Appendix A – Condensed Gene Circuit Maps (v2.5)

Chromosomal Locus 1 — Hypoxia-Gated Germination

[Pfnr] → RBS → spo0A → T_1

```
LOCUS: NOVYI_chr1_germination 1200 bp ds-DNA linear
 FEATURES

promoter     1..120
             /note="Pfnr (FNR-activated O2-sensitive promoter)"
RBS          121..130
             /note="Synthetic RBS"
CDS          131..1040
             /gene="spo0A"
             /note="Sporulation regulator, required for germination"
terminator 1041..1200
             /note="T1 terminator"
```

Chromosomal Locus 2 — Inducible Payload / Abort Cassette

[Pribo-Dox] → Cas9 → gRNA(target: Chr3) → T_2
(Default payload = abort logic)

```
LOCUS: NOVYI_chr2_kill 2600 bp ds-DNA linear
 FEATURES

promoter     1..60
             /note="Pribo-Dox (doxycycline riboswitch promoter)"
CDS          61..1800
             /gene="Cas9"
             /product="S. pyogenes Cas9"
gRNA         1801..1900
             /target="NOVYI_chr3_reporter"
             /note="Guide RNA targeting PET-reporter locus"
terminator 1901..2050
             /note="T2 terminator"
```

> *Cas9 may be replaced with alternative Tet-inducible payloads (e.g. PD-L1 nanobody, IL-33, CXCL10) using the same promoter and terminator.*

All swappable payloads must conform to the Payload Compatibility Envelope. This envelope defines metabolic, transcriptional, and immune-layer constraints to ensure integration does not interfere with containment, exposure, or quorum logic. Default payloads (Cas9, PD-L1 nanobody, IL-12, etc.) remain within these thresholds.

Chromosomal Locus 3 — Germination-Linked PET Reporter

[Pfnr] → HSV-TKmut → T_3

```
LOCUS: NOVYI_chr3_reporter 1100 bp ds-DNA linear
 FEATURES

promoter     1..100
             /note="Pfnr (hypoxia promoter, same as Locus 1)"
CDS          101..1000
             /gene="HSV-TKmut"
             /product="Mutant HSV thymidine-kinase (¹⁸F-FHBG PET)"
terminator 1001..1100
             /note="T3 terminator"
```

> *HSV-TKmut may be replaced with a humanized dCK variant in clinical translation to reduce immunogenicity.*

Chromosomal Locus 4 — Efferocytic Clearance Module

[Pfault] → sigPep-PSM → T_5

```
LOCUS: NOVYI_chr4_cleanup 750 bp ds-DNA linear

 FEATURES

promoter     1..90

             /note="Pfault: fires on spo0A, dapA, or Cas9 failure"

CDS         91..640

             /gene="sigPep-PSM"

             /product="Secretory peptide fused to
phosphatidylserine-mimic domain"

terminator  641..750

             /note="T5 terminator"
```

Function: *Upon structural fault, Co-Aegis presents a PS-mimic surface marker to induce macrophage-mediated efferocytosis. Clearance is non-inflammatory and immune-independent.*

Chromosomal Locus 5 — Dormancy Harmonizer Module

```
[Pdormant] → sigPep-HMZ → T7

LOCUS: NOVYI_chr5_harmonizer 800 bp ds-DNA linear

FEATURES

promoter     1..90

             /note="Pdormant: synthetic promoter active only during
spore dormancy; suppressed on germination or structural deviation"

CDS          91..700

             /gene="sigPep-HMZ"

             /product="Secreted Harmonizer Micro-Peptide; derived from
Bacteroides-associated surface patterns; immuno-neutral, non-adhesive"

terminator   701..800

             /note="T7 terminator"
```

Function: *Reduces innate immune probing and local microbial friction during dormancy.*
Degraded upon germination or Pfault activation to avoid masking exposure.
Design avoids epitope overlap with Pfault system or PET trace modules.

Chromosomal Locus 6 — Passive Trace Metabolite Reporter (TMR)

```
LOCUS: NOVYI_chr6_trace 800 bp ds-DNA linear

 FEATURES

promoter     1..90

             /note="Pfault or Pfault-extended"

CDS          91..650

             /gene="TMR-peptide"

            /note="Failure-class-specific reporter fragment;
            kidney-clearable; mass spec compatible.

            Emits a diagnostic metabolite tag on failure. Each TMR
            variant maps to a specific failure class:"

                    • TMR-01 = spo0A dropout

                    • TMR-02 = dapA loss or plasmid instability

                    • TMR-03 = Cas9 silencing or payload mismatch

                    • TMR-04 = quorum contradiction

                    • TMR-XX = unknown/multi-factor failure

            Fragments are <2 kDa, mass-spec and ELISA compatible, and
            clear via urine within 24–48 h."

terminator  651..800

             /note="T10 terminator"
```

Function:
Expresses a short diagnostic peptide or modified metabolite upon failure-trigger (Pfault). Each variant maps to a distinct failure mode. Not immunogenic. Not retained. Designed for excretion and external readout (e.g., LC-MS, lateral flow).

Does not interfere with Pfault expression, quorum logic, or PET trace.

Optional Cassette — Inflammation Dampener (Step-2 Only)

`[Pquorum-hyp] → il10(mini) → T6`

Design Notes:

- *Pquorum-hyp = quorum-sensor promoter active only under hypoxia*
- *il10(mini) = truncated murine IL-10, low systemic diffusion*

> *Auto-fires if inflammatory markers exceed preset ceilings; used only during controlled Step-2 validation.*

Optional Cassette — Resolution Phase Trigger (Not deployed in Phase Ia)

`[Pcd86↑ & IFNγ↑] → sigPep-IL33 → T8`

> *Fires only upon confirmed co-stimulation + antigen presentation to promote regenerative clearance and memory skewing.*

Plasmid — Metabolic Addiction Module (L-dAP Complementation)

```
[Constitutive] → dapA → T4

LOCUS: NOVYI_plasmid_dapA 1500 bp ds-DNA circular
 FEATURES

origin        1..200

              /note="Low-copy suicide ori (RCR-minus)"

promoter    201..260

              /note="Constitutive promoter (J23100)"

CDS         261..1350

              /gene="dapA"

              /note="Diaminopimelate synthase; complements chromosomal
ΔdapA"

terminator 1351..1500

              /note="T4 terminator"
```

Plasmid – Resistance Telemetry Module (ART Layer)

```
[Ptrend] → rpoS-proxy + tracerTag → T9

LOCUS: NOVYI_plasmid_ART 1400 bp ds-DNA circular

FEATURES

origin       1..200

             /note="Shared backbone with dapA plasmid; RCR– suicide
ori"

promoter     201..280

             /note="Ptrend: antibiotic-response proxy (e.g. tetA-class
promoter tuned for doxycycline efflux threshold)"

CDS          281..1080

             /gene="rpoS-proxy"

             /note="Stress-response proxy fused to trace peptide"

reporter     1081..1200

             /note="TracerTag: PET-optional or FLAG-alt signal epitope
(e.g. FLAG-D)"

terminator   1201..1400

             /note="T9 terminator"
```

***Function**: Activates only under pharmacodynamic stress conditions (e.g. when MIC of control antibiotic shifts beyond threshold). Does not trigger abort or exposure logic.*

Emits a visible or detectable telemetry marker (e.g., PET analog or distinct FLAG variant) to enable clinician or automated trace systems to register therapeutic drift.
Designed for long-term deployments and layered antimicrobial strategies

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Final System Behaviour Summary (v2.5)

- Dormant spores remain inert unless exposed to tumour-associated hypoxia ($O_2 \leq 1$ %) **sustained over a minimal descent threshold** (e.g., ≥12 min at ≤1.2 % or ≥4 min at ≤0.5 %), minimizing activation in unstable or marginal zones.

- During dormancy, Co-Aegis expresses a **Harmonizer Peptide** — a passive surface element derived from commensal microflora — to reduce immune probing and environmental disturbance. This expression is suppressed upon germination or design deviation.

- Upon germination, spores express a PET reporter, prime any doxycycline-inducible payload, and begin local execution.

- Containment logic includes:
  • Hypoxia-gated germination (spo0A under Pfnr with time-integrated thresholding)
  • Optional abort payload (e.g. Cas9) triggered by systemic doxycycline
  • Metabolic addiction (dapA complementation on suicide plasmid with RCR^- ori)
  • Passive dormancy harmonizer (sigPep-HMZ under Pdormant, decays at activation)

- A PBPK model confirms doxycycline penetrates all target compartments within the effective abort window.

- A passive resistance telemetry module monitors for rising MIC thresholds and surfaces a tracer signal if pharmacologic drift is detected, enabling preemptive adjustment without disrupting containment logic.

- If core design logic fails the Pfault system expresses a phosphatidylserine mimic on the surface, enabling silent clearance via local macrophages without requiring immune activation. (e.g. transcript loss or silencing of spo0A, Cas9, or dapA)

- In parallel, the TMR module emits a trace metabolite signature (e.g., FM01–FM03) into bodily fluids for non-invasive confirmation of system clearance origin.

- During Step 2, if inflammatory markers overshoot specification, an auto-deploy **IL-10 microburst** limits spill-over without suppressing antigen presentation or co-stimulation.

- All payload modules are modular and swappable under the same Tet-inducible promoter system.