Engineered bacteria detect tumor DNA

Robert M. Cooper[1*], Josephine A. Wright[2,*], Jia Q. Ng[3], Jarrad M. Goyne[2], Nobumi Suzuki[2,3], Young K. Lee[3], Mari Ichinose[2,3], Georgette Radford[3], Elaine M. Thomas[3], Laura Vrbanac[3], Rob Knight[6,7,8,9], Susan L. Woods[2,3,**], Daniel L. Worthley[2,4,**] and Jeff Hasty[1,5,6,9**].

1. BioCircuits Institute, University of California, San Diego, La Jolla, CA, USA
2. Precision Medicine Theme, South Australia Health and Medical Research Institute, Adelaide, SA, Australia.
3. University of Adelaide, Adelaide, SA, Australia
4. Colonoscopy Clinic, Brisbane, Qld, Australia.
5. Department of Bioengineering, University of California, San Diego, La Jolla, CA.
6. Molecular Biology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA.
7. Department of Pediatrics, University of California, San Diego, La Jolla, CA.
8. Department of Computer Science & Engineering, University of California, San Diego, La Jolla, CA.
9. Center for Microbiome Innovation, University of California, San Diego, La Jolla, CA.

* These authors contributed equally

** Corresponding authors

Word count:

Summary paragraph = 228 words

The main text (summary paragraph plus body text) = 2076 words

Summary

Advances in bacterial engineering have catalysed the development of living cell diagnostics and therapeutics[1–3], including microbes that respond to diseases such as gut inflammation[4], intestinal bleeding[5], pathogens[6] and hypoxic tumors[7]. Bacteria can easily access the entire gastrointestinal tract via oral administration[8], and they can produce outputs that can be noninvasively measured in stool[4] or urine[7]. Cellular memory, such as bistable switches[4,9,10] or genomic rearrangement[11], has been used to allow bacteria to store information over time. However, living biosensors have not yet been engineered to detect specific DNA sequences or mutations from outside the cell. Here, we engineer naturally competent *Acinetobacter baylyi* to detect donor DNA from the genomes of colorectal cancer (CRC) cells and organoids. We characterize the functionality of the biosensors *in vitro* with co-culture assays and then validate *in vivo* with sensor bacteria delivered orally or rectally into mice injected with orthotopic donor CRC organoids. We observe horizontal gene transfer from the tumor to the sensor bacteria *in vivo*, allowing their detection in stool. The sensor bacteria achieved 100% discrimination between mice with and without CRC using both delivery methods. Our findings establish a framework for biosensing applications that require the detection of mutations or organisms within environments that are difficult to sample. In addition, the platform can be readily expanded to include *in situ* production and delivery of therapeutic payloads at the detection site.

Main text

Some bacteria are naturally competent for transformation and can sample extracellular DNA directly from their environment[12]. Natural competence is one mechanism of horizontal gene transfer (HGT), the exchange of genetic material between organisms outside vertical, "parent to offspring" transmission[13]. HGT is common between microbes[13] and from microbes into animals and plants[14]. Genomic analyses have found signatures of HGT from eukaryotes to prokaryotes[15], but the forward engineering of bacteria to detect or respond to human DNA via HGT has not been explored. *Acinetobacter baylyi* is a highly competent and well-studied bacterium[16] that is largely non-pathogenic in healthy humans[17] and can colonize the murine gastrointestinal tract[18]. This combination of traits renders *A. baylyi* an ideal candidate for engineered detection of target DNA *in situ* (Fig. 1). Our strategy delivers bacterial biosensors non-invasively to the gastrointestinal tract, where they sample and genomically integrate target tumor DNA. To systematically demonstrate the concept, we use the sensor to detect engineered tumor cells. Since *A. baylyi* is easily transformable, our approach can be expanded to harness HGT to interact

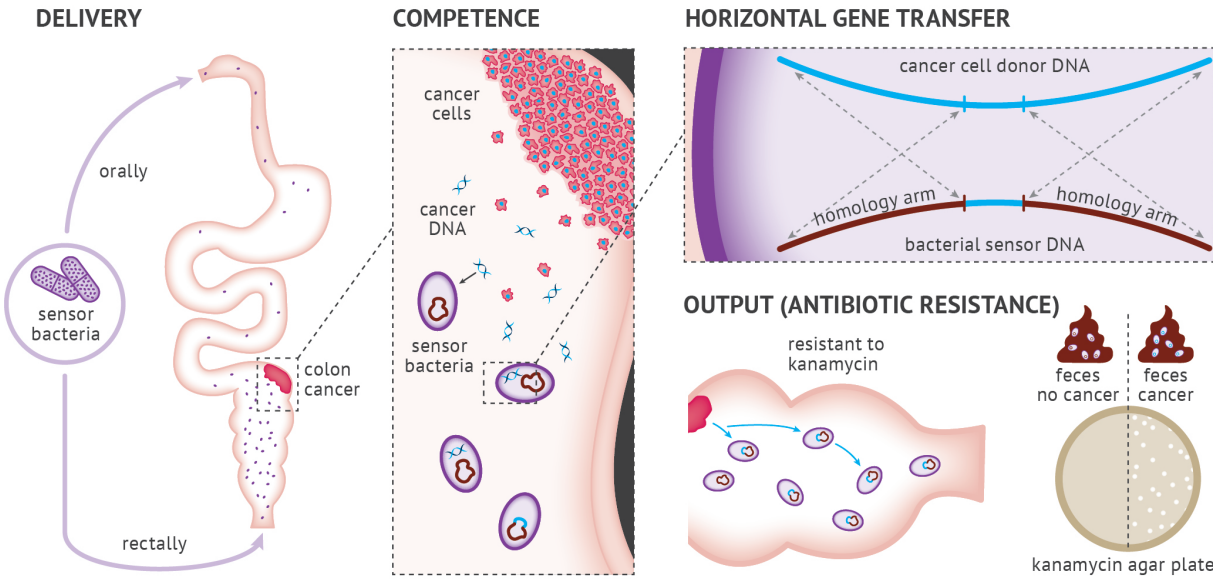

Figure 1. Engineered bacteria to detect tumor DNA. Engineered *A. baylyi* bacteria are delivered orally or rectally in an orthotopic mouse model of CRC. The naturally competent *A. baylyi* take up tumor DNA shed into the colonic lumen. The tumor donor DNA is engineered with a *kanR* cassette flanked by *KRAS* homology arms (HA). The sensor bacteria are engineered with matching *KRAS* homology arms that promote homologous recombination. Sensor bacteria that undergo HGT from tumor DNA, acquire kanamycin resistance and are quantified from stool by serial dilution on kanamycin selection plates.

with genetic elements within the sensor bacteria in a manner that activates downstream output.

Sensor bacteria can detect human cancer DNA

To test the hypothesis that bacteria could detect human tumor DNA, we generated transgenic donor human cancer cells and sensor bacteria (Fig. 2a). The donor cassette comprised a kanamycin resistance gene and GFP (kan^R-*GFP*) flanked by 1 kb homology arms from human *KRAS* (Fig. 2b-c and Extended Data Fig. 1). *KRAS* is an important oncogene in human cancer, and a driver mutation in *KRAS* often accompanies the progression of simple into advanced colorectal adenomas[19]. We stably transduced this donor cassette into both RKO and LS174T human CRC cell lines using a lentiviral vector. To construct the sensor bacteria, we inserted a complementary landing pad with *KRAS* homology arms into a neutral genomic site of *A. baylyi*. We tested both a "large insert" design (2 kb), with a different resistance marker between the *KRAS* arms to be replaced by the donor cassette (Fig. 2b, Extended Data Fig. 2a), and a "small insert" design (8 bp), with the same kan^R-*GFP* cassette as in the tumor donor DNA but interrupted by 2 stop codons in kan^R (Fig. 1 & 2c, Extended Data Fig. 2b & 3). The biosensor output was growth on kanamycin plates, measured as colony-forming units (CFUs).

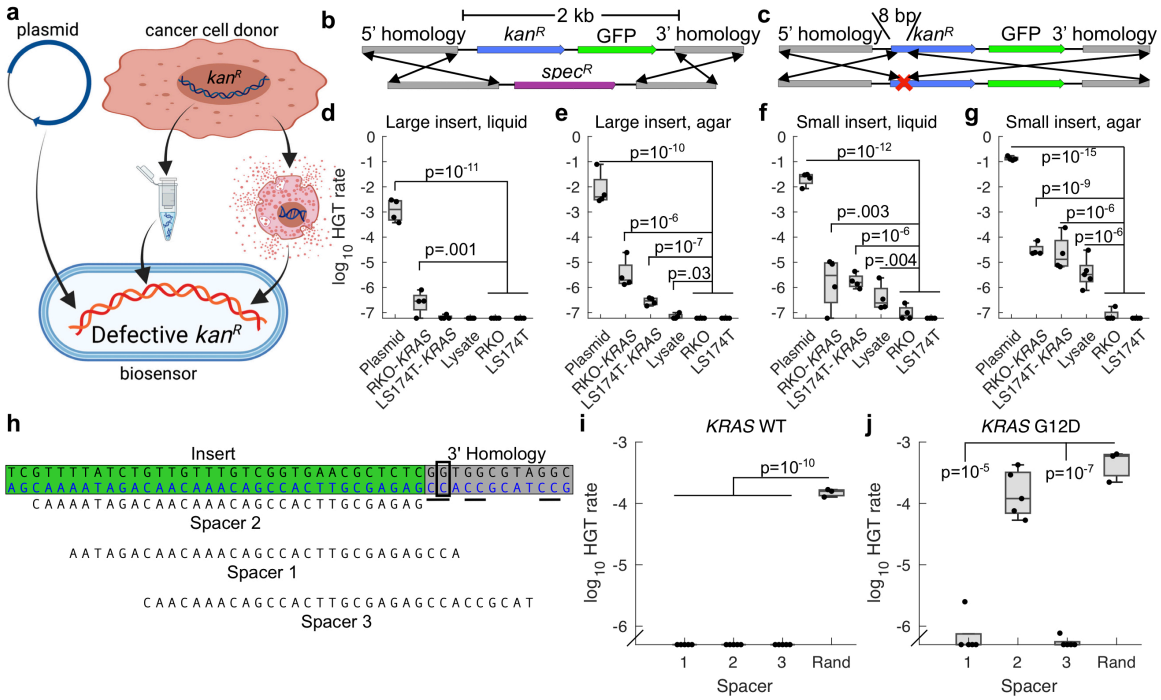

Figure 2: Sensing *KRASG12D* DNA *in vitro*. a-c, Donor DNA consisting of plasmid, purified cancer cell genomic DNA, or raw lysate (top) recombines into biosensor *A. baylyi* cells (bottom), transferring either a large, 2 kb insert (**b**) or a small, 8 bp insert to repair 2 stop codons **(c)** in both cases conferring kanamycin resistance. **d-g**, *A. baylyi* biosensors were incubated with plasmid DNA, purified RKO-*KRAS* or LS174T-*KRAS* genomic DNA, or raw RKO-*KRAS* lysate, all containing the donor cassette, or purified RKO or LS174T genomic DNA as controls. Biosensor cells included either "large insert" (**b,d,e**) or "small insert" (**c,f,g**) designs, and transformations were performed in liquid culture (**d,f**) or on solid agar surfaces (**e,g**). Two-sample t-tests compared data to combined RKO and LS174T genomic DNA controls for the same conditions. **h**, CRISPR spacers targeting the *KRASG12D* mutation (boxed), using the underlined PAMs. **i,j**, Fraction of total biosensor cells expressing the indicated CRISPR spacers that were transformed by plasmid donor DNA with wild type (**i**) or mutant G12D (**j**) *KRAS*. Statistics were obtained using two-sample t-tests. Data points below detection are shown along the x-axis.

We tested both designs using various donor DNA sources, both in liquid culture and on solid agar (Fig. 2a). The "large insert" biosensors detected donor DNA from purified plasmids and genomic DNA both in liquid (Fig. 2d) and on agar (Fig. 2e). On agar, they also detected raw, unpurified lysate, albeit at just above the limit of detection (Fig. 2e). As expected[20], the "small insert" design improved detection efficiency roughly 10-fold, reliably detecting donor plasmid, purified genomic DNA, and raw lysate both in liquid and on agar (Fig. 2f-g, Extended Data Supplemental Movie). Across donor DNA and biosensor design, detection on solid agar was approximately 10-fold more efficient than in liquid culture. Importantly, detection of donor DNA from raw lysate demonstrated that the biosensors do not require *in vitro* DNA purification[21].

A. baylyi can take up DNA at approximately 60 bp/s[22]. Given a human genome of 3.2 x 10^9 bp, each *A. baylyi* cell, including its direct ancestors, can sample roughly 10^{-3} of a human genome in a 24-hour period. Combined with the data shown in Fig. 2g, with a detection rate around 10^{-5} per *A. baylyi* cell for RKO-*KRAS* and LS174T-*KRAS* donor

DNA, this suggests a detection efficiency of around 1% per processed donor sequence. While this calculation assumes a constant DNA processing rate, the result is quite similar to what we found for HGT from *E. coli* to *A. baylyi*[21].

Sensor bacteria can discriminate wild-type from mutant *KRAS* DNA

Mutations in codon 12 of *KRAS* are present in 27% of CRC[23], and are common in solid tumors generally[24]. To test whether sensor bacteria could discriminate between wild-type and mutant *KRAS (KRASG12D)*, which differ by a single G>A transition, we utilized *A. baylyi's* endogenous Type I-F CRISPR-Cas system[25]. We stably transduced an RKO cell line with the *kan^R-GFP* donor cassette flanked by wild-type *KRAS* (RKO-*KRAS*), and a second line with *KRASG12D* flanking sequences (RKO-*KRASG12D*). Next, we designed 3 CRISPR spacers targeting the wild-type *KRAS* sequence at the location of the *KRASG12D* mutation, using the *A. baylyi* protospacer-adjacent motif (PAM) of 5'-CC-protospacer-3' (Fig. 2h). We inserted these as single-spacer arrays into a neutral locus in the "large insert" *A. baylyi* sensor genome.

The sensor bacteria, if effective, should reject wild-type *KRAS* through CRISPR-mediated DNA cleavage. Conversely, the *KRASG12D* sequence should alter the target sequence and evade DNA cleavage. Two of the three spacers blocked transformation by both wild-type and mutant DNA (Fig. 2i-j). However, spacer 2, for which the *KRASG12D* mutation eliminated the PAM site, selectively permitted HGT only with *KRASG12D* donor DNA (Fig. 2E-F). The other common mutations in codon 12 of *KRAS* all eliminate this PAM as well[23]. Thus, sensor *A. baylyi* can be engineered to detect a hotspot mutation in the *KRAS* gene with single-base specificity.

Sensor bacteria can integrate cancer DNA in organoid culture

Ex vivo organoid culture faithfully reflects endogenous tumor biology[26]. We therefore evaluated our sensor and donor constructs in organoid culture (Fig. 3a). We previously used CRISPR/Cas9 genome engineering to generate compound *Braf^{V600E}; Tgfbr2$^{\Delta/\Delta}$; Rnf43$^{\Delta/\Delta}$; Znrf3$^{\Delta/\Delta}$; p16Ink4a$^{\Delta/\Delta}$* (BTRZI) mouse organoids that recapitulate serrated CRC when injected into the mouse colon[27].

We transduced BTRZI organoids with the human *KRAS*-flanked donor DNA construct (*KRAS-kan^R*) to generate donor CRC organoids, and incubated their lysate with the more efficient "small insert" *A. baylyi* biosensors. As with the CRC cell lines, the sensor *A. baylyi* incorporated DNA from donor organoid lysate, but not from control lysates from

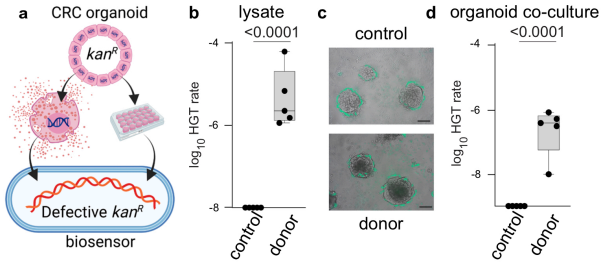

Figure 3: Detection of donor DNA from BTRZI-*KRAS-kan*^{R} organoids. **a**, Schema depicting *in vitro* co-culture of *A.baylyi* sensor bacteria with BTRZI-*KRAS-kan*^{R} (CRC donor) organoid lysates or viable organoids to assess HGT repair of kanamycin resistance gene (*kan*^{R}). **b**, Recombination with DNA from crude lysates enables growth of *A.baylyi* sensor on kanamycin plates with transformation efficiency of $1.4x10^{-5}$ (limit of detection 10^{-8}). **c**, Representative images of GFP-tagged *A.baylyi* sensor surrounding parental BTRZI (control) and BTRZI-*KRAS-kan*^{R} donor organoids at 24h. Scale bar 100µm. **d**, Co-culture of established CRC BTRZI-*KRAS-kan*^{R} donor organoids with *A.baylyi* sensor enables growth of *A.baylyi* sensor on kanamycin plates with transformation efficiency of $3.8x10^{-7}$ (limit of detection 10^{-9}). In **b**, **d**, n = 5 independent experiments each with 5 technical replicates, one sample t-test on transformed data was used for statistical analysis with P values as indicated.

the parental organoids (Fig. 3b, Extended Data Fig. 4a). Next, we co-cultured GFP-expressing sensor *A. baylyi* with BTRZI parental or BTRZI-*KRAS-kan*^{R} donor organoids for 24 hours on Matrigel. The GFP-expressing sensor bacteria surrounded the organoids (Fig. 3c and Extended Data Fig. 4b). Following co-culture with donor, but not parental, organoids, the *A. baylyi* sensor bacteria acquired donor DNA via HGT (Fig. 3d). HGT of kanamycin resistance was confirmed by Sanger sequencing of individual colonies (Extended Data Fig. 4c). Note that these experiments did not test specificity for mutant *KRAS*, but whether organoid-to-bacteria HGT would occur in organoid co-culture.

Sensor bacteria can detect tumor DNA *in vivo*

Given that cancer to bacterial HGT occurred *in vitro*, both in cell lines and in organoid co-culture, we sought to test this system *in vivo*. *A. baylyi* previously survived transit through the mouse gastrointestinal tract in germ-free animals[18]. To confirm this finding and to optimize our experimental protocol, we used mCherry-expressing, kanamycin-resistant *A. baylyi*. One week after antibiotic gut decontamination, we administered 10^{10} *A. baylyi* either by single oral gavage or rectal enema. Mice administered *A. baylyi* by either route maintained gastrointestinal colonization for at least one week, as measured by stool CFU assays and fluorescence (Extended Data Fig. 5). Next, we confirmed that our BTRZI, orthotopic CRC model released tumoral DNA into the fecal stream. In this mouse model of CRC, engineered CRC organoids were injected orthotopically, by mouse colonoscopy, into the mouse colon to form colonic tumors, as previously described[27]. Using digital droplet PCR, we measured *Braf* mutant tumor DNA in stools collected from tumor-bearing and control mice. The BTRZI model reliably released tumor DNA into the colonic lumen (Extended Data Fig. 6).

Having confirmed that sensor bacteria would colonize the mouse gastrointestinal tract and that DNA is released from the tumor, we conducted an orthotopic CRC experiment (Fig. 4a). At week -4, NSG mice were either injected colonoscopically, or not, with

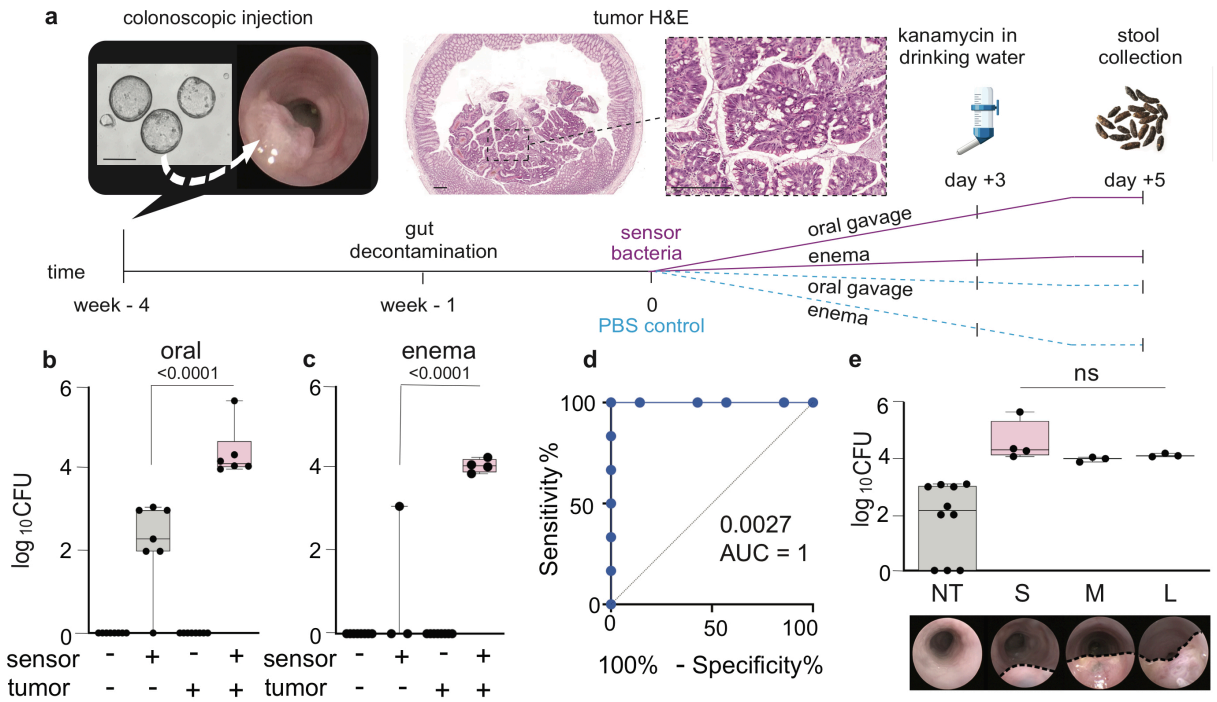

Figure 4. Horizontal gene transfer detected in stool from mice bearing BTRZI-*KRAS-kanR* tumors after oral or rectal dosing of *A. baylyi* sensor bacteria. **a** Schema depicting *in vivo* HGT experiments: generation of BTRZI-*KRAS-kanR* (CRC donor) tumors in mice via colonoscopic injection of CRC donor organoids with tumor pathology validated by H&E histology, administration of PBS control or sensor *A. baylyi* and stool collection. Scale bars 200µm. **b**, oral or **c**, rectal delivery of *A. baylyi* sensor to mice bearing CRC donor tumors results in kanamycin resistant *A. baylyi* sensor in stool via HGT. Average CFU per stool from 2-4 stools per mouse grown on Kanamycin selection plates is shown, n=3-8 mice/group. **d**, ROC curve analysis of HGT CFU following oral gavage. **e**, HGT CFU rate in stool was not affected by donor tumor size in recipient mice, as determined by colonoscopic scoring (S small, M medium, L large). In **b**,**c**,**e**, one-way Anova with Tukey's post-hoc on $\log_{10}$ transformed data was used for statistical analysis with P values shown in the corresponding panels. Limit of detection 80 CFUs

BTRZI-*KRAS-kan*^{R} organoids. At week -1, mice underwent a gut decontamination regimen. A single dose of 10^{10} "small insert" *A. baylyi* biosensors or nonengineered parental bacteria, with additional chloramphenicol resistance for quantification of total *A. baylyi*, was administered by oral gavage or enema to tumor-bearing and non-tumor-bearing mice. Additional control mice with and without tumors that were administered PBS rather than sensor bacteria were included as well (Fig. 4a). All study groups were housed in separate cages. At day 3 after sensor bacteria delivery, mice were administered 2 days of low-dose kanamycin in their drinking water, before having their stools collected at day 5. HGT was measured by serial dilution of stool culture on chloramphenicol and kanamycin agar plates, with results presented as the mean CFU per 2-4 stools collected for each mouse.

Following sensor bacteria delivery, either by oral (Fig. 4b) or rectal (Fig. 4c) delivery, the kan-resistant CFUs were significantly higher in the tumor-bearing mice compared to either non-tumor mice (Fig. 4b,c) or mice with tumors and parental (non-engineered) *A. baylyi* (Extended Data Fig. 7). The sensor bacteria perfectly discriminated tumor from

non-tumor bearing mice (Fig. 4d). The mean stool CFUs were the same regardless of tumor size at the time of stool collection (Fig. 4e). HGT-mediated antibiotic resistance was confirmed by Sanger sequencing of individual colonies (Extended Data Fig. 8). Finally, to ensure that HGT was not occurring on the agar plates *ex vivo*, the collected stool was pre-treated with DNase, which did not reduce the measured CFUs (Extended Data Fig. 9).

Discussion

In this study, naturally competent *A. baylyi* were engineered to sense donor DNA from human tumor cells. The donor-sensor system was optimized *in vitro* and then validated *in vivo* using an orthotopic mouse model of CRC. Furthermore, we engineered a CRISPR-based technique to provide specificity for the mutant *KRASG12D* vs. wild-type *KRAS*. The sensor bacteria described here demonstrate that a living biosensor can detect tumor DNA shed from CRC *in vivo* in the gut, with no sample preparation or processing. The sensor is highly sensitive and specific, with 100% discrimination between mice with and without CRC.

In vitro DNA analysis helps detect and manage important human diseases, including cancer and infection[28]. However, *in vitro* sensing requires potentially invasive removal of samples, and many DNA diagnostics cannot achieve clinically relevant sequence resolution, with more advanced sequencing remaining too expensive for routine use in all settings[29]. Direct sampling of the gut *in vivo* may offer important advantages. The gastrointestinal tract contains significant DNase activity[30], which limits the lifetime of free DNA in both rodents and humans[18,31,32], and may thus reduce the information content of downstream fecal samples[33–35]. Bacterial biosensors located *in situ* could capture and preserve DNA shortly after its release, before degradation by local DNases. In addition, biosensors could amplify target DNA through HGT-induced fitness, intercellular quorum sensing circuits, or intracellular genetic memory switches[9,11]. Perhaps most exciting, however, is that unlike *in vitro* diagnostics, bacterial detection of target DNA could be coupled to direct and genotype-complementary nanobodies, peptides, or other small molecules for the treatment of cancer or infection[36,37]. The sensor may also have important applications in many other settings both clinical and non-clinical, particularly where direct sampling is difficult or too invasive, continuous surveillance is desirable, diagnostic resources are constrained, or a biologically-generated response would be best delivered to the target organism at the time and place of its detection.

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Figure legends

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Figure 3: Detection of donor DNA from BTRZI-*KRAS-kan*^{R} organoids. Schema depicting *in vitro* co-culture of *A. baylyi* sensor bacteria with BTRZI-*KRAS-kan*^{R} (CRC donor) organoid lysates or viable organoids to assess HGT repair of kanamycin resistance gene (kan^R). **b**. Recombination with DNA from crude lysates enables growth of *A. baylyi* sensor on kanamycin plates with transformation efficiency of $1.4\text{x}10^{-5}$ (limit of detection 10^{-8}). **c**. Representative images of GFP-tagged *A. baylyi* sensor surrounding parental BTRZI (control) and BTRZI-*KRAS-kan*^{R} donor organoids at 24h. Scale bar 100μm **d**. Co-culture of established CRC BTRZI-*KRAS-kan*^{R} donor organoids with *A. baylyi* sensor enables growth of *A. baylyi* sensor on kanamycin plates with transformation efficiency $3.8\text{x}10^{-7}$ (limit of detection 10^{-9}). In **b, d,** n = 5 independent experiments each

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Methods

Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files), and raw data files are available upon request.

Bacterial cell culture and cloning to generate biosensors

Acinetobacter baylyi ADP1 was obtained from the American Type Culture Collection (ATCC #33305) and propagated in standard LB media at 30 or 37 °C. *KRAS* homology arms were inserted into a neutral genetic locus denoted *Ntrl1*, replacing the gene remnant ACIAD2826. For the "large insert" design, a spectinomycin resistance gene was placed between the *KRAS* homology arms. For the "small insert" design, two stop codons were placed near the beginning of the kan^R gene of the donor cassette, and the broken cassette was inserted into *A. baylyi*. CRISPR arrays were inserted into a neutral locus used previously, replacing ACIAD2186, 2187 and part of 2185. Ectopic CRISPR arrays were driven by a promoter region that included 684 bp from upstream of the first repeat of the endogenous, 90-spacer array.

In vitro biosensor transformation experiments

A. baylyi were grown overnight in LB at 30 °C. Cells were then washed, resuspended in an equal volume of fresh LB, and mixed with donor DNA. For transformation in liquid, 50 µl cells were mixed with 250 ng donor DNA and incubated in a shaker at 30 °C for 2 hours or overnight. For transformation on agar, 2 µl cells were mixed with >50 ng donor DNA, spotted onto LB plates containing 2% wt/vol agar, and incubated at 30 °C overnight. Spots were cut out the next day and resuspended in 500 µl phosphate buffered saline solution (PBS). To count transformants, cells were 10-fold serially diluted 5 times, and 2 µl spots were deposited onto selective (30 ng/ml kanamycin) and non-selective 2% agar plates, with 3 measurement replicates at each dilution level. Larger volumes of undiluted samples were also spread onto agar plates to increase detection sensitivity (25 µl for liquid culture, 100 µl for resuspended agar spots). Colonies were counted at the lowest countable dilution level after overnight growth at 30 °C, and measurement replicates were averaged. Raw, unpurified lysate was produced by growing donor RKO cells in a culture dish until confluence, trypsinizing and harvesting cells, pelleting them in a 15 ml tube, resuspending them in 50 µl PBS, and placing the tube in a –20 °C freezer overnight to disrupt cell membranes.

In vitro statistics

Hypothesis testing was performed using 2-sample, one-sided t-tests in Matlab after taking base 10 logarithms, since serial dilutions produce log-scale data. Where data points were below the limit of detection, they were replaced by the limit of detection as the most conservative way to include them in log-scale analysis. Comparisons between large vs small inserts or liquid vs solid agar culture were performed using paired t-tests, where data were matched for donor DNA and either culture type (liquid vs agar) or insert size, respectively. For Figure 2, d-g) n=4, i,j) n=5 except for random spacer n=3.

Creation of BTRZI CRC donor organoids

BTRZI (Braf^{V600E};Tgfbr2$^{\Delta/\Delta}$;Rnf43 $^{\Delta/\Delta}$ /Znf43 $^{\Delta/\Delta}$;p16 Ink4a $^{\Delta/\Delta}$) organoids were generated using CRISPR-Cas9 engineering (Lannagan et al, 2019 Gut) and grown in 50 µl domes of GFR-Matrigel (Corning,; 356231) in organoid media: Advanced Dulbecco's modified Eagle medium/F12 (Life Technologies) supplemented with 1x gentamicin/antimycotic/antibiotic (Life Technologies), 10mM HEPES (Gibco), 2 mM GlutaMAX (Gibco), 1x B27 (Life Technologies; 12504-044), 1x N2 (Life Technologies; 17502048), 50 ng/ml mouse recombinant EGF (Peprotech; 315-09), 10 ng/ml human recombinant TGF-β1 (Peprotech; 100-21). Following each split, organoids were cultured in 10 µM Y-27632 (MedChemExpress; HY-10583), 3 µM iPSC (Calbiochem; 420220), 3 µM GSK-3 inhibitor (XVI, Calbiochem; 361559) for the first 3 days.

To create BTRZI CRC donor organoids, lentiviral expression plasmid pD2119-FLuc2 KRasG12D donor was co-transfected with viral packaging vectors, psPAX2 (Addgene; plasmid; 12260) and MD2G (Addgene; plasmid; 12259), into HEK293T cells. At 48 and 72 h after transfection, viral supernatants were harvested, filtered through a 0.45-µm filter, and concentrated using Amicon Ultra Centrifugal Filters (Merck Millipore; UFC910024). Concentrated lentivirus particles were used for transduction. The viral supernatant generated was used to transduce BTRZI organoids by spinoculation. Briefly, organoids were dissociated to single cells using TrypLE. 1x10^5 single cells were mixed with 250 µl organoid media; 10 µM Y-27632; 250 µl concentrated viral supernatant and 4 µg/ml polybrene (Sigma,; H9268) in a 48 well tray before centrifugation at 600 xg for 90 minutes at 32 °C. Meanwhile, 120 µl 50:50 ADMEM:Matrigel mixture was added to a cold 24-well tray before centrifugation of this bottom matrigel layer for 40 minutes at 200xg at room temperature, followed by solidifying the Matrigel by incubating at 37 °C for 30 minutes. After spinoculation, cells were scraped from the well and plated on top of the Matrigel monolayer with organoid media. The following day, the media was removed and the upper layer of Matrigel was set over the organoids by adding 120 µl 50:50 ADMEM:Matrigel and allowing to set for 30 minutes before adding organoid media. 48 hours after transduction, BTRZI donor organoids were selected with 8 µg/ml puromycin for 1 week, then maintained in organoid media with 4 µg/ml puromycin.

Organoid lysate mixed with *A. baylyi* sensor bacteria

BTRZI (parental) and BTRZI donor organoids were grown for 5 days in 50 ml Matrigel domes. Organoids were dissociated to single cells with TrypLE, counted and 6x10^5 single cells were collected in PBS and snap frozen. The CFU equivalence of exponentially growing *A. baylyi* sensor culture at OD_{600} 0.35 was ascertained by serial dilution of 3 independent cultures with 5 technical replicates plated on 10 µg/ml Chloramphenicol LB agar plate to be 2.4 x 10^8 CFU per ml. *A. baylyi* sensor was grown in liquid culture with

10 µg/ml Chloramphenicol to OD_{600} 0.35 before mixing with organoid lysate at a 1:1 ratio and grow overnight on LB agar plates at 30 °C. All bacteria was scraped into 200 µl LB/20% glycerol before spotting 5x 5 µl spots onto kanamycin and chloramphenicol plates and grown overnight at 37 °C. Colonies were counted and the dilution factor was accounted for to calculate CFU per ml. Rate of HGT was calculated by dividing the CFU per ml of transformants (Kanamycin plates) by the CFU per of total *A. baylyi* (chloramphenicol plates) for 5 independent experiments.

Coculture organoids with *A. baylyi* sensor bacteria

For co-culture experiments, 24-well trays were coated with Matrigel monolayers. Briefly, 200 µl 50:50 ADMEM:Matrigel mixture was added to a cold 24-well tray and centrifuged for 40 minutes at 200xg at room temperature, followed by a 30 minute incubation at 37 °C to solidify matrigel. BTRZI (parental) and BTRZI donor organoids were dissociated into small clusters using TrypLE and grown for 5 days on a Matrigel monolayer in organoid media without antibiotics before 50 µl OD_{600} 0.35 *A. baylyi* sensor was added to each well. After 24 hours, organoids were photographed then collected and grown overnight on LB agar plates at 30 °C. All bacteria was scraped into 200 µl LB/20% glycerol before spotting 5x 5 µl spots onto kanamycin and chloramphenicol plates and grown overnight at 37 °C. Colonies were counted and the dilution factor was accounted for to calculate CFU per ml. Rate of HGT was calculated by dividing the CFU per ml of transformants (kanamycin plates) by the CFU per ml of total *A. baylyi* (chloramphenicol plates) for 5 independent experiments.

***A. baylyi* colonisation trial**

This study was approved by the SAHMRI Animal Ethics committee (SAM20.036). NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice (male and female, 10-13 weeks old) were obtained from the SAHMRI Bioresources facility and housed under pathogen-free conditions. NSG mice were administered with antibiotics (2.7mM Ampicillin, Sigma; A1066 and 0.55mM Neomycin, Sigma; N1876) in drinking water a week prior to oral gavage/enema. *A. baylyi*-mCherry/KanR was grown in liquid culture with 50 µg/ml kanamycin to OD_{600} 0.3. *A. baylyi* was washed with PBS before 3 mice received 10^{10} *A. baylyi* via oral gavage, 3 mice received 10^{10} *A. baylyi* via enema and 2 control mice received PBS (1x enema and 1x oral gavage). Oral gavage was administered using a 20G curved feeding needle at a volume of 200 µl per mouse. Enema was performed as per previous publication. Briefly, mice were anaesthetised with isofluorane and colon flushed with 1 ml of room temperature sterile PBS to clear the colon cavity of any remaining stool. A P200 pipette tip coated with warm water was then inserted parallel into the lumen to deliver 50 mL of bacteria into the colon over the course of 30 seconds. After infusion, the anal

verge was sealed with Vetbond Tissue Adhesive (3M; 1469SB) to prevent luminal contents from being immediately excreted. Animals were maintained on anaesthesia for 5 minutes, and then allowed to recover on heat mat and anal canal inspected 6 hours after the procedure to make sure that the adhesive has been degraded. Stool was collected for 2 weeks in 250 µl PBS/20% glycerol, vortexed and stored at -80 °C. Stool slurry (50 µl) was plated onto a LB agar plate and grown overnight at 37 ºC. All bacteria was scraped into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted onto kanamycin plates. Colonies were counted and dilutions were factored to calculate CFU *A. baylyi* per stool.

Horizontal gene transfer *in vivo*

BTRZI donor organoids were isolated from Matrigel and dissociated into small clusters using TrypLE. The cell clusters (equivalent to ~150 organoids per injection) were washed three times with cold PBS containing 10 µM Y-27632 and then resuspended in 20 µl 10% GFR matrigel 1:1000 india ink, 10 µM Y-27632 in PBS and orthotopically injected into the mucosa of the proximal and distal colon of anaesthetised 10-13 week old NSG mice (150 organoids per injection), as previously described (Lannagan et al, 2019 Gut). Briefly, a customised needle (Hamilton Inc. part number 7803-05, removable needle, 33 gauge, 12 inches long, point 4, 12 degree bevel) was used. In each mouse up to 2 injections of 20µl were performed. CRC donor tumor growth was monitored by colonoscopy for 4 weeks and the videos were viewed offline using QuickTime Player for analysis. Colonoscopy was performed using a Karl Storz Image 1 Camera System comprised of: Image1 HDTV HUB CCU; Cold Light Fountain LED Nova 150 light source; Full HD Image1 3 Chip H3-Z Camera Head; Hopkins Telescope, 1.9mm, 0 degrees. A sealed luer lock was placed on the working channel of the telescope sheath to ensure minimal air leakage (Coherent Scientific, # 14034-40). Tumor growth of the largest tumor visualised was scored as previously described using the Becker Scale (Rex et al, 2012 Am J Gastroenterol). Mice were administered antibiotics (2.7mM Ampicillin, Sigma; A1066 and 0.55mM Neomycin, Sigma; N1876) in drinking water a week prior to oral gavage/enema. *A. baylyi* sensor was grown in liquid culture with 10 µg/ml Chloramphenicol to OD_{600} 0.3. *A. baylyi* sensor was washed with PBS before 13 mice received 10^{10} *A. baylyi* sensor via oral gavage (7 mice without tumors and 6 mice with CRC donor tumors), 7 mice received 10^{10} *A. baylyi* sensor via enema (3 mice without tumors and 4 mice with CRC donor tumors). Three days after *A. baylyi* administration, mice received 10 mg/L kanamycin in their drinking water, except 2 mice from the oral gavage *A. baylyi* sensor, CRC donor tumor cohort, 5 mice from the oral gavage *A. baylyi* sensor, no tumor cohort and 2 mice from the enema *A. baylyi* sensor, no tumor cohort . Stool was collected 5 days after *A. baylyi* administration into 250 µl PBS/20% glycerol, vortexed and stored at -80 °C. Stool slurry (50 µl) was plated onto a LB agar plate and grown overnight at 37 °C. All bacteria

was scraped into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted onto chloramphenicol and kanamycin plates. Colonies were counted and dilutions were factored to calculate CFU *A. baylyi* per stool.

Sequencing gDNA from bacterial colonies grown on kanamycin plates

A. baylyi transformants were individually picked from kanamycin plates and grown in liquid culture LB supplemented with 25 µg/ml Kanamycin. gDNA was extracted using purelink genomic DNA minikit (Invitrogen; K182001). Genomic regions of interest were amplified using Primestar Max DNA polymerase (Takara, # R045A) and primers
HGTpcrF: CAAAATCGGCTCCGTCGATACTA;
HGTpcrR: TAGCATCACCTTCACCCTC;
Kan seqF: AAAGATACGGAAGGAATGTCTCC;
Kan seqR: CGGCCGTCTAAGCTATTCGT. Sanger sequencing was conducted by AGRF using the same primers.

DNase treatment of stool

Stool slurry (25 µl) was mixed with 2.5 µl 10x DNase 1 buffer with or without 1 µl DNase 1 (2.7 U/µl) using RNase-free DNase 1 kit (Qiagen,; 79254). Samples were incubated at 37 °C for 30 minutes then the mixture was plated onto LB agar plates and grown overnight at 37 °C. A control to assess DNase 1 activity was set up simultaneously with 25 µl stool (from mouse with no tumor); 1 µl 100 ng/µl KRasG12D donor plasmid DNA (2 ng/ul final concentration); 2.5 µl 10x DNase 1 buffer with or without 1 µl DNase 1 (2.7 U/µl), which was incubated at 37 °C for 30 minutes. Following DNase 1 treatment, controls were mixed with 25 µl of *A. baylyi* sensor liquid culture (OD_{600} 0.35) and incubated at 37 °C 2 hrs before the mixture was plated onto LB agar plates and grown overnight at 37 °C. All bacteria was scraped into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted onto kanamycin plates. Colonies were counted and dilutions were factored to calculate CFU *A. baylyi* per stool.

Acknowledgements: Mr Phil Winning for design assistance with Figure 1. This work was supported by NIH grant R01CA241728. **Professor Barbara Leggett and A/Prof. Vicki Whitehall for the original gift of the parental** RKO and LS174T human CRC cell lines used in this study.

Author contributions: RC, DW & JH conceived of the concept and study plan. RC, JW, JN, JG, NS, YL, MI, GR, ET, LV, SW, DW, & JH were all involved with data acquisition and or interpretation. RC, JW, RK, SW, DW, & JH were involved in writing and revising the final manuscript.

Competing interest declaration: J.H. is a co-founder and board member with equity in GenCirq Inc, which focuses on cancer therapeutics.

Correspondence: Correspondence and requests for materials should be addressed to:
Professor Jeff Hasty: hasty@ucsd.edu
Associate Professor Daniel Worthley: dan@colonoscopyclinic.com.au
Dr Susan Woods: susan.woods@adelaide.edu.au

Extended Data

Extended Data Figure 1: Plasmid donor DNA used to transfect mammalian cell lines and as positive control donor DNA for *in vitro* experiments.

Extended Data Figure 2: "Large insert" (**a**) and "small insert (**b**) designs for the biosensors. *KRAS* homology arms are shown in striped gray with surrounding genomic context outside them. Note that large and small inserts refers to the size of the donor DNA region that must transfer to confer kanamycin resistance, not to the size of the region between homology arms in the biosensor. Two single-base changes introducing nearby stop codons at the beginning of kan^R are shown for the small insert design (**b**).

Extended Data Figure 3: "Small insert" biosensor design. Donor DNA in the cancer cell genome (top) contains a kanamycin resistance gene *kanR*, surrounded by GFP and human *KRAS* homology arms of about 1 kb (*KRAS* HA). The bacterial biosensor genome contains the exact same construct, except that 2 stop codons are introduced to kan^R with 2 single-base mutations within 8 bases. Upon homologous recombination with

the donor DNA, the 2 stop codons are repaired, and the biosensors acquire kanamycin resistance.

Extended Data Figure 4: Sensor detection of donor DNA from BTRZI CRC organoids. *A. baylyi* sensor bacteria are constitutively chloramphenicol resistant, hence c*hlorR* CFUs provide a read-out of total *A. baylyi* present. In contrast, kanamycin resistant sensor bacteria rely on incorporation of donor DNA from CRC organoids to correct the defective *kan* gene and enable growth on kanamycin selection plates. **a** Recombination with lysate from CRC donor organoids enables growth of *A. baylyi* sensor on kanamycin plates. Shown here with representative plates and CFU analysis. **b** After co-culturing established CRC donor organoids with *A. baylyi* sensor, recombination with donor DNA from CRC donor organoids enables growth of *A. baylyi* sensor on kanamycin plates. Shown here with representative images and CFU analysis. Scale bars 200 µm. **a, b,** Fig 3 contains the same data as shown here but presented as HGT rate (kanamycin resistant CFU *A.baylyi* per ml/chloramphenicol CFU *A.baylyi* per ml*)*, n = 5 independent experiments each with 5 technical replicates. **c** Representative Sanger sequencing chromatograms of PCR amplicon covering the region of the *kan* gene containing informative SNPs, to highlight the difference in sequence in gDNA isolated from parental *A. baylyi* sensor bacteria compared to *A.baylyi* colonies isolated from kanamycin plates following mixing with donor organoid lysates or viable organoids.

Extended Data Figure 5: *A. baylyi* is detected in stool for 2 weeks after oral gavage or enema. a, Schematic illustrating the experimental pipeline of colonisation trial, n=6 mice administered *A.baylyi* mCherry-*kanR* bacteria (constitutively kanamycin resistant), n=2 PBS control mice. Representative bright-field and fluorescent image of *A.baylyi* mCherry-*kanR* CFU from stool. **b,** *A. baylyi* mCherry-*kanR* is detected in stool from mice. Data points represent the average CFU per stool grown on kanamycin selection plates from 1-3 stools/mouse, with results from each mouse (84.2a, 84.2b, 84.2c, 84.2g) plotted separately.

Extended Data Figure 6: High sensitivity digital droplet PCR (ddPCR) detection of CRC mutation (*BrafV600E*) in stool DNA isolated from tumour bearing animals (n=3-4 mice/group). a, Representative images of ddPCR data. **b,** CRC mutation (*BrafV600E)* positive droplets as a % of total droplets. Analysis of no template negative control samples and stool DNA samples from non-tumour bearing animals was used to determine the sensitivity threshold of the assay. Positive control samples contain 10% *BrafV600E* gDNA spiked into stool DNA sample from non-tumour

bearing animal. NT, no tumour; Ts, small tumour; Tm, medium tumour; Tl, large tumour; NTC, no template PCR negative control.

Extended Data Figure 7: Efficient horizontal gene transfer detected in stool from tumor bearing mice requires both engineered biosensor bacteria, as opposed to parental *A. baylyi*, and tumor. Average CFU per stool from 2-4 stools per mouse grown on kanamycin selection plates is shown, n=4-10 mice/group. Combined data for oral and rectal dosing of biosensors. One-way Anova with Tukey's post-hoc on log10 transformed data was used for statistical analysis with P values shown in the corresponding panel.

Extended Data Figure 8: *A. baylyi* sensor in stool from mice bearing BTRZI CRC donor tumors become kanamycin resistant via HGT. a, Representative Sanger sequencing chromatograms of PCR amplicon covering the region of the *kan* gene containing informative SNPs, to highlight the difference in sequence in parental *A. baylyi* sensor bacteria (defective *kanR*) in comparison to colonies isolated from kanamycin plates from stool of tumor bearing mice administered *A. baylyi* sensor bacteria (corrected *kanR* oral and rectal). **b** Representative CFU plates.

Extended Data Figure 9: DNase treatment of stool homogenates to remove unincorporated donor DNA prior to CFU analysis did not alter number of *A. baylyi* sensor bacteria that were kanamycin resistant. Stools from mice bearing BTRZI donor tumors and administered *A. baylyi* sensor bacteria were incubated with and without DNase before CFU analysis on kanamycin plates. 1-4 stools from 5 mice were analysed. No statistical difference was evident between the number of kanamycin resistant colonies from CRC donor tumor stools treated with or without DNase. As a positive control for DNase treatment efficacy, stool from a non-tumour bearing mouse was mixed with *A. baylyi* sensor and CRC donor plasmid, then treated with or without DNase. In this case, kanamycin resistant colonies were only evident in the absence of DNase. This suggests that the HGT evident in stool from the experimental animals likely occurred *in vivo* or in stools, but prior to CFU plating. Paired t-test was used for statistical analysis.

Extended Data Movie 1: *A. baylyi* biosensors taking up plasmid donor DNA.
A. baylyi were grown overnight, washed into fresh LB, mixed with saturating pLenti-KRAS donor DNA, and sandwiched between an agar pad and a glass bottom dish. Images

were taken every 10 minutes. GFP fluorescence indicates that the cells have taken up and genomically integrated the donor DNA cassette.

Extended Data DNA Files:

DNA cassettes and surrounding regions corresponding to the "large insert" and "small insert" designs for

A. baylyi, and the plasmid donor DNA, as shown in Extended Data 1,2, in Genbank format.