

STUDY PROTOCOL

QG-AGR

Quranic Acoustic–Genomic Response

A Controlled, Blinded and Preregistered Framework for Testing Whether Qur’anic Recitation
Produces a Distinct Molecular Response

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Protocol status

Prospective concept and experimental protocol. No QG-AGR molecular results are claimed in this manuscript. Any future empirical findings must be generated under the preregistered procedures described below and replicated independently.

Scientific scope

The protocol tests a narrow, falsifiable hypothesis: whether Qur’anic recitation induces a cellular molecular response that differs from carefully matched linguistic and acoustic controls. It does not assume that DNA contains Qur’anic text, that cells understand semantic meaning, or that a supernatural mechanism has been demonstrated.

Keywords: audible sound, mechanotransduction, Qur’anic recitation, RNA-seq, gene expression, psychoacoustics, sonobiology, preregistration

Abstract

Audible acoustic waves can act as mechanical stimuli at the cellular level, and recent work has shown that controlled sound exposure can alter gene expression in mammalian cells. Separately, studies of Qur'anic listening have predominantly examined neural or psychophysiological outcomes, with substantial heterogeneity and risk of bias. This protocol proposes QG-AGR (Quranic Acoustic–Genomic Response), a blinded and preregistered experimental framework designed to test whether Qur'anic recitation produces a molecular response distinguishable from responses to acoustically and linguistically matched control stimuli. The study intentionally separates three competing explanations: a generic sound effect, an effect of acoustic-temporal organization, and a human semantic/neurobiological effect. The core cellular arm uses standardized recordings, calibrated exposure, randomized sample coding, RT-qPCR screening, RNA-seq discovery, false-discovery-rate control, and an independent replication stage. The primary comparison is Qur'anic recitation versus matched non-Qur'anic Arabic speech produced by the same speaker; secondary contrasts include a temporally disrupted surrogate, a spectral surrogate, and silence. Acoustic features are quantified independently of biological outcomes. The protocol is designed to support either a positive or negative conclusion and explicitly prohibits interpreting a transcriptomic difference as evidence that DNA 'recognizes' Qur'anic meaning. A positive result would establish only that, under specified experimental conditions, Qur'anic recitation generated a reproducible molecular response not fully explained by the prespecified controls. The framework is intended as a rigorous bridge between sonobiology, psychoacoustics and transcriptomics.

1. Background and rationale

Audible sound as a biological stimulus

Experimental work from Kyoto University has shown that audible acoustic stimulation can influence mechanosensitive gene expression in cultured mammalian cells. A 2018 study reported cell-type-specific transcriptional responses to audible sound, with effects dependent on waveform and pressure level. In 2025, a follow-up study using RNA sequencing identified sound-associated differential expression after controlled acoustic exposure and implicated focal adhesion kinase and PTGS2/COX-2 signaling in the response. These findings support a plausible pathway from acoustic mechanical perturbation to cellular signaling and gene regulation [1–3].

The emerging field of sonobiology therefore provides a mechanistic basis for testing whether different acoustic structures can produce different cellular responses. Importantly, this mechanism does not require a cell to understand language. It requires only that sound-specific physical properties interact differently with mechanosensitive cellular systems.

Qur'anic listening research

A 2025 systematic review identified 22 eligible EEG studies of Qur'anic listening and reported a recurring association with increased alpha and theta activity, while also emphasizing major heterogeneity, incomplete reporting and high risk of bias in most included studies [4]. This literature motivates more rigorous experimental work but does not establish a molecular or genomic mechanism. QG-AGR is designed to address a different question: whether the acoustic signal itself can produce a reproducible cellular gene-expression response under controlled conditions.

Research gap and novelty

A targeted literature search conducted for this protocol identified studies of audible-sound mechanotransduction and studies of Qur'anic listening, but did not identify a directly comparable cell-transcriptomic experiment that contrasts Qur'anic recitation with same-speaker, linguistically matched Arabic speech together with temporal and spectral surrogates. This observation is not equivalent to a formal systematic prior-art review; therefore, any claim of “first in the world” should be reserved until a structured search of major bibliographic databases is completed.

2. Research question, hypotheses and estimands

Primary research question

Under calibrated in-vitro acoustic exposure, does Qur'anic recitation produce a reproducible transcriptomic response that differs from the response to non-Qur'anic Arabic speech matched for speaker, duration, vocal intensity, speaking rate and broad phonetic composition?

Primary hypotheses

- H0 (null): after controlling for specified acoustic and linguistic variables, the transcriptomic response to Qur'anic recitation does not differ from matched Arabic speech beyond expected experimental variation.
- H1 (alternative): Qur'anic recitation produces a reproducible transcriptomic response that differs from matched Arabic speech under the prespecified experimental conditions.

Secondary hypotheses

- Temporal-organization hypothesis: the response to intact recitation differs from a temporally disrupted surrogate despite similar global spectral properties.
- Generic-acoustic hypothesis: any observed response is explained by acoustic energy or broad spectral characteristics and is reproduced by a spectral surrogate.
- Cell-type specificity hypothesis: effects vary by cell model, consistent with known cell-type-specific mechanosensitivity.

Interpretive boundary

A statistically significant molecular difference would not demonstrate that DNA contains Qur'anic text, that cells comprehend semantic meaning, or that a supernatural mechanism has been established. It would support only the experimentally bounded statement that the tested acoustic condition produced a reproducible molecular response distinct from the prespecified controls.

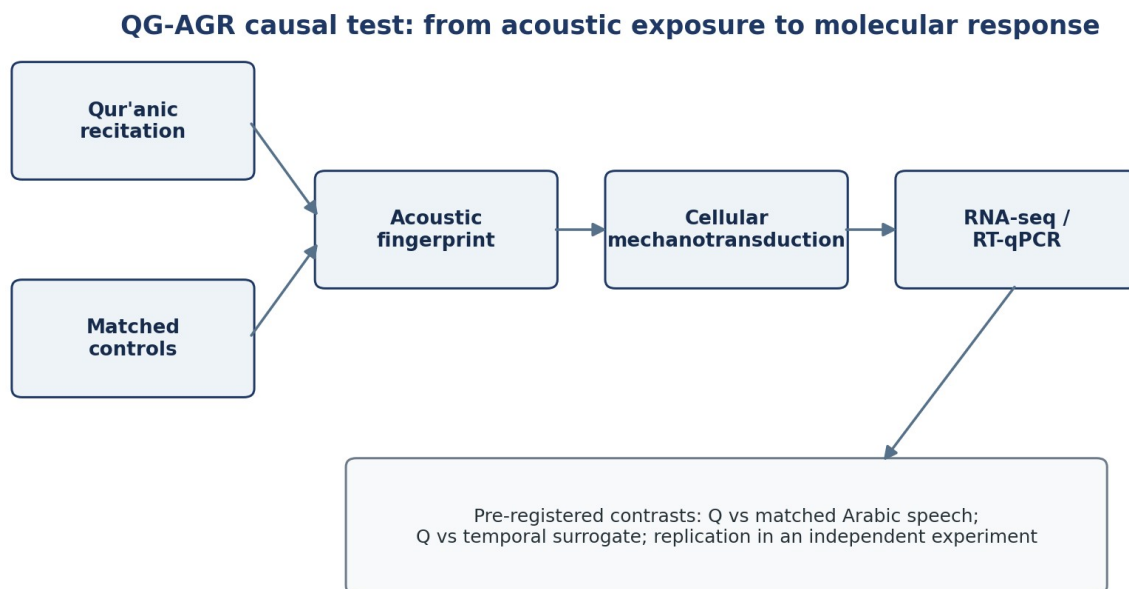


Figure 1. QG-AGR causal test. Acoustic conditions are characterized independently, delivered under calibrated exposure, and followed by molecular measurement and preregistered statistical contrasts. The design deliberately separates acoustic physics from semantic interpretation.

3. Study design

Overview

The core study is a randomized, blinded, controlled in-vitro experiment with staged validation. Phase 0 characterizes and matches audio. Phase 1 validates the acoustic-exposure system using known sound-responsive molecular markers. Phase 2 performs discovery RNA-seq. Phase 3 independently replicates pre-specified targets or pathway scores. Human listening studies, if undertaken, constitute a separate protocol and should not be mixed with the in-vitro causal analysis.

Code	Condition	Purpose
Q	Qur'anic recitation with standard tajwid	Test condition
A	Matched non-Qur'anic Arabic prose, same speaker	Primary linguistic/acoustic control
T	Temporally disrupted recitation preserving broad acoustic content	Tests temporal organization
S	Spectral surrogate preserving long-term spectrum/amplitude statistics	Tests broad spectral-energy explanation
0	Silence / sham handling	Baseline control

Stimulus selection

Qur'anic passages must be selected before biological data are generated. Selection should not be based on a presumed health effect, gene association or theological interpretation. Multiple passages should be used across discovery and replication so that a single recording cannot define the entire result. A same-speaker matched Arabic condition should approximate duration, phoneme distribution, syllabic rate, mean fundamental frequency and vocal intensity as closely as practicable.

Acoustic standardization

All stimuli will be normalized and characterized before wet-lab unblinding. Sound pressure or equivalent mechanical input at the culture surface will be physically measured rather than inferred from loudspeaker settings. The exposure level will be chosen through a viability-preserving pilot and held constant across active conditions. The protocol will record frequency spectrum, root-mean-square amplitude, modulation spectrum, fundamental-frequency trajectory, pause distribution, spectral centroid, spectral entropy, harmonic-to-noise ratio, MFCC features and temporal recurrence metrics.

Randomization and blinding

Audio conditions and biological samples will be assigned opaque random codes. Personnel performing RNA extraction, library preparation and primary bioinformatic processing will remain blinded to condition labels until the prespecified quality-control and primary-analysis steps are complete. Plate or batch assignments will be randomized and balanced across conditions and time points.

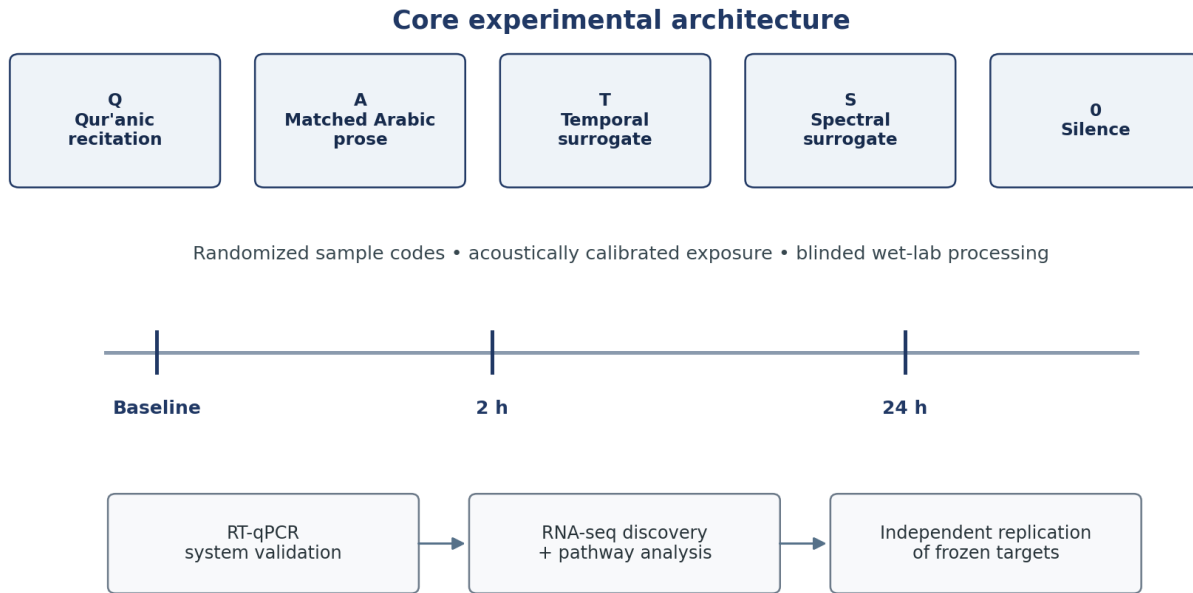


Figure 2. Core experimental architecture. Phase 1 screens the exposure system; Phase 2 performs transcriptome-wide discovery; Phase 3 independently tests a frozen set of targets or pathway scores.

4. Experimental system

Cell models

The initial positive-mechanosensitivity validation should use a mammalian cell model with published audible-sound responsiveness, such as C2C12 myoblasts, to confirm that the exposure apparatus reproduces a measurable response [1,2]. The central QG-AGR claim should then be tested in at least one human-derived cell model selected prospectively on the basis of viability, reproducibility and a validated response to a standardized acoustic control. The choice of human model should be documented before the Qur'anic-versus-control comparison is unblinded.

Exposure apparatus and calibration

The apparatus must deliver stable, reproducible acoustic stimulation to the culture environment while minimizing confounds from heat, airflow, vibration of the incubator or uncontrolled plate movement. Calibration should be performed at the location of the culture vessel. Temperature and other relevant environmental variables should be logged. A pilot experiment should verify cell viability and exclude gross cytotoxicity across the selected exposure range.

Time points

Two time points are proposed for the discovery study: 2 hours to capture relatively early signaling/transcriptional responses and 24 hours to capture sustained or secondary transcriptional responses. This choice is anchored in prior audible-sound RNA-seq work, which reported differential expression at both 2 and 24 hours [1]. The 24-hour Q-versus-A contrast is designated as the primary transcriptomic contrast; the 2-hour comparison is secondary.

5. Molecular measurements

Phase 1: RT-qPCR system validation

Before transcriptome-wide sequencing, the apparatus should be validated using a small panel of mechanosensitive or previously sound-responsive genes, with PTGS2/COX-2 included as a mechanistically motivated positive marker based on prior work [1]. The purpose is not to establish Qur'anic specificity but to confirm that the sound-delivery system can elicit a reproducible biological signal.

Phase 2: RNA-seq discovery

The discovery stage will use genome-wide RNA sequencing. Library preparation, read depth and sequencing platform should be standardized across all samples and balanced across batches. Raw read quality, mapping rate, library complexity and sample-level outliers will be assessed according to a written QC plan before condition unblinding.

Replicates

The planned core design uses eight independent biological replicates per condition per time point, subject to pilot-based power refinement. Benchmark studies consistently show that biological replication contributes more to differential-expression power than simply increasing sequencing depth, and at least six biological replicates have been recommended for robust RNA-seq designs under many conditions [5,6]. Eight replicates is therefore a defensible starting point rather than a universal guarantee of power.

Element	Planned specification
Core conditions	Q, A, T, 0; S may be included in the full design or a dedicated secondary experiment
Time points	2 h and 24 h
Biological replicates	8 per condition per time point, refined by pilot dispersion estimates
Primary contrast	Q vs A at 24 h
Key secondary contrasts	Q vs A at 2 h; Q vs T; active sound vs silence
Discovery threshold	Benjamini-Hochberg FDR < 0.05; report effect sizes and confidence intervals
Replication	Independent biological experiment using a frozen target/pathway set

6. Statistical analysis plan

Pre-registration

Before the first discovery RNA-seq dataset is unblinded, the protocol will freeze: stimulus files and hashes, primary and secondary contrasts, inclusion/exclusion rules, batch covariates, QC thresholds, normalization method, statistical model, multiple-testing procedure and replication criteria. Any exploratory analyses added afterward will be labeled explicitly as exploratory.

Differential expression

Gene-level counts will be analyzed using a negative-binomial framework such as DESeq2 [7]. The model will include condition, time, their interaction when appropriate, and prespecified batch terms. The primary analysis will compare Q versus A at 24 hours. Genome-wide P-values will be adjusted using the Benjamini-Hochberg false-discovery-rate procedure [8]. Results will report adjusted P-values, log2 fold changes, uncertainty intervals and normalized expression, rather than relying on significance labels alone.

Global transcriptomic structure

Principal-component and sample-distance visualizations will be used for descriptive QC, not as standalone evidence of a treatment effect. A prespecified multivariate test may be used as a complementary global endpoint, but gene-level inference and independent replication remain necessary.

Pathway analysis

Gene-set or pathway enrichment will be performed only after the primary gene-level analysis using curated databases. Mechanotransduction, focal-adhesion, calcium-signaling, cytoskeletal, YAP/TAZ-associated and prostaglandin-related pathways are biologically motivated areas of interest, but they will not be declared positive unless they meet the prespecified statistical criteria. The protocol will distinguish confirmatory from exploratory pathway analyses.

Acoustic-to-genomic modeling

A secondary innovation of QG-AGR is to model the relationship between measured acoustic features and molecular response without assigning semantic meaning to the signal. Acoustic feature vectors $A(t)$ will be linked to gene- or pathway-level response vectors G through regularized regression or other cross-validated predictive models. Model development and evaluation must be separated by held-out recordings or experiments to prevent overfitting.

Replication rule

A discovery result will not be considered established until an independent experiment tests a frozen set of genes, pathway scores or predictive outputs. Replication requires prespecified directional concordance and statistical support in the independent dataset. Exploratory post hoc target selection will not count as confirmatory replication.

7. Criteria for interpreting outcomes

Outcome	Interpretation
$Q \approx A$; both differ from silence	Consistent with a generic speech/sound response; no evidence of Qur'anic-specific acoustic effect.
Q differs from A but resembles T	Possible phonetic/spectral difference; temporal organization not supported as the driver.
Q differs from A and T ; effect replicates	Evidence that intact recitation has a reproducible molecular effect not captured by the specified controls; mechanism remains unresolved.
Q differs in discovery but not replication	Initial finding is not considered established.
No active condition differs from silence	Exposure system or chosen cell model may be biologically insensitive under the tested parameters.

Strong-evidence criteria

1. The Q -versus- A effect survives genome-wide multiplicity correction or meets a preregistered global endpoint.
2. The direction and magnitude are reproducible in an independent biological experiment.
3. The finding is not explained by measured loudness, broad spectral content, batch or viability differences.
4. The effect is robust to at least one alternative passage and, ideally, more than one reciter.
5. Predictive acoustic-to-genomic models generalize to held-out recordings rather than merely fitting the discovery data.

8. Reciter- and passage-generalization experiments

A single recording cannot support a general claim about Qur'anic recitation. If the core experiment is positive, a second-stage factorial design should cross multiple reciters with multiple passages. The strongest evidence for a recitation-structure effect would occur if the same passage or recitation class produced convergent molecular patterns across different voices while same-speaker non-Qur'anic controls did not. Conversely, if effects track

the individual speaker rather than the recited material, the interpretation should shift toward generic voice acoustics.

Verse Molecular Fingerprint (exploratory concept)

A later exploratory program may represent each passage by an acoustic feature vector and each exposure by a transcriptomic response vector, then test whether passage identity predicts molecular-response similarity across reciters. This proposed “Verse Molecular Fingerprint” should remain exploratory until the core Q-versus-A effect is independently replicated.

9. Future human-validation arm

Any human listening study should be conducted under a separate ethics-approved protocol. A randomized crossover design could compare Qur’anic recitation, matched Arabic prose and acoustic controls while measuring EEG, heart-rate variability, respiration and selected peripheral biomarkers. Inclusion of Arabic-speaking and non-Arabic-speaking participants could help distinguish acoustic from semantic/cognitive pathways. The current in-vitro protocol cannot adjudicate semantic effects because isolated cells do not possess linguistic comprehension.

10. Bias control, reproducibility and open science

- Preregister primary hypotheses, contrasts and analysis code before unblinding.
- Archive original audio files, processing scripts, acoustic fingerprints and cryptographic hashes.
- Randomize plate position, treatment order and sequencing batch.
- Blind wet-lab and primary bioinformatics personnel to condition labels.
- Publish negative as well as positive results.
- Deposit raw and processed transcriptomic data in an appropriate public repository when ethically and legally permitted.
- Provide executable analysis code and a machine-readable protocol version.
- Seek replication by an independent laboratory before making broad biological or theological claims.

11. Limitations

The protocol faces several important limitations. First, acoustic matching across natural speech conditions can never be perfect; residual differences must therefore be measured and modeled rather than assumed absent. Second, in-vitro sound delivery may include substrate vibration or other mechanical pathways that differ from ordinary human listening. Third, cell-line effects may not generalize to human tissues or whole organisms. Fourth, transcriptomic differences do not by themselves identify the proximal physical mechanism. Fifth, multiple acoustic features are highly correlated, creating substantial overfitting risk. Finally, religious significance is a property of human interpretation and cannot be inferred from a cell-culture response.

12. Novelty statement

QG-AGR is intentionally framed as a falsifiable experimental architecture rather than a claim of discovery. The potentially novel element is the combination of (i) Qur’anic recitation, (ii) same-speaker matched Arabic linguistic control, (iii) temporal and spectral surrogate controls, (iv) calibrated in-vitro acoustic exposure, (v) transcriptome-wide readout, (vi) blinded processing, and (vii) independent replication. A formal systematic search and prior-art review should be completed before submission if the manuscript intends to use language such as “first study” or “first demonstration.”

13. Expected scientific contribution

A negative result would be scientifically informative by establishing that, under the tested conditions, Qur’anic recitation does not produce a molecular response distinguishable from matched speech or acoustic controls. A positive and independently replicated result would justify mechanistic investigation of which acoustic dimensions produce the effect. In either case, the protocol shifts the discussion from numerical coincidence or unverifiable claims to experimentally testable biology.

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14. Author contribution statement

Nabi Mamazhan: conceptualization; origin of the QG-AGR research hypothesis; development of the core comparative framework; study-design direction; interpretation framework; manuscript concept and supervision of the proposed research program. Experimental, bioinformatic and statistical contributors should be added as co-authors or acknowledged in accordance with their actual contributions and the policies of the eventual target journal.

15. Ethics, funding and conflicts

Ethics: The core protocol described here is an in-vitro study and does not involve human participants. Any future human arm requires prior institutional ethics approval and informed consent. Funding: to be declared before submission. Competing interests: to be declared before submission.

16. Data and code availability plan

Before data collection, the final protocol, stimulus hashes and statistical analysis plan should be time-stamped in a public or institutionally controlled preregistration repository. Following publication, de-identified raw sequencing data, processed count matrices, acoustic features and analysis code should be made available to the extent permitted by applicable agreements and repository policies.

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Submission note

This manuscript is a study protocol/concept paper prepared for public online dissemination and future journal submission. Corresponding author: Nabi Mamazhan (Nabi.mamazhan@gmail.com). Before formal journal submission, the author should add any target institutional affiliation, finalized funding/conflict statements, and the final preregistration identifier. Journal-specific requirements for disclosure of AI-assisted drafting or editing should also be followed.