HOST-DIALOG

INTEGRATED FUNCTIONAL ASSAY SUITE

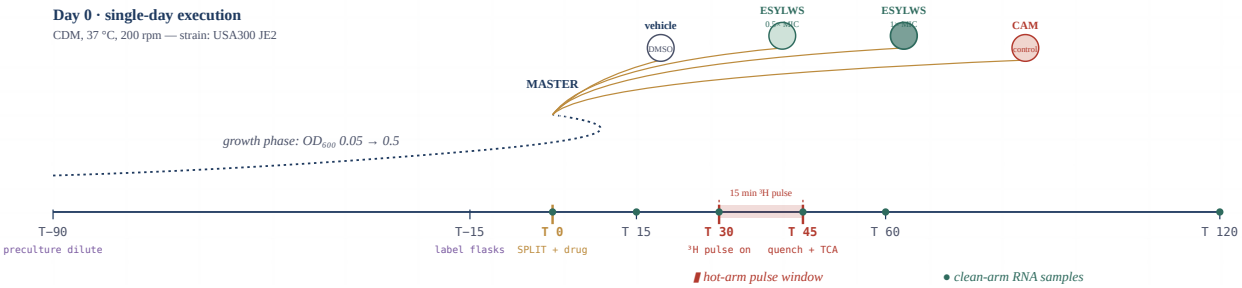
Visual Protocol Overview · for benchside reference

SOP-HD-001 · v1.0
April 2026 · DRAFT
Karlsen / Bollvåg et al.

> One master culture, split four ways, sampled through three orthogonal axes — *radiolabel incorporation*, *tRNA charging state*, and *small RNA profile* — converging onto a single mechanistic readout of GatCAB-mediated tRNA-Asn maturation in *S. aureus*.

01 Timeline · split-culture, drug application, sampling

all times relative to drug addition (T = 0)



02 Three orthogonal readouts · downstream workflow

samples at T+45 (hot) and T+15/30/45/60/120 (clean)

ARM A · HOT

Translation flux per residue

SOP 2 · ³H-Asp/Asn/Leu/Gly

1. Add ³H-amino acid at T+30
   5 µCi/mL final, 4 tracers in parallel
2. 15 min pulse · ice quench
   3 × 1 mL into 10 % TCA on ice
3. Filter onto GF/C · hot-TCA wash
   90 °C, 20 min — strips RNA signal
4. Scintillation count (DPM)
   Tri-Carb · ³H or dual ³H/¹⁴C channel

DELIVERABLE

[³H-Asn] / [³H-Leu] ratio

Per condition × replicate.
GatCAB block: ratio drops < 0.6
at sub-MIC ESYLWS.

ARM B · ACID-UREA

Direct tRNA charging state

SOP 3 · low-pH PAGE northern

1. Acid-fix pellet · pH 4.5
   0.3 M NaOAc cold — preserves aa-bond
2. Acid phenol extract · ethanol pp.
   Everything cold; do not heat-denature
3. 8 M urea PAGE · 100 mM NaOAc pH 5
   8 V/cm, 4 °C, 4–6 h
4. Northern · ³²P-tRNA-Asn probe
   + tRNA-Leu specificity control

DELIVERABLE

3-band charging spectrum

Asp-tRNA-Asn band rises = direct GatCAB block.

↑ Asn-tRNA-Asn
↓ uncharged

ARM C · SMALL RNA SEQ

tRF & sRNA regulatory profile

SOP 4 · CIP+PNK + AlkB + NEBNext

1. TRIzol + miRNeasy hybrid
   Retains 15–200 nt fraction
2. CIP + T4 PNK end-repair
   Removes 2′,3′-cyclic-P (tRF-essential)
3. AlkB demethylation
   Strips m1A/m3C/m1G — RT-permissive
4. NEBNext lib · 50 bp SE @ Nord U.
   10 M reads/sample · MINTmap pipeline

DELIVERABLE

tRF-3b^Asn quantification

Sense to DMBT1-mRNA window.
Stringent-response sRNA cohort,
global regulatory landscape.

INTEGRATED DATASET

Mechanistic concordance across three axes

flux ⇄ charging state ⇄ regulation — single biological state, three orthogonal probes

03 Mechanistic readouts · diagnostic fingerprints

expected three-axis signatures per scenario

HYPOTHESIS CONFIRMED

OFF-TARGET

CELL-WALL AXIS

THE FULL MECHANISM

Clean GatCAB block

ESYLWS hits transamidation specifically. Asn codons stall while Asp codons stay open. The headline result.

Asn/Leu	Asp-tRNA	tRF-3b
↓	↑↑	↑

General translation hit

Looks like chloramphenicol. All four tracers drop in proportion; no charging-state shift on the northern.

All equal	No shift	Stringent
↓		↑

MurG/MurF arm dominant

Self-activation hypothesis branch: Gly into peptidoglycan drops selectively, protein synthesis preserved.

Asn/Leu	No shift	Cell-wall sRNA
≈ 1		

Dual hit · the strongest case

GatCAB *and* MurG/MurF both engaged. Asp-tRNA-Asn rises, Gly-PG drops, tRF-3b^Asn enriched.

Asn/Leu	Asp-tRNA	tRF + PG sRNA
↓	↑↑	

HOT ARM · radiation ACID-UREA · clean RNA SMALL RNA SEQ SHARED master / split

All arms originate at T = 0 from one synchronised culture.

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Karlsen, Bollvåg, Emblem, Lau Bårdsen, Brekke, Knutsen, Landsem, Mårli, Joakimsen, Mollnes
Department of Laboratory Medicine, Nordland Hospital Trust · Bodø

“One culture, three witnesses. If all three say the same thing,
we have a mechanism. If they disagree, we have a paper.”