Laboratory Notebook — Cell Signalling Project

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Volume 1: March

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Monday, 3 March

1 Cell culture maintenance

Thawed a fresh vial of HEK293 cells (passage 4) into a T25 flask with 5 mL DMEM + 10% FBS + 1% penicillin/streptomycin. Cells looked healthy under the microscope at 4 h: mostly round, few adherent yet. Incubator at 37°C, 5% CO_2.

2 Plasmid transfection trial

Prepared a small pilot to check transfection efficiency before committing the full plate. Seeded 2×10^5 cells per well in a 6-well plate.

Well	Plasmid	Reagent ratio
A1	pEGFP-C1	1:2
A2	pEGFP-C1	1:3
A3	empty vector (control)	1:2

Table 1: Pilot transfection plate layout

Transfection reagent mixed per manufacturer protocol, added dropwise. Plate returned to incubator; check at 24 h under GFP filter.

Tuesday, 4 March

1 Plasmid transfection trial

24 h check under the fluorescence microscope. A2 (1:3 ratio) shows the clearest GFP signal, estimated 35–40% of cells positive by eye. A1 is dimmer, around 15%. A3 negative as expected.

Decision: proceed with the 1:3 ratio for the full-plate experiment on Thursday.

2 Cell culture maintenance

Cells at ∼70% confluence. Passaged 1:4 into two new T25 flasks for propagation.

Thursday, 6 March

1 Plasmid transfection trial

Full 12-well plate transfected at 1:3 ratio, 4 conditions in triplicate: untreated, empty vector, target construct, target construct + inhibitor (10 µM, added 1 h pre-transfection).

2 Western blot for target protein

Harvested lysates from yesterday's pilot wells (A1–A3) for a trial blot. Protein concentration by BCA assay:

Sample	Concentration (µg/µL)
A1	2.1
A2	2.4
A3	1.9

Table 1: BCA protein quantification, pilot lysates

Loaded 20 µg per lane on a 10% SDS-PAGE gel. Running at 120 V. Transfer and blocking scheduled for tomorrow morning.

Friday, 7 March

1 Western blot for target protein

Transferred to PVDF (wet transfer, 100 V, 1 h). Blocked 1 h in 5% milk-TBST. Primary antibody (1:1000, anti-target) incubated overnight at 4°C on a rocker.

Note to self: the ladder lane looks smeared again — check whether the pre-stained ladder has expired before the next run.