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Automated 6-Well Plate Transfection

A Flexible Architecture for CHO Protocols on Lynx Liquid Handlers

30mL Tips Compatible with Lynx 5mL 24-Channel ST or VVP Head

Introduction

As protein targets increase in structural complexity and become more difficult to express, laboratories require transfection workflows that support automated execution of CHO cell transfection workflows. This application note describes an automated CHO cell transfection workflow on the Lynx LM liquid handler for Thomson 6-well plates at volumes 10-40mL per well, supporting automated mid-scale transfection workflows beyond traditional small-volume formats.

The method architecture presented here is compatible with both the VVP and ST head configurations on the Lynx and is designed to accommodate different lab-specific reagent approaches — including master mix formulations and cell culture media-based protocols — without requiring changes to the underlying method logic.

System Requirements

The methods described here are compatible with the following hardware and software:

Parameter	Specification
Instrument	Lynx LM or ST series
Software	MethodManager 4 (MM4)
Pipetting head	5mL VVP (24-channel) or ST head (24-channel)
Tips	Mammoth™ 30mL Automation Tips
Destination labware	Thomson 6-well plates (P/N 931170); Universal Rapid Clear plate (P/N 921547)
Source labware	Reservoir or 6-well plate depending on reagent format
Labware holder	Thomson Universal Rack (P/N 1212605-399)
Cell line	CHO (Chinese Hamster Ovary) or equivalent suspension cell

Workflow Overview

The workflows described here use the same three-stage architecture, all transfection protocols typically share the same three-stage workflow. The specific volumes, source labware, and media used at each stage differ between labs, but the method logic remains constant.

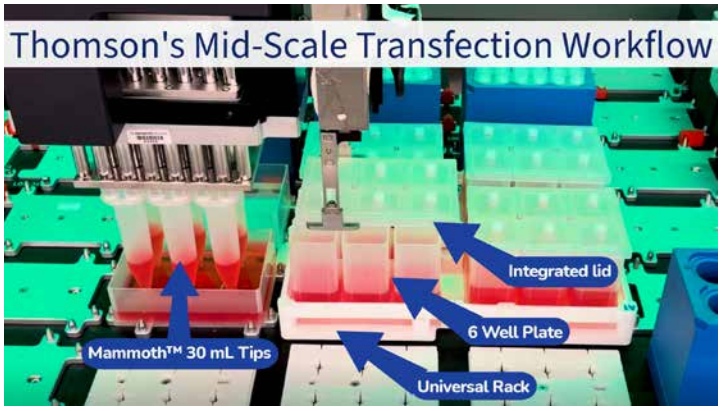
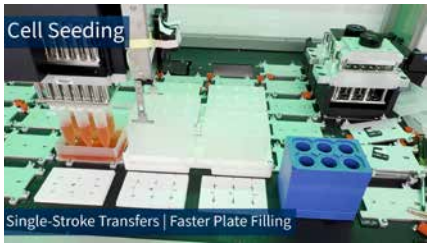


Figure 1. Deck setup for the mid-scale transfection workflow: Mammoth™ 30mL Tips, integrated lid, 6-well plate, and Universal Rack.

1. Plates seeded with 20mL of cells in preparation for addition of transfection complex.
2. Transfection complex prepared according to appropriate concentrations prior to placement on deck.
3. The Universal 4-Position Rack supports movement of multiple plates at once.
4. Once transfected, cells are moved to shaker incubator until first feed.
5. For Day 3 and Day 5 feeds, plates are returned to the deck for the automated feeding protocol.
6. Transfer samples into 24-Well Universal Rapid Clear® Filter Plates for at-line characterization." "Real-time.
7. Fresh feed added according to experimental parameters.
8. Harvest – centrifuge 6-well plate at 3,000 rpm, in a swinging bucket rotor ready for lysate or supernatant processing.



Cell seeding and transfection-complex addition on the Lynx platform

Batch handling with the Universal 4-Position Rack and shaker incubation..

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Stage 1–3 Workflow

Stage 1 — Seed Plate (Day 0)	Stage 2 — Add Transfection Complex (Day 0)	Stage 3 — Feeding (Day 3/ Day 5)
Load Mammoth tips	Load Mammoth tips	Load Mammoth tips
Aspirate freshly diluted cell culture from reservoir	Aspirate DNA/transfection complex or master mix from source	Aspirate fresh feeding media from source
Dispense media to 6-well plate	Dispense complex to 6-well plate	Dispense to 6-well plate
Eject tips ➔ replace lid	Optional: Mix after dispense	Optional: Mix after dispense
	Eject tips	Eject tips
	Replace lid ➔ move rack to incubator	Replace lid ➔ move to incubator

All stages use the same Mammoth™ tip format. Multi-pass dispensing is applied automatically by MM4 when total volume per well exceeds the 20mL per-pass software limit.

DNA Complex Preparation

The transfection complex is prepared by pre-diluting DNA and transfection reagent separately in fresh cell culture media, then combining them prior to addition.

Format	How Complex Is Prepared	Diluent/Media Used	Typical Source Labware
Cell Culture Media-Based	DNA and transfection reagent pre-diluted separately in fresh cell culture media, then combined	Standard cell culture media (e.g., CD CHO, ExCell, HyClone)	Two separate source reservoirs (DNA+media and reagent+media), combined on-deck or pre-combined in a third vessel

This method runs on the same MM4 method structure regardless of how the master mix is prepared. The operator specifies source positions and volumes at runtime via User Input prompts in MM4; no method re-authoring is required to adapt the protocol to a different reagent preparation approach.

MM4 Method Architecture

All three stages are implemented as discrete ".met" files within MM4. This modular design allows each stage to be run independently or sequenced together in a master method and makes it straightforward to adapt individual stages to a new reagent format without modifying the others.

Formula-Driven Volume Calculation

The operator enters two runtime values via User Input prompts at the start of each method run:

- PlateNum — number of 6-well plates to process (1–4)
- VolTransfer — volume to deliver per well, entered inmL MM4 Expression steps automatically derive all downstream volumes from these inputs:

Expression Variable	Calculation	Purpose
VolTransfer_uL	VolTransfer × 1000	ConvertsmL operator input to µL for MM4 pipetting steps
NumPasses	CEILING(VolTransfer_uL / 20000)	Splits transfers > 20mL into multiple aspirate/dispense cycles
PassVolume	VolTransfer_uL / NumPasses	Equal volume per pass delivered to each active channel
ComplexVolume*	Site-defined ratio of VolTransfer_uL (e.g., 16.5% for master mix format)	Scales Stage 2 volume proportionally to the total transfer volume

*ComplexVolume ratio is set by the lab at method setup time and stored as a constant in the Expression step. It does not require operator input at runtime.

Worked Example

For a 30mL/well transfer:

- VolTransfer = 30mL
- VolTransfer_uL = 30,000 µL
- NumPasses = CEILING(30,000 / 20,000) = 2
- PassVolume = 15,000 µL
- Result: two 15mL transfers per well

Plate Loop and Dynamic Labware Naming

Each method iterates through the specified number of plates using a plate loop in MM4. String Builder steps inside the loop construct labware names dynamically (e.g., Cells_01, Cells_02) so the correct deck position is referenced for each plate without hardcoding. This enables processing of 1–4 plates from a single method file with no manual editing between runs.

Multi-Pass Dispensing

The Mammoth™ Automation Tip aspirates up to 20mL. MM4 software caps aspiration at 20mL per pass. For total well volumes above 20mL, the NumPasses expression automatically breaks the transfer into equally divided cycles within the same loop iteration, delivering the full target volume before advancing to the next plate.

Deck Configuration

The deck is organized into three functional zones. Source positions for cells, complex, and media are defined at method setup and can be adjusted without modifying the method logic.

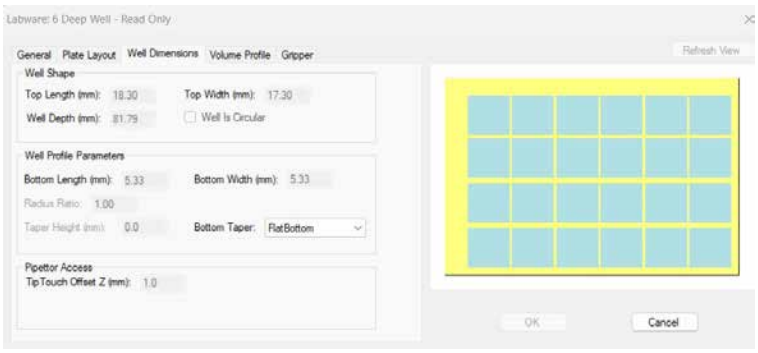
Zone	Labware	Description
Left	Mammoth™ 30mL Automation Tip boxes (Stage 1, cells)	One box per plate, loaded sequentially through the plate loop. Both VVP and ST heads are 24-channel; 6 tips are loaded and the 6-well plate is defined as a 24-well labware in MM4 to satisfy pipettor access requirements.
Center	Thomson 6-well destination plates (DwP_01–04)	Target plates for all three stages. Well geometry accommodates Mammoth tip directly, eliminating intermediate vessel transfers.

Zone	Labware	Description
Center	Cell source (reservoir or 6-well plate)	Cell stock in suspension. Mixing is triggered before aspiration to ensure homogeneity.
Center	Complex/master mix source	DNA and transfection reagent sources – pre-diluted in media, or premixed off-deck as a master mix. Reservoir or 6-well plate.
Right	Mammoth™ 30mL Automation Tip boxes (Stage 2/3)	Separate tip supply for complex and media transfer steps. Active tip box is advanced automatically through the plate loop.

Head Compatibility

The workflow is compatible with both the VVP 24-channel and ST head configurations on the Lynx. Method structure remains the same across both head types, but volume logic differs: the VVP head supports independent variable-volume dispensing across all 24 channels, while the ST head delivers the same fixed volume across all 24 channels per stroke. The 6-well plate is defined identically in MM4 for both head types.

Head Type	Tips Loaded	6-Well Labware Definition
VVP 24-channel (5mL)	6 tips loaded into the 24-channel head	Due to the unique tip geometry and pipettor access constraints in MM4, the Thomson 6-well plate must be defined as a 24-well labware. Each physical well is represented as a 2×2 block of virtual positions (A1, A2, B1, B2), where all four positions correspond to a single well. One tip physically enters the well; the full 2×2 block declaration is required for the software to correctly resolve pipettor access.
ST head (24-channel)	6 tips loaded into the 24-channel head	Same 24-well labware definition applies. Each physical well is declared as a 2×2 block of virtual positions (A1, A2, B1, B2) to satisfy MM4 pipettor access requirements. One tip physically enters the well per transfer.



Figures 2a–b. MM4 Labware Manager — Thomson 6-well plate defined as a 24-position labware (General tab and Well Dimensions tab).

Technical Data Sheet

Field	Value	Description
Labware Name	6 Deep Well	Name as defined in MM4 labware library
Manufacturer (Mfg.)	TIC	Thomson Instrument Company
Columns	6	Matches 3-column physical layout ×2 virtual sub-positions per well
Rows	4	Matches 2-row physical layout ×2 virtual sub-positions per well
Length (mm)	127.8	Plate outer length
Width (mm)	85.5	Plate outer width
Height (mm)	88.0	Plate height
Z Clear (mm)	10.0	Z clearance above plate for head movement
Stack Height (mm)	20.3	Used for stacked plate offsets
Top Length (mm)	18.30	Subdivided well opening length
Top Width (mm)	17.30	Subdivided well opening width
Well Depth (mm)	81.79	Full physical well depth, unchanged by virtual subdivision
Well Is Circular	No	Square well geometry
Bottom Length (mm)	5.33	Subdivided bottom profile length
Bottom Width (mm)	5.33	Subdivided bottom profile width
Bottom Taper	FlatBottom	Bottom taper type as defined in MM4
Taper Height (mm)	0.0	No taper height for flat bottom
TipTouch Offset Z (mm)	1.0	Pipettor access Z offset

Key Performance Attributes

- Walk-away execution after two operator inputs at run start supports total working volumes of 10–40mL per well, with transfers above 20mL automatically divided into multiple aspiration/dispense cycles by MM4 – no manual calculation required
- Dynamic plate naming supports 1–4 plate runs from a single ".met" file
- Works with cell culture media-based or master mix formats
- Compatible with both VVP and ST 24-channel head configurations
- Thomson 6-well plate geometry accommodates Mammoth tip directly – no intermediate transfers
- Modular ".met" file structure allows individual stages to be run or adapted independently

Conclusion

Demand for mid-scale expression workflows continues to grow as biologics programs pursue more difficult protein targets. Thomson 6-well plates operating at 10–40mL per well, combined with Mammoth™ Automation Tips, make this workflow practical on a automated liquid-handling platform. By requiring only minimal operator input and using formula-driven calculations with automatic multi-pass dispensing, the method uses formula-driven calculations and automated multi-pass dispensing to support consistent method execution. The result is a flexible, automated CHO cell transfection workflow that better connects early discovery processes with larger-scale protein production needs.

Ordering Information

Product	Part Number
Thomson 6-Well Plate	931170
Mammoth™ 30mL Automation Tips	9453030
24-Well Universal Rapid Clear® Filter Plate	921547
24-Well Plate I Square Well Round Bottom	931565-G-1X
24-Well Plate I Square Well Round Bottom	931568
24-Well Plate I Square Well Pyramid Bottom	931569-G-1X
24-Well Plate I Square Well Pyramid Bottom	931571
Universal 4-Position Rack	1212605-399

For technical support or to request a method demo, contact Thomson Instrument Company at www.htslabs.com