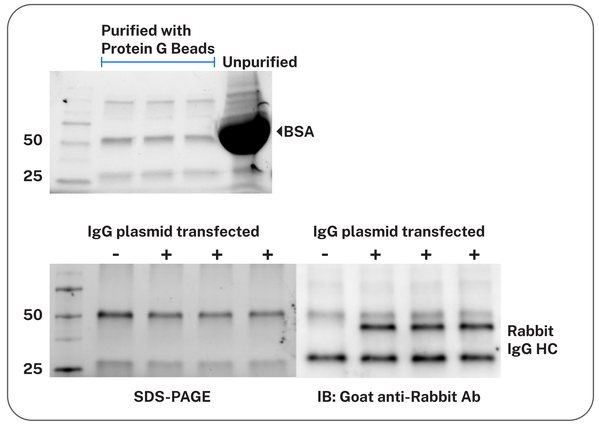


Figure 2. Media harvested 72 hours after transfection were processed on Flex for IgG isolation using Dynabeads Protein G, and eluates subjected to SDS-PAGE and Western Blot. Upper: purified vs. unpurified medium; Lower: the presence of rabbit IgG detected by using IRDye® 680RD Goat anti-Rabbit IgG (LI-COR Biosciences, Lincoln, NE)

PROTOCOL PROTEIN DIGESTION & CLEAN-UP

Platform: Opentrons Flex with Thermocycler Module
Workflow:

1. Reduction: with DTT for 30 minutes at 37 °C
2. Alkylation: with IAA for 30 minutes at room temperature
3. Digestion: with Trypsin for 16 hours at 37 °C
4. Clean-up: digested samples desalted by single-pot, solid phase, sample preparation (SP3) using Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Cytiva, Marlborough, MA)
5. Eluates collected and vacuum dried (off deck)

Final products subjected to LC-MS analysis

BCA protein assay for purified IgG in the final product performed by automated Protein Quantification protocol on Flex platform (Figure 3).

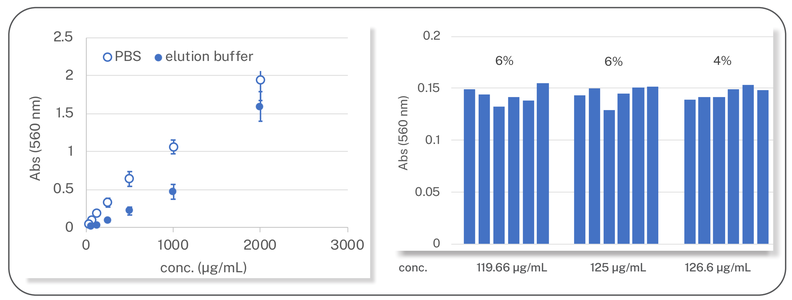
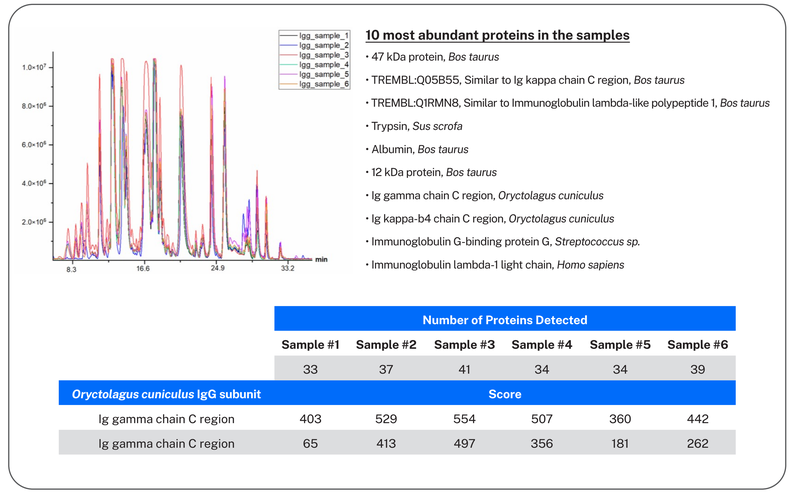


Figure 3. Standard curves were plotted by preparing serial 2-fold dilutions of BSA in PBS or in elution buffer used for Protein Purification protocol (starting concentration: 2000 µg/mL), running the BCA protein assay using 25 µL of diluted BSA (both on Flex), and then absorbances measured using a plate reader. Mean and STD obtained based on n = 4 (Left). Eluates collected by Protein Purification protocol were subjected to BCA protein assay, and protein concentrations estimated. Mean and CV obtained based on n = 6 (Right)

- **Samples processed by Protein Digestion & Clean-up protocol exhibited well aligned base peak ion chromatography patterns (Figure 4)**
- **Rabbit IgG detected in all peptide samples**



	Number of Proteins Detected					
	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
	33	37	41	34	34	39
Oryctolagus cuniculus IgG subunit	Score					
Ig gamma chain C region	403	529	554	507	360	442
Ig gamma chain C region	65	413	497	356	181	262

Figure 4. Six purified rabbit IgG were processed for analysis by Bruker's maXis-II ETD ESI-QqTOF/Dionex Ultimate-3000 LC system at the CUNY Advanced Science Research Center (New York, NY). (Left) Base peak intensity chromatograms (ion current vs. retention time) and (right) proteins detected.

CONCLUSION

A panel of automated protocols utilizing robotic liquid handlers streamlines critical steps in the workflow for recombinant mAb production: protein expression, purification and quantification and sample preparation for proteomics. Our preliminary work has demonstrated both the feasibility and advantages of automating the processes, which include minimal hands-on time and the capability of high throughout sample preparation

B. Lin and K. Watson are current employees of Opentrons Labworks Inc.