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Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR) for Recombinant Proteins: Litre-Scale Expression of SARS-CoV-2 Nucleocapsid C-terminal oligomerization domain for Structural Biology and Drug Design (SBDD)

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We use this protocol and it's working

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Disclaimer

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Abstract

This study presents a scalable method for recombinant expression of SARS-CoV-2 nucleocapsid protein C-terminal oligomerization domain using single-use bubble column reactors (suBCRs) for structural biology and drug discovery applications. The nucleocapsid protein is essential for viral assembly, RNA packaging, and regulation of viral transcription/replication, making it a critical therapeutic target for COVID-19 interventions. The expression system utilized parallel 1L E. coli batch cultivation with auto-induction Terrific Broth media, controlled aeration (1 lpm), and optimized temperature management transitioning from 37°C growth phase to ambient temperature for overnight protein expression.

The protocol successfully achieved high-density cultures with final OD_{600} values of 11.8, yielding 149.1g wet cell weight from 6L total culture volume within 20-24 hours from inoculation to harvest. Quality control via magnetic bead purification and SDS-PAGE analysis confirmed successful overexpression of the target construct. This suBCR approach offers significant advantages over traditional shake-flask methods by reducing labor requirements while enabling parallel processing of multiple protein variants. The resulting protein quantities are sufficient for crystallography studies, structural biology research, and structure-based drug design efforts, providing a robust platform for therapeutic target validation and development of COVID-19 interventions targeting this essential viral component.

Attachments

PAGE24-01220 (1).pdf

1.6MB

Materials

Media (auto-induction):

Formedium AIMTB0310 **https://formedium.com/product/aim-terrific-broth-base-including-trace-elements/**

Ni-NTA Magnetic Beads (42179.02) from Serva (**https://www.serva.de/**)

Lysis reagent: FastBreak (V857C or V8571) from Promega (https://www.promega.co.uk/)

Protocol materials

SARS-CoV-2 isolate : Wuhan-Hu-1 Nucleocapsid Protein C terminal **addgene Catalog #**228641

Troubleshooting

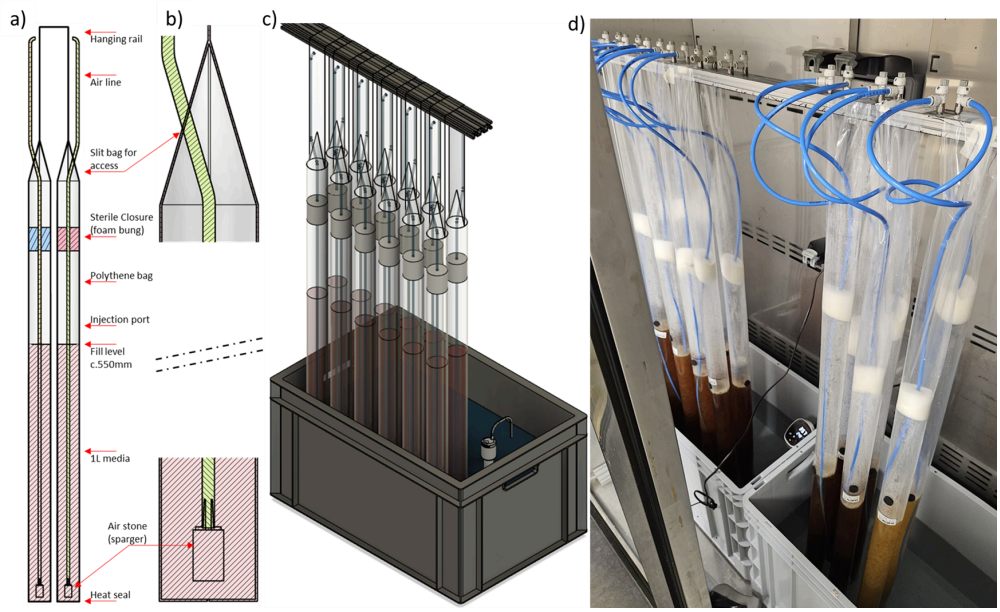
Before start

The reader should be familiar with the companion protocol: "The Manufacture and Setup of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD)."

Culture Main Bags

4h 32m

1



Bag setup: a) Guidance for Bag manufacture and assembly, b) Arrangement of the Bags over the hanging rail, c) A Bag Array in operation.

Bag set-up a) Bags removed from water bath for illustrative purposes, b) a typical bubble swarm and foam layer, c) close-up of the Bags in the water bath with heaters, d) a heat map showing water bath heating of the Bags;

with heaters, d) a heat map showing water bath heating of the Bags, left-to-right Bags acclimating.

2 **Prepare and autoclaved Terrific Broth Base including Trace elements (Formedium AIMTB310)**

2.1 To each litre of sterilised media:
Add 1 mL 10% Antifoam 204
Add 1 mL 1000x antibiotic stock (e.g. Kanamycin 50mg/ml)
Add 20 g glycerol

3 **Equilibrate the incubator bath:**

3.1 Start the immersion heater program 37 °C 06:00:00

3.2 Allow incubator to reach set temperature.

4 **Loading and equilibrating the BCR bags:**

4.1 Add 1 L prepared AIM TB media to each BCR bag.

4.2

The bag with airline inserted, and a funnel being used to demonstrate the filling process.

4.3 Transfer BCR bags to incubator.

4.4 Insert sparger stones into BCR bag, making sure they are at the bottom of the BCR.

4.5 Attach sparger stones to air control valves. Set air flow, 1 lpm.

4.6 Allow bags to equilibrate.

5 **Inoculate Media:**

5.1

Note

If cultures are innoculated by late morning biomass growth can be monitored in person. If choosing to manually induce protein expression by IPTG this can be done before the end of the work day.

Expression can run unattended overnight.

5.2 Add 10 mL starter culture, per L main culture.

6 **Biomass Growth Phase:**

6.1 Continue incubation 37 °C , 04:00:00 (approximately). 4h

Note

Incubation at 37 °C should continue until the culture has entered the exponential growth phase. Typically this takes 4-6 hours given the conditions described. Autoinduction media will trigger protein expression once all the glucose in the media has been consumed, this happens at OD600 of between 3-5 in most cases. The incubation temperature should be reduced before the autoinduction point to promote protein solubility.

7 **For protein expression we used the following plasmids:**

SARS-CoV-2 isolate : Wuhan-Hu-1 Nucleocapsid Protein C terminal **addgene Catalog #**228641

7.1 Incubate (ambient) Overnight (approximately 16 h)

- [EndPoint] ΔOD600≈0

Note

Without external cooling cultures' metabolic activity will heat the culture to 25-27 °C . If working with proteins with temperature sensitive expression, an external recirculating chiller can be connected to the water bath.

7.2

Note

- A typical culture, having been innoculated at between 10-12 pm on day 1, can be harvested in the morning of day 2. This gives a total culture time of 20-24 hours.
- Culture times of up to 36 hours have been tried successfully, but this is likely to be cell/protein specific.
- Short expression experiments can be performed with a c.3 hour induction period following the addition of IPTG.
- Culture biomass can be measured by taking Optical Density readings at 600 nm in a spectrophotometer. The relationship between OD600 and biomass is non-linear at high concentrations. If using a fixed path-length or cuvette, dilute samples such that readings remain below 1 OD unit.
- If using autoinduction media and cell growth appears to have stalled below OD600=5, IPTG can be added to ensure induction has occurred.

7.3

Note

- In rich media biomass will continue to increase after induction.
- Once OD600 values plateau late log phase has occurred, and cell harvest must occur before OD600 begins to drop when death phase begins.

8 **Harvest:**

8.1 Take final OD600 reading

8.2 Collect 1 mL culture samples for diagnostic testing

8.3 Transfer culture to 1 L centrifuge bottles

Note

The Bags are long and flexible, decanting by pouring culture out from the top of bags can lead to spillage.

Place Bags into a large jug or similar. With a sharp implement, make a small incision in the bag and allow it to drain into the jug.

There is a lot of hydrostatic pressure on the liquid at the bottom of the Bags when full. To avoid splashing make incisions towards the top of the liquid level at first. As the culture drains, larger incisions can be made at the bottom to drain all liquid and sediments.

8.4 Pellet the cell, 4000 rcf, 4°C, 00:30:00 30m

8.5 Drain spent media and sterilise with 1 tablet/L Virkon

8.6 Transfer cell pellet to cryo-safe sample bags. Mark with full and clear tracking information.

8.7 Record wet cell weight (g)

8.8 Flash freeze the cell pellet in liquid nitrogen.

8.9 Store cell pellet -80 °C

9 **Overexpression QC:**

9.1

Note

Using Nickel Magnetic Beads perform a rapid over-expression assay. The protocol described here is abridged and achieves only incomplete cell lysis, but can be used to varify over-expression in 5-10 minutes, plus gel running time.

9.2 Transfer 1 ml of culture sample to a microcentrifuge tube go to step #8.2

9.3 Add 100 µL lysis reagent to the sample

Note

If biomass is low or protein expression levels are expected to be low, take a larger culture sample and pellet it first. This will increase the target protein yield but you may need to check sufficient lysis has occurred.

9.4 Incubate Room temperature 00:02:00 in a tube rotor

9.5 Pellet the cell debris at maximum speed in a microtube centrifuge
16000 rpm, 00:02:00

Note

There is no need to spin at 4 °C unless you plan to use the eluted protein for other purposes than QC SDS-PAGE.

9.6 Aspirate soluble fraction (**mixed proteins**) with pipette

9.7 Add 50 µL magnetic IMAC beads to the soluble fraction (**mixed proteins**)

9.8 Incubate Room temperature 00:05:00 in a tube rotor

9.9 Incubate Room temperature 00:01:00 in a magnetic separator stand

9.10 Aspirate soluble fraction (**unbound material**), reserve for SDS-PAGE analysis.

9.11 Wash the retained beads 2 mL binding buffer 00:01:00

9.12 Incubate Room temperature 00:01:00 in a magnetic separator stand

9.13 Aspirate soluble fraction (**unbound material**), reserve for SDS-PAGE analysis.

9.14 Add 50 µL elution buffer to the mag beads 2m

9.15 Incubate 00:02:00 Room temperature

9.16 Incubate 00:01:00 in a magnetic separator stand

9.17 Aspirate soluble fraction (**eluted protein**) with pipette

9.18 Measure the A280 using a spectrophotometer (e.g. NanoDrop)

9.19 Run an SDS-PAGE gel, to check for over-expression and quality control

Results:

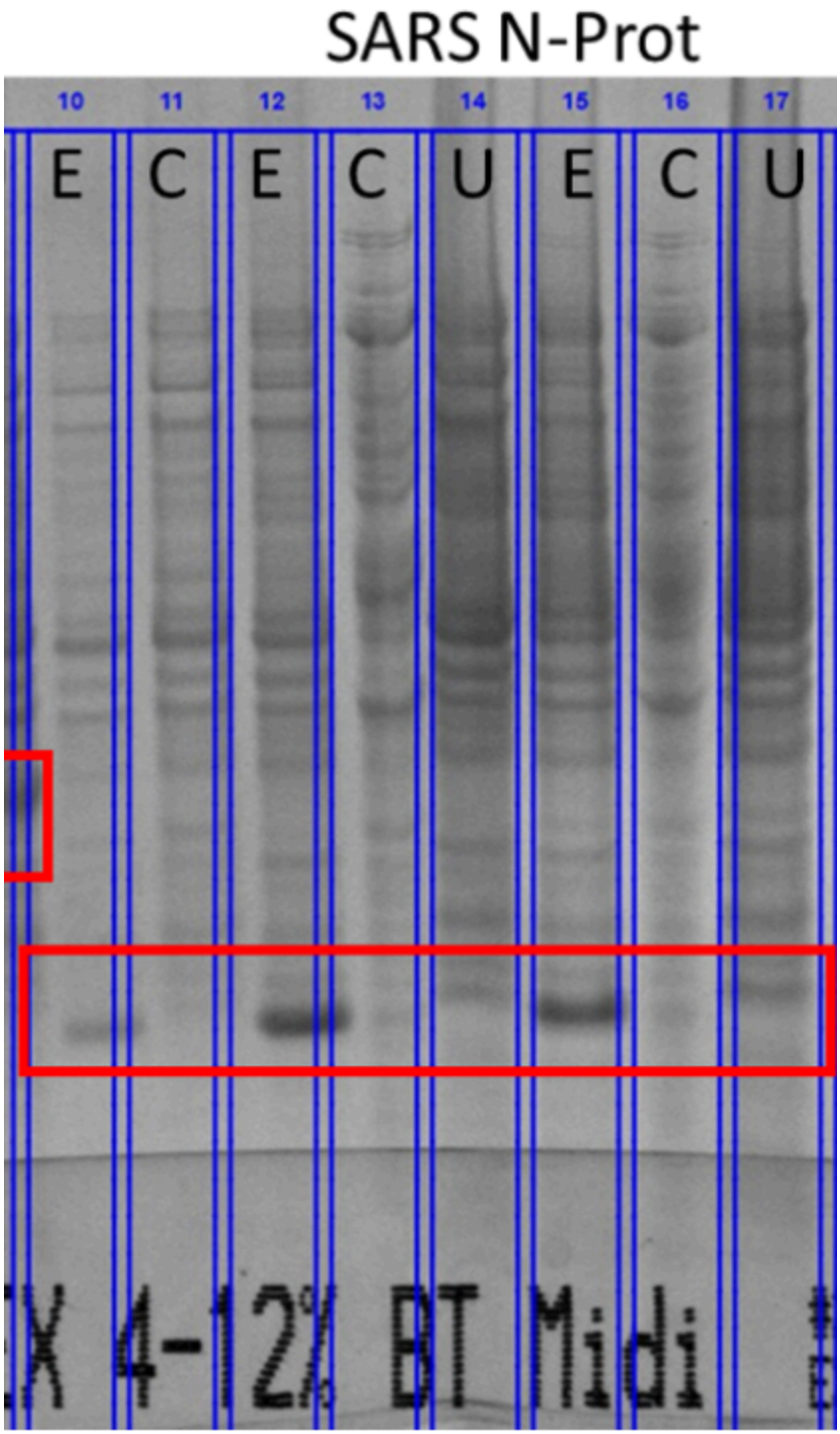
10 1220Tracking IDs and Yields:

	A	B	C	D	E	F	G	H	I	J
	ELN title	Target ID	Clone ID	Construct ID	Expression ID	Culture Total Volume (L)	OD600	Pellet wet cell weight (g)	SDS-Page: Test purification mag prep (pass)	Expression growth (pass)
	PAGE25-01220	CVNPRO	CVNPRO-k001	CVNPRO-c001	CVNPRO-e001	6	11.8	149.1	Pass	Pass

Id for internal reference and yields achieved for both N Protein C terminus construct used in this protocol.

11 Annotated SDS-PAGE gel of Mag Prep test purifications for SARS-CoV-2 N Protein C-terminal domain protease:

Expected result



Bands showing N Protein C-terminal domain SDS-PAGE gel for 3-12% BT