AB Bioscience Internship Reflection – Acquisition of antibody variable region sequence from hybridoma

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Table of Contents

Abstract

Hybridoma-derived monoclonal antibodies (mAbs) remain foundational in discovery, but long-term supply is risky due to genetic drift and the presence of myeloma-derived aberrant chains from fusion partners such as FO cells. This project sequenced the variable regions (VH/VL) from five hybridoma antibodies acquired from Leadgene's collection (Sample IDs 054, 080, 122, 140, 147), confirmed productivity and monoclonality, and prepared CHO-ready constructs by cloning VH into a CMV-driven pHDM-G backbone and VL into pCAGGS (CAG promoter). To date, eight of the ten chains are sequence-verified: three pairs (054, 080, 122) are ready to proceed, and two heavy chains (140H, 147H) remain. This paper presents the project objective, lab workflow (PCR, directional restriction cloning, colony screening, Sanger/IgBLAST), troubleshooting (temperature-sensitive PCR, impure amplicons, failed transformations, and an internal EcoRI site), and reflections. The rationale—locking the exact binder, avoiding hybridoma liabilities, and leveraging CHO's scalability.

Introduction & Background

Hybridomas are immortal antibody-secreting cell lines created by fusing an antigen-specific B cell to a myeloma (plasma-cell) fusion partner, that enabled monoclonal antibodies of predefined specificity. The myeloma partner is typically engineered so only fused hybrids survive and the commonly used partner is the FO mouse myeloma line. While hybridomas are invaluable for discovery, they are imperfect for manufacturing because they can drift genetically and phenotypically and may co-express aberrant light-chain transcripts from the myeloma partner, which can mis-pair with the heavy chain and compromise specificity and product homogeneity. Accordingly, best practice is to sequence VH/VL, confirm a single productive pair (monoclonality), and re-express recombinantly in a well-characterized mammalian host. Chinese Hamster Ovary (CHO) cells have become the dominant host for therapeutic mAb production due to robust suspension growth, high titers in serum-free media, and human-compatible glycosylation profiles that are familiar to regulators. These attributes also make CHO an excellent fit here: once we lock the authentic VH/VL from each hybridoma, we can express them in a scalable, reproducible system.

Project Aim

The aim is to “lock” the exact binder for each clone by obtaining sequence-verified, productive VH/VL and demonstrating monoclonality (one IGH plus one IGK/IGL). Sequences are annotated with

IgBLAST to call V(D)J segments, define framework (FR) and complementarity-determining regions (CDRs), and confirm productivity (in-frame, no premature stops).

Methods

For sequencing, light-chain targets were amplified with designed primer pairs (1014/1015) and heavy-chain targets with primers 1016/1018, using 2× Q5 hot-start high-fidelity polymerase. Gradient or touchdown annealing (typically 55 °C, with targeted exploration of 52–60 °C for difficult templates). Products were checked on 1% agarose for the expected ~320–360 bp (VL) and ~350–400 bp (VH) bands, cleaned by gel extraction or spin-column as needed, and bidirectionally Sanger sequenced. IgBLAST was used to assign V/J (or V/D/J). For cloning, VLs were inserted into pCAGGS (CAG promoter) and VHs into pHDM-G (CMV-IE promoter) using a directional restriction-enzyme scheme (VL: SacI/XhoI; VH: EcoRI/SalI) to enforce orientation and reduce background. Ligation products were transformed by 42 °C heat shock into high-efficiency E. coli, plated on ampicillin, and isolated single colonies were picked. Plasmid minipreps were quality-checked (NanoDrop, diagnostic digests and/or colony PCR) and verified by Sanger sequencing of the full insert in the expression backbone.

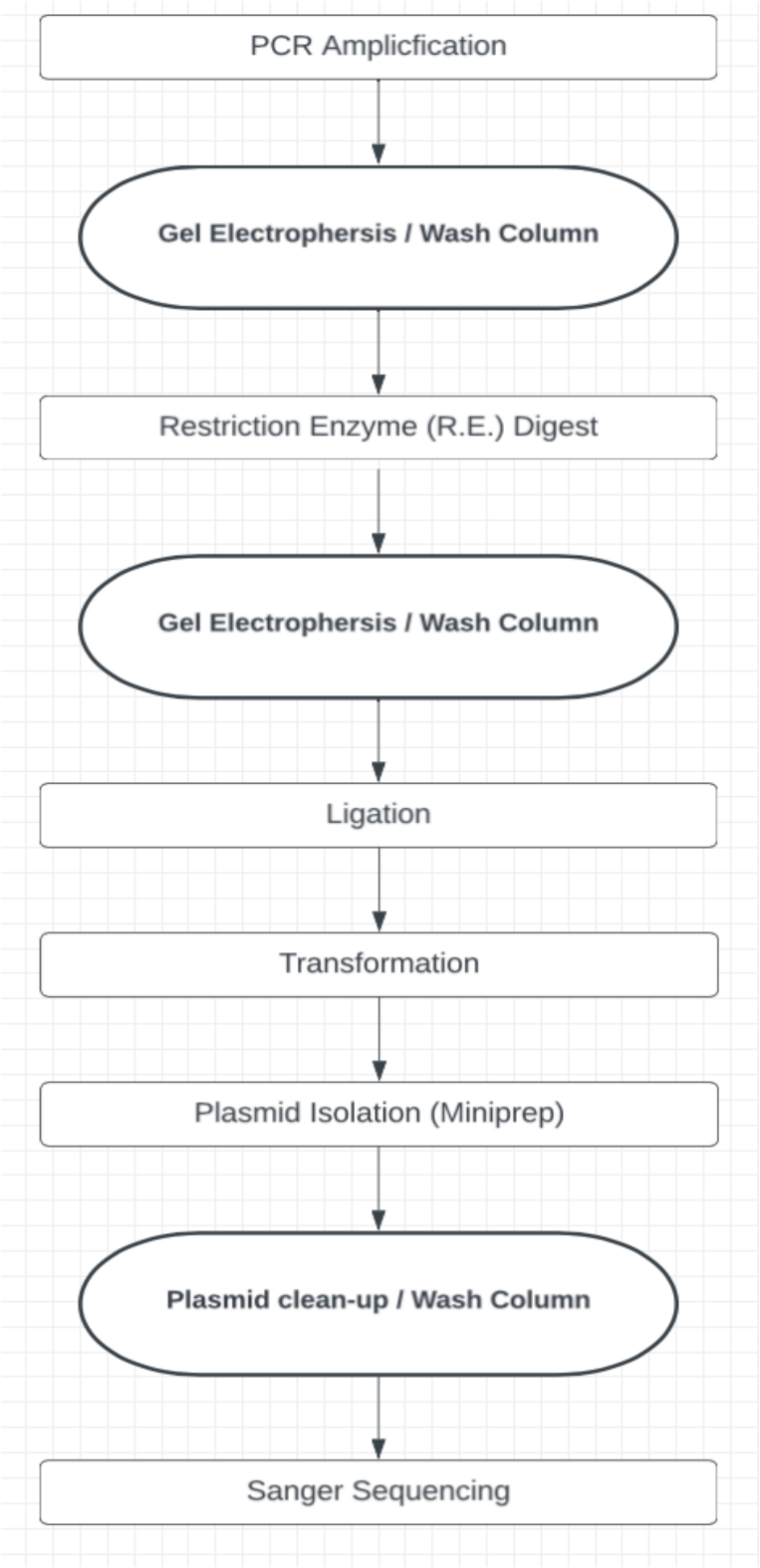

Figure 1. Flow Chart of procedure

1. PCR Amplification

a. Preparation

i. Primers (eg. 1014/1015 [10mM] for light chain and 1016/1018[10mM] for heavy chain)

ii. cDNA

iii. H2O

iv.

Reagent	Volume
NEB 2x Q5 HF	12.5 uL
1014 (10mM) || 1016 (10mM)	1.25 uL
1015 (10mM) || 1018 (10mM)	1.25uL
H20	9.5 uL (+ 0.5 ~ 1uL)
cDNA / H20 (for negative control)	0.5 uL * each (This step can be added later)
Total	24.5 uL (24.5 + 0.5 (DNA)) // (24.5 + 0.5 (H_2O)

* Per reaction * (usually we do at least 2 reactions, because one for sample and one for negative control.)

v. Total should have x PCR tubes with ~ 25 uL in each

vi. Run the PCR on the machine (igG_PCR) ~ 1:30hr

2. Purify PCR product

a. Protocol

i. Add 5 loading dye to each mixture above; 25 uL mixture + 5 uL loading dye = 30uL.

ii. Run gel

- 5uL	+ 5uL	Introgen 1kb plus ladder	+ 12. 5uL	+ 12.5 uL

iii. ~ 365 wavelength
iv. Snap photo and cut out DNA

3. PCR clean up

a. Protocol
   i. Add 5x of GEX to the cut out area of the gel reading and place it on the hot plate until all gel dissolves.
   ii. Centrifuge 500uL of BL buffer to spin column
   iii. Decant all flow through of 500 ul BL buffer
   iv. Once gel has dissolved pipette all mixture from the tube into the spin column and centrifuge at 5000rpm for 1 min
   v. Decant flow through
   vi. Wash 500 uL of PW buffer and decant flow through
   vii. Repeat step vi.
   viii. Decant once more without adding buffer
   ix. Air dry for 1 minute
   x. Elute 25 RO water into the silica gel, dropwise. *make sure to cover the silica gel with TE buffer to yield best results

4. Restriction enzyme Digest

a. Protocol
   i. Buffer 2.1, Xho1, Sac1 (for light chain), Sal1 (for heavy chain), H_2O, DNA (pCAGGS || pHDM-G) * [Enzymes are from New England Biolabs, NEB]
   ii.

Reagent	Volume (uL)
Buffer 2.1	2
Sac1 || Sal1	1
EcoR1	1
DNA	16
Total	20uL

   iii. Incubate @37ºC for 1 hour
   iv. Run gel, 1% agarose in 0.5X TAE
   v. Snap photo and cut out dna

5. Gel verification and digested products

a. Protocol
   i. Add 5x of GEX to the cut out area of the gel reading and place it on the hot plate until all gel dissolves.
   ii. Centrifuge 500uL of BL buffer to spin column
   iii. Decant all flow through of 500 ul BL buffer

iv. Once gel has dissolved pipette all mixture from the tube into the spin column and centrifuge at 5000rpm for 1 min
v. Decant flow through
vi. Wash 500 uL of PW buffer and decant flow through
vii. Repeat step vi.
viii. Decant once more without adding buffer
ix. Air dry for 1 minute
x. Elute 25 RO water, dropwise. *make sure to cover the silica gel with TE buffer to yield best results

6. Ligation

a. Protocol
   i. Prepare a competent cell on ice in the -80°C, third row, third metal box, second slot.
   ii. Prepare 5x ligase buffer (NEBNext Quick Ligation Reaction Buffer (5X) , pCAGGS Sac1 & Xho, turbo ligase enzyme (NEB Turbo Ligase Enzyme), and insert (DNA) from the digested products. (eg. DNA - 140)
   iii. Make sure that when transferring enzymes to place it in the ice box at all times.
   iv. Store the insert once completed with this step
   v.

Reagent	Volume (uL) - exp	Volume (uL) - negative control
5x ligase buffer	2	2
pCAGGS Sac1 & Xho1 or pHDMG Sal1 & EcoR1	1	1
Turbo ligase enzyme	1	1
H20	0	6
Insert	6	0
Total	10	10

   vi. Incubate for 30 minutes on ice and prepare for heat shock

7. Transformation & Plate on LB +AMP

a. Protocol
   i. Prepare an amp plate, store at room temperature.
   ii. Turn on the hotplate to 42°C and load tube with competent cell + ligation mixture (exp and negative control) for 45 seconds
   iii. Incubate on ice for 2 mins
   iv. Load the mixture on amp plate and use a spreader to homogenize the mixture on the plate

v. Place the plates inside the 37°C for overnight

8. Colony picking

a. Protocol
   i. Take the plates out in the morning and let it sit in RT
   ii. Around ~ 3:30PM pick single colonies into 40 mL of LB to the 13 mL culture tubes and place it on the shaker for overnight

9. miniprep

a. Protocol
   i. From the 4mL LB + plasmid tube, take 2mL into a 2mL tube
   ii. Spin down 3 minutes @ 8,000rpm
   iii. Decant supernatant
   iv. Get P1/RNase and P3 3M K.Acetate from the fridge
   v. Add 200uL P1 to tubes vortex nicely
   vi. Add 200uL P2 to tube with plasmid gently rock 6~8 times , air dry for 5 minutes
   vii. Add 350uL P3, gently rock gently invert back and forth 6 ~ 8 times
   viii. Place in freezer for 30 min and set up tubes (can leave it in the fridge overnight if necessary)
   ix. Centrifuge @ maximum speed for 10 minutes
   x. Add 600 ul of 100% isopropanol to new tubes
   xi. Add **supernatant** from the tubes in the centrifuge to the tubes with isopropanol
   xii. Allow reaction to precipitate in the freezer for > 20 minutes
   xiii. Centrifuge max at 10 min, discard supernatant and add 500ul of 70% of EtOH in the freezer.
   xiv. Spin down again and decant all EtOH
   xv. Prepare a solution of RNase with TE buffer
      1. For 9 samples → add 9uL of RNase with 450 uL of TE buffer (50uL/sample)
   xvi. Vortex and incubate at 37°C for ~20mins or leave it in the fridge and incubate it when ready for "12. Restriction enzyme check"

10. Restriction enzyme check

a. Protocol
   i. Buffer 2.1, Xho1, EcoR1, H_2O, DNA → for 140L
      1. *use EcoR1 because it is cheaper than Sac1 *

Reagent	Volume (uL)
Buffer 2.1	2
Xho1	1
EcoR1	1
H20	12
DNA	4

Total	20uL

ii. Incubate @37°C for 1 hour
iii. Run gel (ladder + loading dye [~4ul])
iv. Snap photo and cut out dna ~ 350 band

11. Gel filtration

a. Protocol
   i. 500 uL of BL buffer into the spin column + tube
   ii. Centrifuge 1 min @ 12,000 rpm, decant flow through
   iii. Thaw plasmid
   iv. Add 5x GEX to plasmid (eg. 50 uL of plasmid : 250 uL of GEX)
   v. Vortex, transfer to spin column + tube
   vi. Spin down (5000rpm , 1 min) , decant flow through
   vii. PW buffer wash, 500 uL of PW to the spin column + tube. Spin down (12,000 rpm 1 min)
   viii. Repeat vii
   ix. Air dry 1 min and prepare 1.5 mL tubes for elution with TE buffer
   x. Elute 30 uL of TE buffer **dropwise to the white silica gel"
   xi. Spindown 12,000 ~ 14,000 gradually increase the speed from ~6 to max.
   xii. Discard the spin column and keep the liquid at the bottom as it is DNA + TE buffer

b. Measuring
   i. Take the DNA samples to the nanodrop machine and measure absorbance at 260/280

c. Prepare for sequence
   i. 50ng/uLof 10uL/rxn (eg. sample 490ng/ul → 1 uL sample : 9 ul H_2O)
   ii. 5 (rxn + 1) ul of primer (pCAGGS - 1839 || pHDM-G - 1840)

12. Sequencing

Experimental Results

Eight of ten chains (VH/VL) have been successfully sequenced and annotated. We now have three CHO-ready VH/VL pairs—054, 080, and 122—each composed of a single productive IGH with a single productive IGK/IGL. The remaining samples are 140H (in progress) and 147H (pending). Representative gels showed VH and VL amplicons at their expected sizes. Cloning and transformation yielded numerous

isolated colonies, and screening (colony PCR or diagnostic digests) identified multiple correct clones per construct. An internal EcoRI site within a VH led to split inserts in EcoRI-containing digests; to resolve this, we confirmed sequence identity directly from the PCR product by Sanger sequencing..

Challenges & Troubleshooting

Temperature sensitivity across samples was addressed with gradient PCR, exploring annealing temperatures from ~52–60 °C to improve specificity and yield. Impure PCR products (extra bands or smears) were mitigated by raising annealing temperature and by gel-extracting the correct band prior to cloning. Occasional failed transformations were corrected by confirming complete double digests, using fresh high-efficiency competent cells, employing an optimized insert:vector ratio, and running proper positive/negative controls. The internal EcoRI site in a VH required modified screening logic to avoid misinterpretation of diagnostic digests; confirmatory Sanger sequencing of PCR ensured correctness.

Discussion

Sequencing both heavy and light chains establishes authoritative records for each variable domain, confirms productivity (in-frame, no premature stop codons), and reveals any extra myeloma-derived chains so they can be excluded. IgBLAST provides standardized V(D)J calls, framework (FR) and CDR boundaries, and the amino-acid CDR3, ensuring that the recombinant antibody reconstructs the original six-CDR binding site at the VH–VL interface. To date, we have completed eight of the ten chains: the full 054, 080, and 122 pairs (heavy and light) and the 140 and 147 light chains. The remaining items are the 140 heavy chain and the 147 heavy chain. Both have been investigated and partially optimized, and additional experiments are planned.

Below are the plasmid cleanup gel images generated prior to submitting samples to Genewiz for Sanger sequencing. Light-chain constructs in pCAGGS were cloned with SacI/XhoI and heavy-chain constructs in PHDM-G with SalI/EcoRI; for miniprep QC, we used diagnostic digests (e.g., EcoRI/XhoI for HC) to verify insert size and orientation. Overall, light chains proceeded with minimal difficulty compared with heavy chains.

Among the six representative gels, the 054 heavy chain appeared atypical because the banding pattern did not show a single fragment at the expected ~350–400 bp insert size that was observed from the 054H PCR product. Troubleshooting and sequence analysis of the 054H PCR product showed an internal EcoRI site within VH. Consequently, the EcoRI used during plasmid cleanup split the insert, yielding smaller fragments and multiple bands. We resolved this by confirming sequence identity via Sanger both on the PCR product and on the final plasmid selected for transfection.

Another notable case was the 122 heavy chain, where only three single colonies appeared after transformation from the seed plates. Initial hypotheses included suboptimal heat-shock timing, outgrowth duration, or inoculum volume. A repeat transformation on a separate construct produced no colonies, prompting a step-back review of reagents (including ligase buffer, which tested functional on other samples) and then of the insert:vector ratio. Nanodrop measurements indicated that the ratio we were using was too low on insert. After increasing the insert volume and decreasing the vector volume to achieve an appropriate excess of insert, transformations returned to normal with numerous single colonies suitable for screening.

During PCR optimization, we also observed that 140H and 147H were particularly sensitive to annealing temperature. Running controlled gradients across 52–60 °C consistently improved specificity and yield; after that, standard cleanup and cloning proceeded as usual.

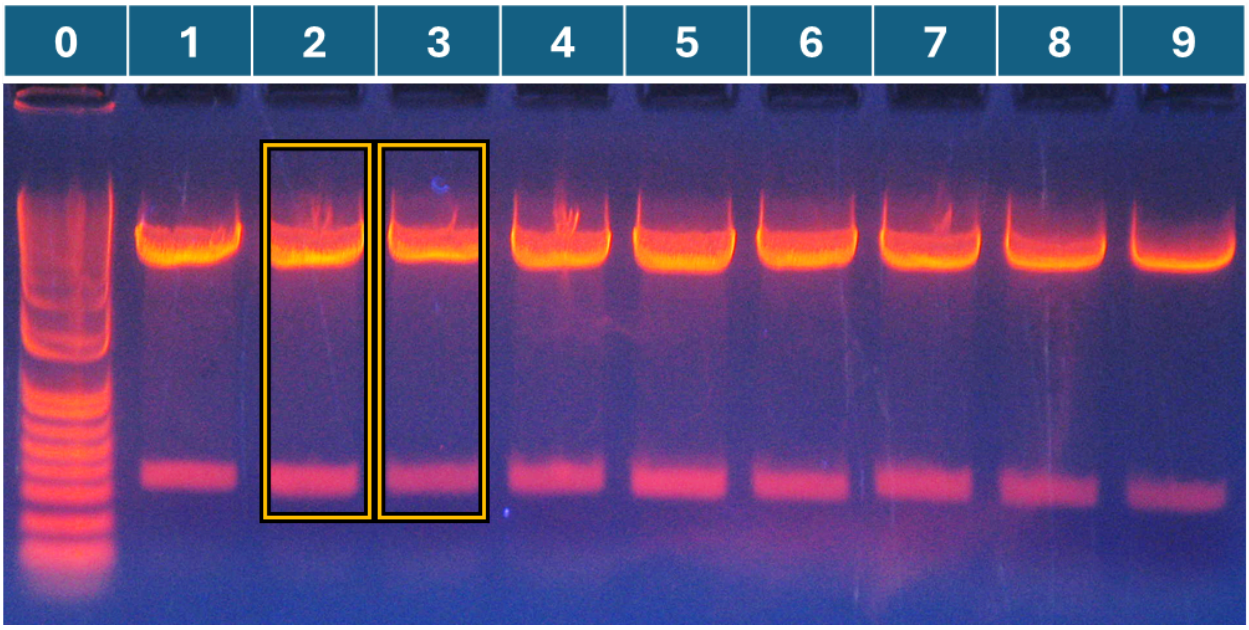

Image 1. 080 light chain in pCAGGS vector

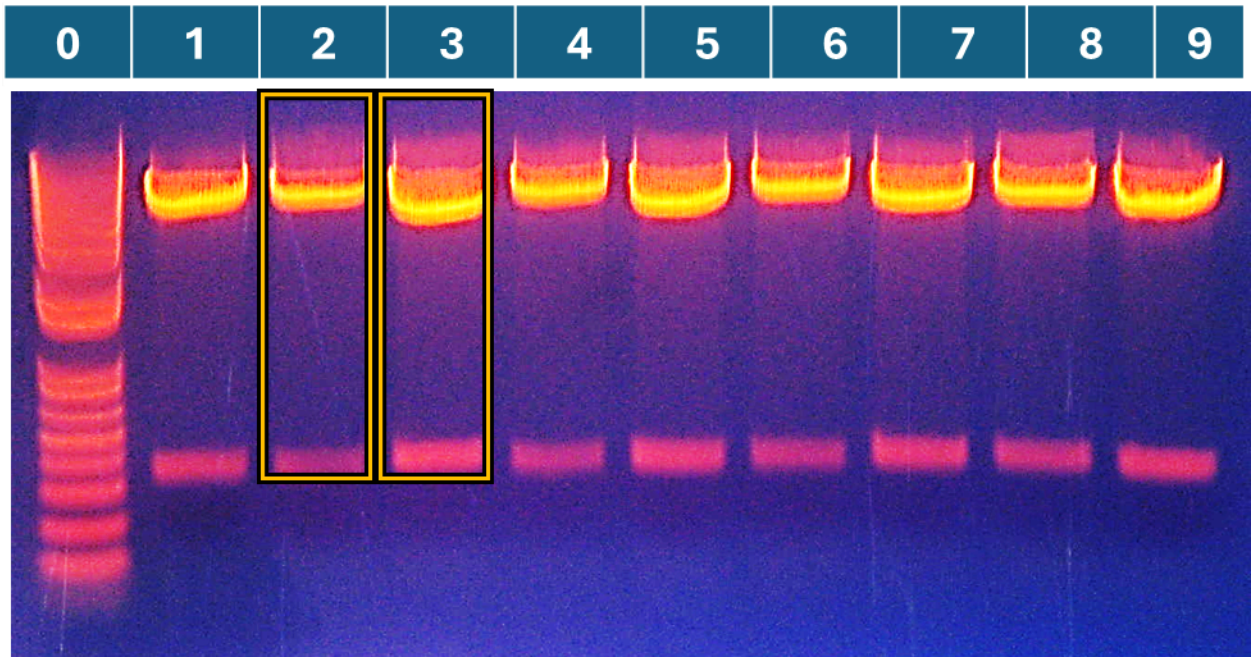

Image 2. 122 light chain in pCAGGS vector

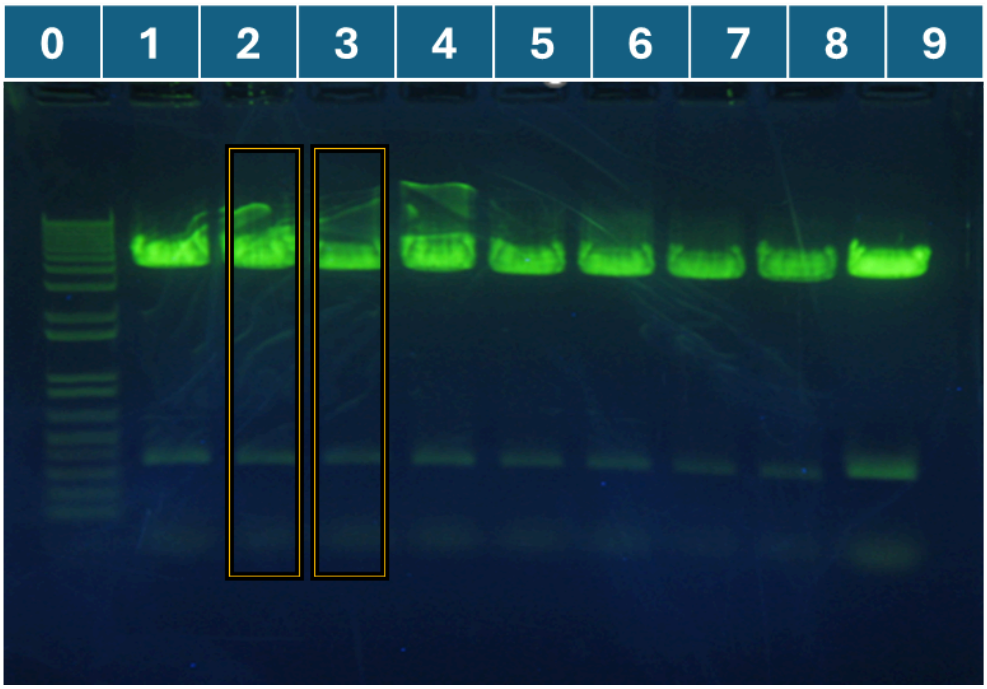

Image 3. 140 light chain in pCAGGS vector

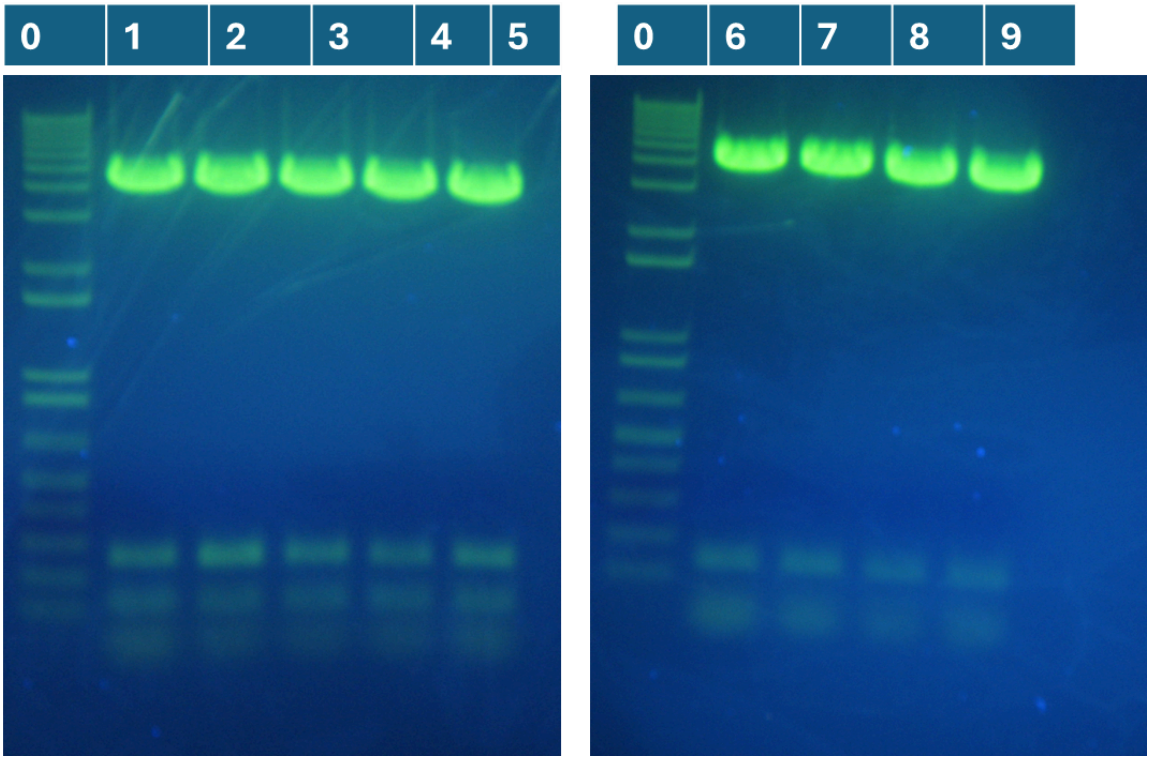

Image 4. 054 heavy chain in pHDM-G vector

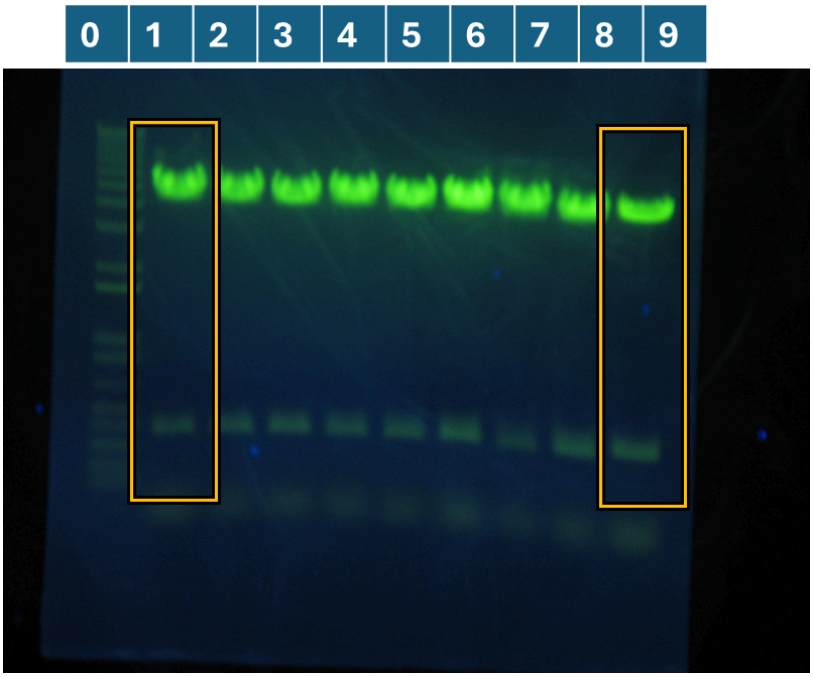

Image 5. 080 heavy chain in pHDM-G vector

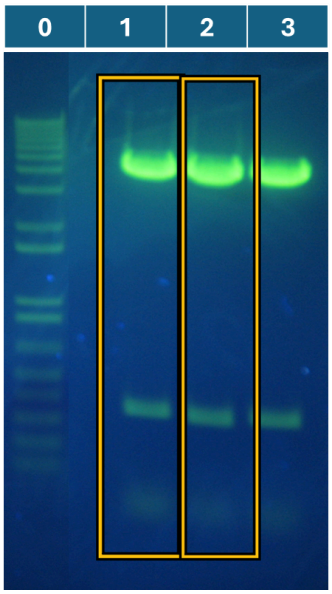

Image 6. 122 heavy chain in pHDM-G vector

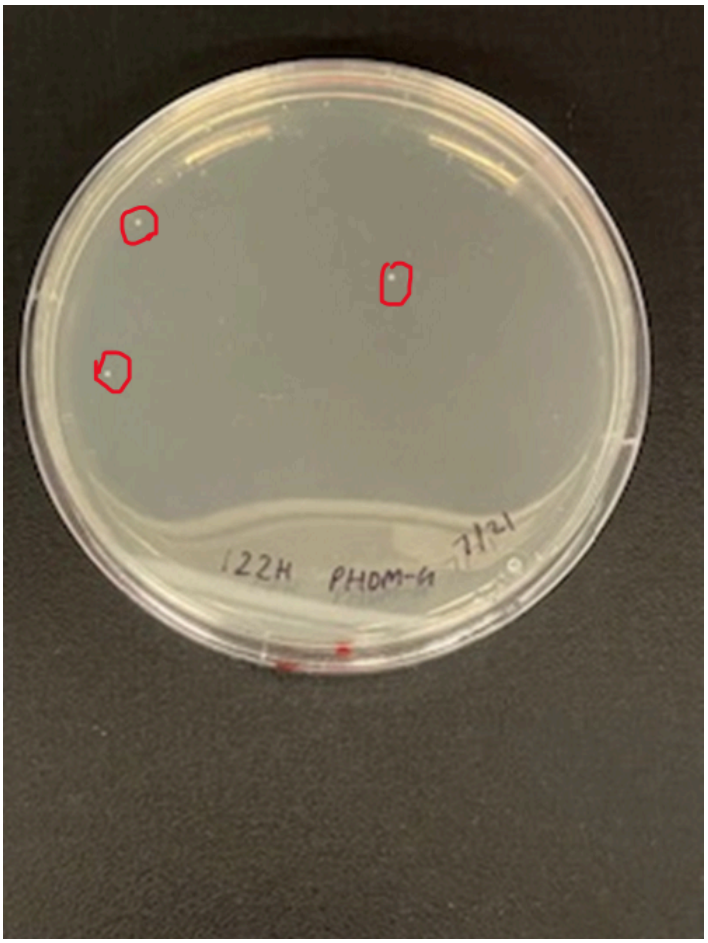

Image 7. 122 heavy chain insert + pHDM-G vector plate

Future Work

We will complete sequence verification of 140H and 147H, finalize cloning of any remaining constructs into pHDM-G/pCAGGS, and reconfirm plasmid integrity by full-insert Sanger in the expression backbones. Transient co-expression of each VH/VL pair will be performed to verify assembly and secretion, followed by binding/functional confirmation (e.g., ELISA or antigen-specific assay). Successful pairs will advance to CHO transient expression and then to stable CHO line development for consistent supply, with additional analytics to de-risk manufacturing.

Relevance & Impact

By locking the exact VH/VL and moving to recombinant CHO expression, we eliminate hybridoma drift and aberrant chain risks while delivering batch-to-batch consistency at scale—key attributes of development-ready monoclonals in industrial settings.

Conclusion

We have sequenced eight of ten chains across five hybridoma antibodies and confirmed three CHO-ready VH/VL pairs (054, 080, 122). The remaining heavy chains (140H, 147H) are being finalized. By excluding myeloma-derived transcripts, verifying productivity, and flagging liabilities before cloning, we have established a clean recombinant path: pCAGGS-LC and pHDM-G-HC for transient validation, followed by stable CHO expression for reliable supply. This workflow aligns with best practices in antibody discovery and biomanufacturing and positions the project for rapid progression to functional validation and scale-up.

Reflection

Interning at AB Biosciences this summer moved me from following protocols to operating as an independent researcher. The experience deepened my interest in biology and immunology and let me practice core methods while learning new techniques. With an engineering background and time at the Levine Lab (BioE), I recognized useful overlaps as well as areas to grow. Over three months, I developed a realistic view of high-level research: planning and executing end-to-end experiments, setting appropriate controls and QC checkpoints, maintaining rigorous records, and communicating results clearly. I became

comfortable troubleshooting when things went off script, making data-driven decisions, and balancing independence with timely feedback. This experience transformed me from a student who mainly prepared reagents and assisted on partial runs into an early-career scientist who owns experiments. I discovered that I genuinely enjoy the day-to-day work of science—designing runs, working carefully at the bench, and turning messy data into clear conclusions. The internship helped integrate my engineering mindset with biology, strengthening both my technical skills and my confidence, and it confirmed my next step: to continue in substantive research roles and pursue advanced study in science and engineering so I can contribute meaningfully to the field and the community.

References

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