First-Signal Law (FSL) — Biofilm Research Plan (High-Level)

Purpose

Test whether Restraint ≥ Alignment ≥ Persistence (R≥A≥P) predicts late-window biofilm stability across controlled environmental constraints, coordination signaling, and matrix maturation, using non-actionable, high-level observational measurements.

Conceptual Variables (map to proxies)

• Restraint (R): environmental/therapeutic constraints that limit chaotic growth (e.g., nutrient limitation duty cycle, maximum antibiotic exposure ceiling, shear stress bounds). Normalize proxies to [0,1].

• Alignment (A): coherence of community signaling and coordinated behavior (e.g., quorum-sensing coherence index from reporter signal; metabolic synchrony metrics). Normalize to [0,1].

• Persistence (P): temporal memory/inertia (e.g., EPS maturity window, matrix density/viscoelastic proxy, time-since-inoculation normalized to [0,1]).

Primary Outcome (stability target)

Late-window stability = mean biomass/viability proxy over final observation window × (1 − variance). Scale to [0,1] across the study. (Alternative: mean cooperative behavior proxy × (1 − variance).)

Design Sketch (non-procedural)

Use parallel conditions spanning R, A, P ranges without step-by-step lab instructions. Example axes:

(1) Constraint sweep: vary nutrient duty cycle and set a conservative antibiotic ceiling;

(2) Alignment modulation: compare strains/consortia/reporters with different quorum-signal coherence;

(3) Persistence modulation: observe across early/mid/late matrix maturation windows.

Record high-level observations only; avoid actionable wet-lab detail.

Predictions (locked FSL, qualitative)

• Correct ordering (R≥A≥P) → highest stability;

• Misorder (P>A or A>R) → "stable-but-brittle" or quick fade;

• Added "release" analogs (e.g., intermittent stress relief) can prolong stability only when R is credible and A is coherent.

Data Schema (CSV columns)

domain, context_id, R_proxy, A_proxy, P_proxy, target_stability, notes

(Use the template you already have: FSL_biofilm_template.csv).

Analysis Plan (high■level)

1) Normalize proxies to [0,1].

2) Compute FSL prediction with synergy and ordering penalties (locked model).

3) Plot reliability (binned predicted vs measured), report r/MAE/RMSE.

4) Slice by ordered vs unordered to test the law's core hypothesis.

5) Report gaps and adjust proxy mapping—not lab conditions—to improve calibration.

Ethical/Safety Note

This plan intentionally omits actionable wet■lab instructions, quantities, organism specifics, or protocols. Focus is on conceptual modeling, measurement mapping, and statistical testing to keep within safe, high■level guidance.