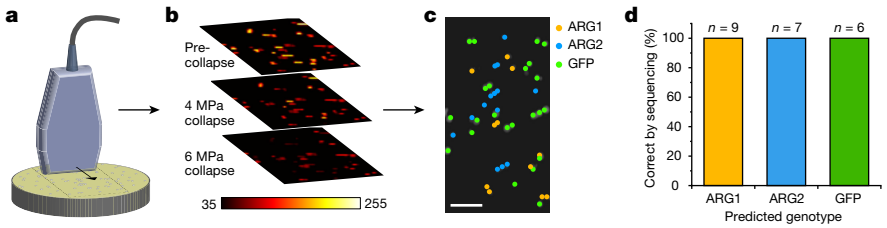

Figure 5 | High-throughput screening of acoustic phenotypes. **a**, Illustration of acoustic colony screening. **b**, Colony ultrasound images of a mixed population of *E. coli* colonies expressing *arg1*, *arg2*, and GFP. Images were acquired before collapse and after collapse at peak acoustic pressures of 4 and 6 MPa. This imaging experiment was performed once; each colony was treated as a biological replicate. **c**, Predicted genotypes of each colony based on the acoustic phenotype seen in the images in **b**. Scale bar, 10 mm. **d**, Confirmation of predicted genotypes by colony picking and sequencing. *n*, number of sequenced colonies of each type.

image these cells after injection into tumours (Extended Data Fig. 9 and Supplementary Note 3).

Finally, to facilitate future genetic engineering of ARGs, we assessed the amenability of these constructs to high-throughput screening. In fluorescent protein engineering, directed evolution has served as an effective approach to identify variants with new spectral and biochemical properties[31,32], often using mutant bacterial colonies as a convenient platform for high-throughput screening[32]. To determine whether a similar approach could be used with ARGs, we developed a method to scan bacterial colonies with ultrasound (Fig. 5a). In this method, colonies are immobilized on agar plates with an over-layer of agarose, then scanned with an ultrasound transducer translated by a computer-controlled robot. This results in a series of transverse images that can be reconstructed to form an in-plane image of the plate (Fig. 5b). We used this technique to image a mixed plate of *E. coli* transformed with *arg1*, *arg2* or GFP. Serial acoustic collapse imaging (Fig. 5b) revealed three distinct colony populations (Fig. 5c and Extended Data Fig. 10), allowing the genotypes to be distinguished from each other with 100% accuracy (Fig. 5d). This result suggests that colony screening can discriminate acoustic phenotypes with sufficient accuracy to serve as a high-throughput assay for acoustic protein engineering.

Our study establishes engineered gas vesicle gene clusters as reporter genes for ultrasound, giving this widely used noninvasive imaging modality the ability to visualize genetically modified bacteria inside living animals. Future work will build on the *in vitro* and *in vivo* proofs of concept presented in this study to answer scientific and translational questions. This research will benefit from the development of ultrasound techniques to detect ARG signals and distinguish them from background (Supplementary Note 4), further genetic engineering to optimize the stability and host burden of ARG constructs, and expression of these reporters in a broader range of microbial species (Supplementary Note 5). In addition, it is ultimately desirable to express ARGs in mammalian cells.

We anticipate that the ARGs presented in this work are a starting point for future engineering of ultrasound reporter genes. Since their initial discovery as optical reporters, fluorescent proteins have been engineered, evolved and used in thousands of unforeseen optical imaging applications. Our findings that genetic engineering can be used to generate ARGs with distinct acoustic properties and that ARGs are amenable to colony-based high-throughput screening suggest that a similar trajectory may be available for this new technology.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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