E X R N A - A G

Bacterial Extracellular RNA-Mediated Reprogramming of Quinoa (*Chenopodium quinoa*) Seed Germination

Target Analysis · Mechanistic Models · Validation Strategy

31 Gene Targets Analyzed

REPORT PREPARED BY

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CONFIDENTIAL

ExRNA Research Report: Quinoa

Chenopodium quinoa — Bacterial Extracellular sRNA Target Analysis [CONFIDENTIAL]

> **CONFIDENTIAL** — Generated 2026-02-19 **Family**: Amaranthaceae | **Assembly**: ASM168347v1 **Treatment**: M-9 bacterial EPS solution **Analysis Status**: targets_identified

Executive Summary [CONFIDENTIAL]

This report presents the analysis of **31 predicted exRNA targets** in Chenopodium quinoa (quinoa). These transcripts were identified as potential targets of bacterial extracellular small RNAs (exRNAs) that may improve seed germination and seedling vigor when seeds are treated with M-9 bacterial EPS solution.

Target Distribution

PRIORITY	COUNT
High	4
Medium	4
Low	23
Total	**31**

Pathway Distribution

PATHWAY	TARGETS
Unknown	23
Defense Immunity	3

PATHWAY	TARGETS
Metabolic Priming	1
Transport Ion Homeostasis	1
Signaling	1
Photosynthesis	1
Hormone Signaling	1

High-Priority Targets [CONFIDENTIAL]

GENE ID	ANNOTATION	PATHWAY
AUR62010943	CqHAK5 - High-Affinity K+ transporter 5 (HAK/KUP/KT family)	transport_ion_homeostasis
AUR62044372	CqCNGC14 - Cyclic Nucleotide-Gated Channel 14 (Ca2+ signalin	signaling
AUR62015391	CqGUN4 - Tetrapyrrole-binding protein, chloroplastic (Mg-che	photosynthesis
AUR62003502	CqBRL2/VH1-like - BRI1-Like Receptor Kinase 2 (LRR-RLK; vasc	hormone_signaling

Pathway Analysis Summary [CONFIDENTIAL]

Pathway Analysis Index

TL;DR: Gene targets grouped by 14 biological pathways. Key pathways include hormone signaling, defense/immunity, epigenetics, ROS/redox, transport, and metabolic priming. Last Updated: 2026-02-18

Pathways [CONFIDENTIAL]

PATHWAY	FILE	TARGETS	SUMMARY
Cell Wall Remodeling	cell_wall_remodeling.md	3	Of course. As a plant systems biologist, I will analyze the coordinated downregu
Defense Immunity	defense_immunity.md	5	Of course. As a plant systems biologist, I will analyze this "Defense Immunity"
Dna Repair Replication	dna_repair_replication.md	6	Of course. As a plant systems biologist, I will analyze this pathway by integrat
Epigenetic Regulation	epigenetic_regulation.md	6	Of course. As a plant systems biologist, I will analyze this set of epigenetical
Hormone Signaling	hormone_signaling.md	3	Of course. As a plant systems biologist, I will analyze the coordinated function
Metabolic	metabolic_priming.md	15	Of course. As a plant systems biologist, I will analyze this set of downregulate
Organelle Biogenesis	organelle_biogenesis.md	6	Of course. As a plant systems biologist, I will analyze this "Organelle Biogenes
Protein Turnover	protein_turnover.md	11	Of course. As a plant systems biologist, I will analyze the coordinated downregu
Rna Processing	rna_processing.md	10	Of course. As a plant systems biologist, here is a pathway-level analysis of the
Ros Redox	ros_redox.md	3	Of course. As a plant systems biologist, here is a detailed pathway-level analys
Signaling	signaling.md	11	Of course. As a plant systems biologist, here is a pathway-level analysis of the
Transport Ion Homeostasis	transport_ion_homeostasis.md	18	Of course. As a plant systems biologist, I will analyze the "Transport Ion Homeo

PATHWAY	FILE	TARGETS	SUMMARY
Transposon Related	transposon_related.md	5	Of course. As a plant systems biologist, here is a detailed pathway-level analys
Unknown	unknown_function.md	7	Of course. As a plant systems biologist, I will analyze this gene set. The key c

Cross-Pathway Analysis [CONFIDENTIAL]

See cross_pathway_interactions.md

Theme Analysis Summary [CONFIDENTIAL]

Themes Index

> TL;DR: 6 cross-cutting biological themes extracted from target analysis. Last Updated: 2026-02-18

Themes [CONFIDENTIAL]

THEME	FILE	SUMMARY
Defense Downshift	defense_downshift.md	Theme analysis for defense downshift
Epigenetic Remodeling	epigenetic_remodeling.md	Theme analysis for epigenetic remodeling
ROS Optimization	ros_optimization.md	Theme analysis for ros optimization
Hormone Nodes	hormone_nodes.md	Theme analysis for hormone nodes
Transport / Ion Homeostasis	transport_ion_homeostasis.md	Theme analysis for transport / ion homeostasis
Metabolic Priming	metabolic_priming.md	Theme analysis for metabolic priming

Theme Interactions [CONFIDENTIAL]

See individual theme files for cross-theme analysis.

Synthesis: Ranked Targets [CONFIDENTIAL]

Ranked Target Analysis — Quinoa (Chenopodium quinoa)

Definitive Ranked Target Analysis: Bacterial exRNA-Mediated Germination Improvement in *Spinacia oleracea* (with Quinoa Context)

> **Methodological Note**: All targets are spinach (*Spinacia oleracea*) genes being analyzed for their contribution to germination/vigor phenotypes. The crop header states *Chenopodium quinoa*; given the close phylogenetic relationship (both Chenopodiaceae/Amaranthaceae), mechanistic inferences are largely transferable, but direct quinoa ortholog validation is required before application. This discrepancy is flagged as a critical confounder throughout.

Executive Summary [CONFIDENTIAL]

This target landscape represents a remarkably broad, multi-pathway intervention by bacterial extracellular small RNAs (exRNAs), encompassing 110+ predicted gene targets across 14 functional pathway groupings. The dominant biological theme is the **suppression of the dormancy-defense-growth tradeoff**: the seed's default state maintains costly epigenetic silencing, immune readiness, and stress-responsive signaling that collectively act as brakes on germination. The bacterial exRNAs appear to function as a systemic "all-clear" signal that simultaneously dismantles multiple repressive layers. The highest-confidence targets are those with direct, mechanistically established roles in hormone signaling (ethylene receptor, AHP-like cytokinin relay, LOX/JA biosynthesis), epigenetic reprogramming (DNA methyltransferase, HIRA, SUVR5), and ion/osmotic homeostasis (CCC cotransporters, CNGC channels), as these pathways have strong Arabidopsis precedent and occupy central nodes in the germination regulatory network.

A critical systems-level observation is that the target set is not a random sample of the transcriptome but shows strong enrichment for **regulatory and signaling genes** rather than core metabolic enzymes. This pattern is consistent with a genuine cross-kingdom RNA interference mechanism rather than non-specific transcriptional noise. However, several important confounders must be acknowledged: (1) the bacterial exopolysaccharide (EPS) matrix used for osmopriming may independently improve germination through water potential manipulation; (2) bacterial polysaccharides can act as elicitors that prime plant immunity in complex ways; (3) the spinach-to-quinoa translational gap requires explicit ortholog mapping; and (4) many targets (reverse transcriptases, unknown proteins, pesticidal crystal protein annotation) likely represent annotation artifacts or off-target effects with minimal phenotypic contribution.

The ranking below integrates four criteria: mechanistic centrality to germination regulation, strength of Arabidopsis/plant homolog evidence, pathway priority scores from the provided analyses, and the degree to which the target's downregulation provides a parsimonious explanation for the observed vigor phenotype. Targets are penalized for annotation uncertainty, potential misannotation, or placement in pathways with ambiguous directional effects on germination.

Ranking Methodology [CONFIDENTIAL]

CRITERION	WEIGHT	RATIONALE
Mechanistic centrality	35%	Does the gene occupy a rate-limiting or master regulatory node in a germination-relevant pathway?
Evidence strength	30%	Quality of Arabidopsis/plant homolog data; conservation of function in Chenopodiaceae
Pathway priority score	20%	High/medium/low priority as assigned in pathway analyses, cross-validated against literature
Annotation confidence	15%	Penalty for unknown proteins, potential misannotations, transposon-related genes

Directional logic applied: All targets are predicted to be *downregulated* by exRNAs. For a target to rank highly, its downregulation must have a *clear, positive* effect on germination rate, uniformity, or seedling vigor. Targets where downregulation has ambiguous or potentially negative effects are penalized.

Tier 1: Critical Targets (Expected Large Phenotypic Effect)

[CONFIDENTIAL]

These targets occupy master regulatory nodes with strong mechanistic precedent. Their downregulation alone is predicted to produce measurable germination improvement.

1. SOV3g000150.1 — Ethylene Receptor

- **Mechanism**: Ethylene receptors (ETR1/ERS family) are **negative regulators** of ethylene signaling — when the receptor is present and unoccupied, it actively suppresses ethylene responses via CTR1 kinase. Downregulation of the receptor therefore **constitutively activates ethylene signaling** without requiring ethylene ligand. Ethylene

promotes germination by antagonizing ABA signaling, reducing ABA sensitivity, and promoting endosperm cap weakening. In Arabidopsis, *etr1* loss-of-function mutants show enhanced germination under ABA-inhibitory conditions. [KNOWN]

- **Evidence strength**: **Strong**. ETR1 function as a negative regulator of ethylene signaling is one of the best-characterized mechanisms in plant biology (Chang et al., 1993, *Science* 262:539; Bleecker & Kende, 2000, *Annu Rev Cell Dev Biol* 16:1). Ethylene's role in promoting germination via ABA antagonism is established (Linkies & Leubner-Metzger, 2012, *Plant Cell Environ* 35:1727). [KNOWN]
- **Key references**: AtETR1 (AT1G66340); *etr1-1* Arabidopsis mutants; Linkies et al. 2009 *Plant Cell* 21:3803 (ethylene promotes endosperm rupture in *Lepidium*)
- **Confounders**: [INFERRED] The specific receptor subtype (ETR1 vs. ERS1 vs. ETR2) determines the magnitude of effect. If this is an ERS-type receptor with redundant paralogs, single-gene downregulation may have attenuated effect. Spinach has multiple ethylene receptor genes; paralog compensation is possible. [SPECULATIVE] EPS osmopriming may independently modulate ethylene sensitivity.
- **Confidence**: **High**

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2. SOV4g032870.1 — Histidine-Containing Phosphotransfer Protein 1 (AHP-like)

- **Mechanism**: AHP proteins are relay components of the two-component cytokinin signaling cascade (AHK receptor → AHP → ARR response regulator). Critically, AHPs also function as **positive relays in ABA signaling** — AHP1 in Arabidopsis phosphorylates and activates type-A ARRs that suppress cytokinin responses, but AHPs also interact with ABA signaling components. More directly relevant: AHP6 in Arabidopsis is a **pseudo-AHP that inhibits cytokinin signaling** and promotes ABA responses. Downregulation of an AHP-like protein would be predicted to **reduce ABA-promoting cytokinin-independent signaling**, shifting the hormonal balance toward germination. [INFERRED] Additionally, the two-component pathway cross-talks with ethylene signaling, creating a synergistic effect with SOV3g000150.1 downregulation. [SPECULATIVE]
- **Evidence strength**: **Moderate-Strong**. AHP function in hormone crosstalk is established (Hutchison et al., 2006, *Plant Cell* 18:3073; Mahonen et al., 2006, *Curr Biol* 16:1116). The specific role of this particular AHP in ABA vs. cytokinin signaling during germination requires functional validation in spinach. [INFERRED]
- **Key references**: AtAHP1-6 (AT3G21510, AT3G29350, AT5G39340, AT3G16360, AT1G03430, AT1G80100); Arabidopsis two-component signaling review: To & Kieber, 2008, *Trends Plant Sci* 13:533
- **Confounders**: AHP proteins have partially redundant functions; the net effect of downregulating one family member depends on which specific AHP this represents and its expression domain in the seed.
- **Confidence**: **High** (pathway priority: High)

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3. SOV3g035520.1 — Lipoxygenase (LOX)

- **Mechanism**: LOX enzymes catalyze the first committed step in jasmonic acid (JA) biosynthesis (13-LOX pathway: linolenic acid → 13-HPOT → allene oxide → JA). JA is a potent **inhibitor of germination**, acting synergistically with ABA to suppress radicle emergence. In Arabidopsis, JA-insensitive mutants (*jar1*, *coi1*) show enhanced germination under stress conditions. Downregulation of LOX wou [... truncated]

Synthesis: Causal Models [CONFIDENTIAL]

Causal Models — Quinoa (Chenopodium quinoa)

Alternative Causal Models: Bacterial exRNA-Mediated Germination Improvement in *Chenopodium quinoa*

> **Critical Note on Species Translation**: The target gene IDs (SOV prefix) derive from *Spinacia oleracea* (spinach). Both spinach and quinoa belong to the Amaranthaceae s.l. (formerly Chenopodiaceae), sharing substantial synteny and gene family conservation. Mechanistic inferences are transferable at the pathway level, but direct ortholog validation in quinoa is essential before any translational application. All models below assume conserved function of spinach orthologs in quinoa. [INFERRED]

Model 1: The Epigenetic Derepression Cascade ("Unlock the Blueprint") [CONFIDENTIAL]

Core hypothesis: Bacterial exRNAs primarily target the seed's epigenetic silencing machinery, causing a genome-wide chromatin relaxation event that derepresses dormancy-locked germination programs, with downstream hormonal and metabolic changes occurring as secondary consequences of transcriptional activation.

Causal chain:

1. **Bacterial exRNA enters seed cells via vesicle-mediated uptake during imbibition.** During the initial hours of seed hydration, bacterial outer membrane vesicles (OMVs) or free exRNAs (protected by protein complexes or secondary structure) are taken up by the rehydrating embryo cells through endocytosis or direct membrane fusion. The compromised membrane integrity characteristic of early imbibition facilitates entry. [SPECULATIVE — uptake mechanism in seeds is uncharacterized; extrapolated from cross-kingdom RNAi studies in *Arabidopsis* roots and mammalian gut epithelium (Cai et al., 2018, *Nature Plants*; Liu et al., 2012)]
2. **Epigenetic silencing machinery is dismantled (primary targets):**
3. **DNA (cytosine-5)-methyltransferase (SOV1g033340.1)** is downregulated → maintenance methylation at CG and CHG contexts fails during the first round of DNA replication post-imbibition → passive demethylation of promoters controlling GA biosynthesis genes (e.g., *GA3ox*, *GA20ox*), cell wall loosening enzymes, and cell cycle regulators. [KNOWN that maintenance methyltransferases are required to propagate methylation patterns; INFERRED that their loss derepresses germination-specific loci based on Arabidopsis methylome data from Narsai et al., 2017]

4. **SUVR5-like histone methyltransferase (SOV4g015450.1)** is downregulated → H3K9me2 deposition at heterochromatic loci ceases → transposable element (TE)-adjacent genes, which in plants are frequently developmental regulators, become accessible. [KNOWN that SUVR5 deposits repressive marks; INFERRED for germination context]
5. **HIRA histone chaperone (SOV6g036290.1)** is downregulated → altered H3.3 variant deposition dynamics → replication-independent histone turnover is disrupted, favoring retention of activating marks deposited by endogenous germination signals (GA-responsive chromatin remodelers). [KNOWN that HIRA deposits H3.3 at active genes; SPECULATIVE that its downregulation paradoxically favors germination — this could alternatively impair gene activation, representing a key weakness]
6. **GIS2 zinc finger protein (SOV4g038060.1)** is downregulated → stress-responsive transcriptional repression is lifted → growth-promoting genes (e.g., trichome/epidermal differentiation, cell expansion) are derepressed. [INFERRED from Arabidopsis GIS family function in trichome/growth regulation]
7. **PHD-domain protein (SOV4g030590.1)** is downregulated → reduced recruitment of Polycomb Repressive Complex 2 (PRC2) to H3K4me3-marked loci → PRC2-mediated H3K27me3 silencing of germination genes is attenuated. [INFERRED — PHD domains read histone marks; specific substrate unknown]
8. **Chromatin relaxation enables hormone pathway activation (secondary effects):**
9. Derepressed GA biosynthesis genes produce active gibberellins → DELLA repressor proteins are targeted for degradation via the GID1-SCF^SLY1 pathway → GA-responsive transcription factors activate α-amylases, cell wall hydrolases, and expansins. [KNOWN pathway in Arabidopsis and cereals]
10. Simultaneously, the ethylene receptor (SOV3g000150.1) — a negative regulator — is downregulated by exRNAs → constitutive ethylene signaling → EIN3/EIL1 transcription factors stabilized → activation of ethylene-responsive genes that antagonize ABA signaling (e.g., *ERF1* family members). [KNOWN: ethylene receptors are negative regulators; *etr1* loss-of-function enhances germination]
11. LOX (SOV3g035520.1) downregulation → reduced JA biosynthesis → relief of JA-mediated growth suppression and reduced JA-ABA synergistic dormancy maintenance. [KNOWN that JA and ABA synergize to maintain dormancy in many species; Arabidopsis *lox* mutants show altered germination]
12. **Transposon silencing is maintained through exRNA-mediated redundancy:**
13. The five retrotransposon-related genes (SOV2g004250.1, SOV4g025520.1, SOV3g033520.1, SOV1g003910.1, SOV4g035390.1) are directly silenced by bacterial exRNAs → despite the global reduction in host epigenetic silencing machinery, TEs remain suppressed → this prevents the metabolic drain and insertional mutagenesis that would otherwise accompany chromatin relaxation. [SPECULATIVE — this is a key prediction of Model 1 that distinguishes it from Models 2 and 3; it implies the bacterial exRNAs provide a "safety net" for TE control]
14. **Net phenotypic outcome**: Faster, more uniform germination driven by genome-wide transcriptional activation of dormancy-repressed gene programs. Seedling vigor is enhanced because the epigenetic "reset" produces a transcriptional state equivalent to a seed that has undergone complete, natural dormancy release (after-ripening), but achieved in hours rather than weeks/months. Predicted: earlier radicle protrusion (6–12 h advantage), more synchronous germination across a seed lot, and enhanced hypocotyl/radicle elongation rates.

Supporting evidence: - DNA methylation dynam [... truncated]

Synthesis: Confounder Analysis [CONFIDENTIAL]

Confounder Analysis — Quinoa (Chenopodium quinoa)

Critical Analysis of Potential Confounders in the exRNA Germination Improvement System

Preamble [CONFIDENTIAL]

This analysis assumes the following experimental setup: *Chenopodium quinoa* seeds are treated with an exopolysaccharide (EPS) solution derived from an M-9 bacterial strain, and the solution contains extracellular small RNAs (exRNAs) proposed to act via antisense targeting of 31 plant transcripts. The observed phenotype is improved germination rate, vigor, and early seedling growth. My goal is to rigorously evaluate what fraction of this phenotype could arise from mechanisms **other than** sequence-specific antisense RNA silencing.

1. EPS Osmopriming Effect [CONFIDENTIAL]

Mechanism

[KNOWN] Osmopriming is one of the most well-established seed priming techniques in agriculture. Seeds exposed to osmotic solutions (PEG, mannitol, or polysaccharide solutions) undergo controlled hydration without completing germination (Phase II imbibition arrest), allowing pre-germinative metabolic activation — including DNA repair, mitochondrial biogenesis, mRNA accumulation, and antioxidant system upregulation — before the seed is placed in germination conditions (Paparella et al., 2015, *Plant Cell Reports*; Ibrahim, 2016).

[KNOWN] Bacterial EPS solutions are inherently viscous, high-molecular-weight polysaccharide solutions that exert significant osmotic effects. EPS from *Pseudomonas*, *Bacillus*, *Rhizobium*, and other genera typically have water potentials in the range of −0.5 to −1.5 MPa depending on concentration, which falls squarely in the effective osmopriming range (Chen et al., 2007).

Expected Magnitude vs. Observed Effect

[KNOWN] Osmopriming alone routinely produces: - **10–30% increases in germination rate** under normal conditions - **20–50% increases under stress conditions** (salinity, drought) - Significant improvements in germination uniformity (reduced T50) - Enhanced early seedling vigor metrics (root length, shoot length, seedling dry weight)

[INFERRED] This magnitude of effect substantially overlaps with the phenotype described in the quinoa exRNA system. Without knowing the precise fold-change in germination metrics, **osmopriming alone could plausibly account for a large fraction — potentially the majority — of the observed improvement**, particularly if the EPS solution was applied at concentrations that produce meaningful water potential depression.

Controls Needed

CONTROL	PURPOSE
Heat-denatured EPS solution (autoclaved, same concentration)	Retains osmotic properties, destroys RNA
Size-matched polysaccharide solution (e.g., xanthan gum, dextran at matched viscosity/osmolality)	Osmopriming without bacterial-specific elicitor or RNA
PEG 6000 at matched water potential	Pure osmopriming control
Water-only imbibition at matched duration	Hydropriming baseline
EPS solution + RNase A/RNase III treatment	Destroys RNA while retaining all other EPS properties

Evidence level for confounding: [KNOWN] — This is the single most likely confounder and must be rigorously excluded.

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2. Polysaccharide Elicitor Effects [CONFIDENTIAL]

Known Defense/Growth Priming by Bacterial Polysaccharides

[KNOWN] Bacterial EPS and lipopolysaccharides (LPS) are well-characterized microbe-associated molecular patterns (MAMPs) that trigger pattern-triggered immunity (PTI) in plants. Key findings:

- **EPS from plant-beneficial bacteria** (e.g., *Bacillus subtilis*, *Pseudomonas fluorescens*) can prime systemic defense responses including upregulation of PR genes, callose deposition, and ROS burst modulation (Trdá et al., 2015, *Plant Physiology*; Desaki et al., 2018).
- **β-glucans, peptidoglycans, and other polysaccharide fragments** are recognized by plant LysM-type receptors (e.g., CERK1/LYK5 in Arabidopsis), triggering MAPK cascades and defense gene expression (Gust et al., 2012, *Annual Review of Phytopathology*).
- [KNOWN] Bac [... truncated]

Synthesis: Validation Plan [CONFIDENTIAL]

Validation Plan — Quinoa (Chenopodium quinoa)

Comprehensive 4-Tier Validation Plan: Bacterial exRNA-Mediated Germination Improvement in *Chenopodium quinoa*

> **Document Status**: Research design framework | All experimental predictions labeled by epistemic confidence | Species translation gap (SOV → CqV2 orthologs) treated as a primary experimental variable throughout

Preamble: Logical Architecture of the Validation Plan

[CONFIDENTIAL]

The validation plan is structured around a **falsification-first principle**: Tier 1 is designed explicitly to rule out the three most parsimonious alternative explanations (osmopriming, elicitor priming, non-specific RNA effects) before any target-specific mechanistic work begins. Resources should not advance to Tier 2 unless Tier 1 produces affirmative evidence for sequence-specific exRNA activity. Each tier builds on the previous, with explicit decision gates.

Overarching null hypothesis: The observed germination improvement in quinoa seeds treated with bacterial EPS/exRNA solution is entirely attributable to (a) osmopriming by the EPS matrix, (b) MAMP-triggered immunity/priming by bacterial polysaccharides, and/or (c) non-sequence-specific RNA effects, with no contribution from sequence-specific antisense silencing of the 31 predicted target transcripts.

Positive hypothesis: Bacterial exRNAs enter quinoa seed cells during imbibition, are loaded into ARGONAUTE-containing RISC complexes, and direct sequence-specific cleavage or translational repression of quinoa orthologs of the 31 spinach target genes, producing measurable germination improvement through the causal pathways described in the models.

Pre-Validation Requirements (Before Any Tier Begins)

[CONFIDENTIAL]

These are not optional preliminary experiments — they are **prerequisites** without which the entire validation program is uninterpretable.

PRE-1: Quinoa Ortholog Mapping

Rationale: All 31 target gene IDs carry SOV (Spinacia oleracea) prefixes. The entire mechanistic framework assumes conserved function in quinoa. [KNOWN] The CqV2 genome assembly (Jarvis et al., 2017, *Nature*; updated Pan et al., 2021) provides a reference for ortholog identification. [INFERRED] Given ~85–90% amino acid identity for conserved regulatory genes between spinach and quinoa (both Amaranthaceae), functional conservation is likely but cannot be assumed without verification.

Protocol: 1. Extract all 31 SOV protein sequences from SpinachBase or NCBI 2. Perform reciprocal best-hit BLAST against CqV2 protein models (E-value < 1×10^{-20}, identity > 60%) 3. Validate synteny using MCScanX with at least 5-gene collinear blocks flanking each target 4. For targets with multiple quinoa paralogs (e.g., LOX family, DNA methyltransferases), perform phylogenetic tree construction (IQ-TREE, LG+G model) to identify the true ortholog versus paralogs 5. Confirm expression in quinoa seeds using existing RNA-seq datasets (e.g., NCBI SRA: SRP150830 — quinoa seed development transcriptome)

Output required: A curated ortholog table with CqV2 gene IDs, percent identity, synteny confirmation status, and expression evidence for each of the 31 targets. Targets without confirmed quinoa orthologs expressed in seeds are **deprioritized** for all downstream validation.

Timeline: 3–4 weeks (bioinformatics) **Difficulty**: Medium **Cost**: ~$2,000 (computational resources, personnel time)

PRE-2: exRNA Characterization

Rationale: The composition, size distribution, and sequence identity of the bacterial exRNAs must be established before any mechanistic claims can be made. [KNOWN] Bacterial extracellular RNAs are heterogeneous, including tRNA fragments (tRFs), rRNA fragments, regulatory small RNAs (sRNAs), and mRNA fragments, with different biogenesis and functional properties (Ghosal et al., 2015, *Nucleic Acids Research*).

Protocol: 1. Isolate exRNAs from bacterial culture supernatant by sequential centrifugation (300×g, 2,000×g, 10,000×g [... truncated]

Methodology [CONFIDENTIAL]

1. **Target Identification**: Bacterial exRNA sequences aligned against Chenopodium quinoa transcriptome
2. **Gene Analysis (Stage 1)**: Individual gene function analysis via Gemini 2.5 Flash
3. **Pathway Mapping (Stage 2)**: Pathway-level grouping and interaction analysis via Gemini 2.5 Pro
4. **Literature Dive (Stage 3)**: Homolog research and deep literature review
5. **Theme Extraction (Stage 4)**: Cross-cutting biological theme identification
6. **Synthesis (Stage 5)**: Claude-powered ranking, causal modeling, and validation design

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Generated by ExRNA Autonomous Research Platform Gemini (bulk research) + Claude (synthesis & critical review)