Engineered bacteria detect tumor DNA

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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

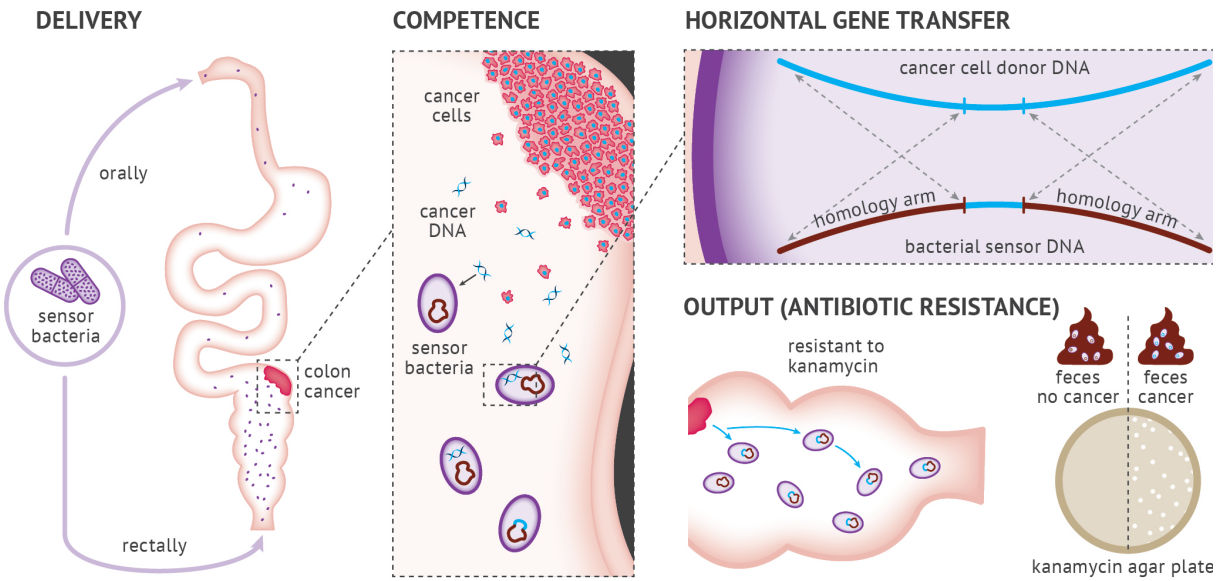

Figure 1. Engineered bacteria to detect tumor DNA. Engineered *A. baylyi* bacteria are delivered orally or rectally in an orthotopic mouse model of CRC. The naturally competent *A. baylyi* take up tumor DNA shed into the colonic lumen. The tumor donor DNA is engineered with a *kanR* cassette flanked 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).

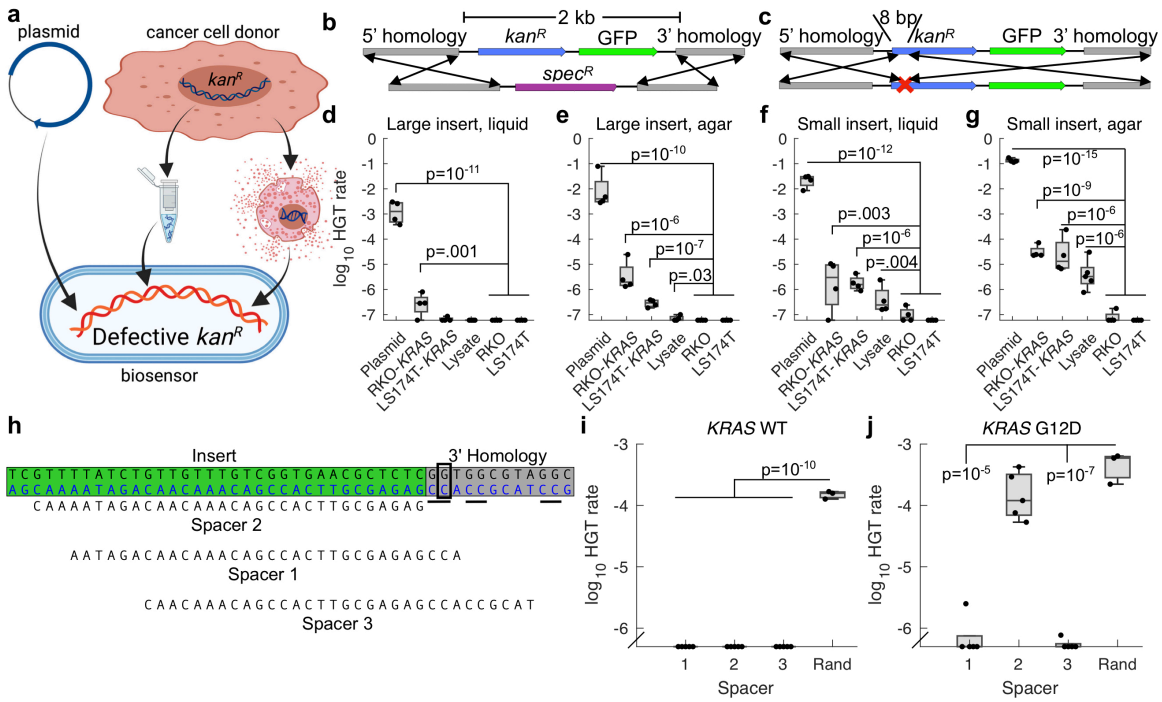

Figure 2: Sensing *KRASG12D* DNA *in vitro*. a-c, Donor DNA consisting of plasmid, purified cancer cell genomic DNA, or raw lysate (top) recombines into biosensor *A. baylyi* cells (bottom), transferring either a large, 2 kb insert (**b**) or a small, 8 bp insert to repair 2 stop codons (**c**) in both 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

102 DNA, this suggests a detection efficiency of around 1% per processed donor sequence.
103 While this calculation assumes a constant DNA processing rate, the result is quite similar
104 to what we found for HGT from *E. coli* to *A. baylyi*[21].
105
106 **Sensor bacteria can discriminate wild-type from mutant *KRAS* DNA**
107
108 Mutations in codon 12 of *KRAS* are present in 27% of CRC[23], and are common in solid
109 tumors generally[24]. To test whether sensor bacteria could discriminate between wild-type
110 and mutant *KRAS (KRASG12D)*, which differ by a single G>A transition, we utilized
111 *A. baylyi's* endogenous Type I-F CRISPR-Cas system[25]. We stably transduced an RKO
112 cell line with the kan^R-*GFP* donor cassette flanked by wild-type *KRAS* (RKO-*KRAS*),
113 and a second line with *KRASG12D* flanking sequences (RKO-*KRASG12D*). Next, we
114 designed 3 CRISPR spacers targeting the wild-type *KRAS* sequence at the location of
115 the *KRASG12D* mutation, using the *A. baylyi* protospacer-adjacent motif (PAM) of 5'-
116 CC-protospacer-3' (Fig. 2h). We inserted these as single-spacer arrays into a neutral locus
117 in the "large insert" *A. baylyi* sensor genome.
118
119 The sensor bacteria, if effective, should reject wild-type *KRAS* through CRISPR-
120 mediated DNA cleavage. Conversely, the *KRASG12D* sequence should alter the target
121 sequence and evade DNA cleavage. Two of the three spacers blocked transformation by
122 both wild-type and mutant DNA (Fig. 2i-j). However, spacer 2, for which the *KRASG12D*
123 mutation eliminated the PAM site, selectively permitted HGT only with *KRASG12D*
124 donor DNA (Fig. 2E-F). The other common mutations in codon 12 of *KRAS* all eliminate
125 this PAM as well[23]. Thus, sensor *A. baylyi* can be engineered to detect a hotspot mutation
126 in the *KRAS* gene with single-base specificity.
127
128 **Sensor bacteria can integrate cancer DNA in organoid culture**
129
130 *Ex vivo* organoid culture faithfully reflects endogenous tumor biology[26]. We therefore
131 evaluated our sensor and donor constructs in organoid culture (Fig. 3a). We previously
132 used CRISPR/Cas9 genome engineering to generate compound $Braf^{V600E}$; $Tgfbr2^{\Delta/\Delta}$;
133 $Rnf43^{\Delta/\Delta}$; $Znrf3^{\Delta/\Delta}$; $p16Ink4a^{\Delta/\Delta}$ (BTRZI) mouse organoids that recapitulate serrated
134 CRC when injected into the mouse colon[27].
135
136 We transduced BTRZI organoids with the human *KRAS*-flanked donor DNA construct
137 (*KRAS*-kan^R) to generate donor CRC organoids, and incubated their lysate with the more
138 efficient "small insert" *A. baylyi* biosensors. As with the CRC cell lines, the sensor *A.*
139 *baylyi* incorporated DNA from donor organoid lysate, but not from control lysates from

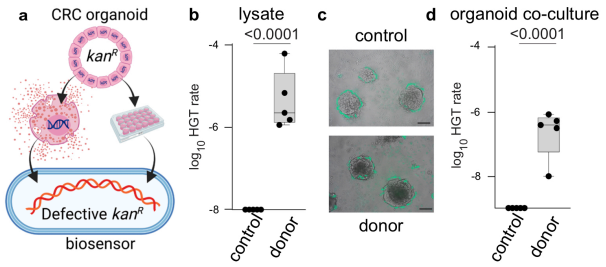

Figure 3: Detection of donor DNA from BTRZI-*KRAS-kan*^{R} organoids. **a**, Schema depicting *in vitro* co-culture of *A.baylyi* sensor bacteria with BTRZI-*KRAS-kan*^{R} (CRC donor) organoid lysates or viable organoids to assess HGT repair of kanamycin resistance gene (*kan*^{R}). **b**, Recombination with DNA from crude lysates enables growth of *A.baylyi* sensor on kanamycin plates with transformation efficiency of 1.4×10^{-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 of 3.8×10^{-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

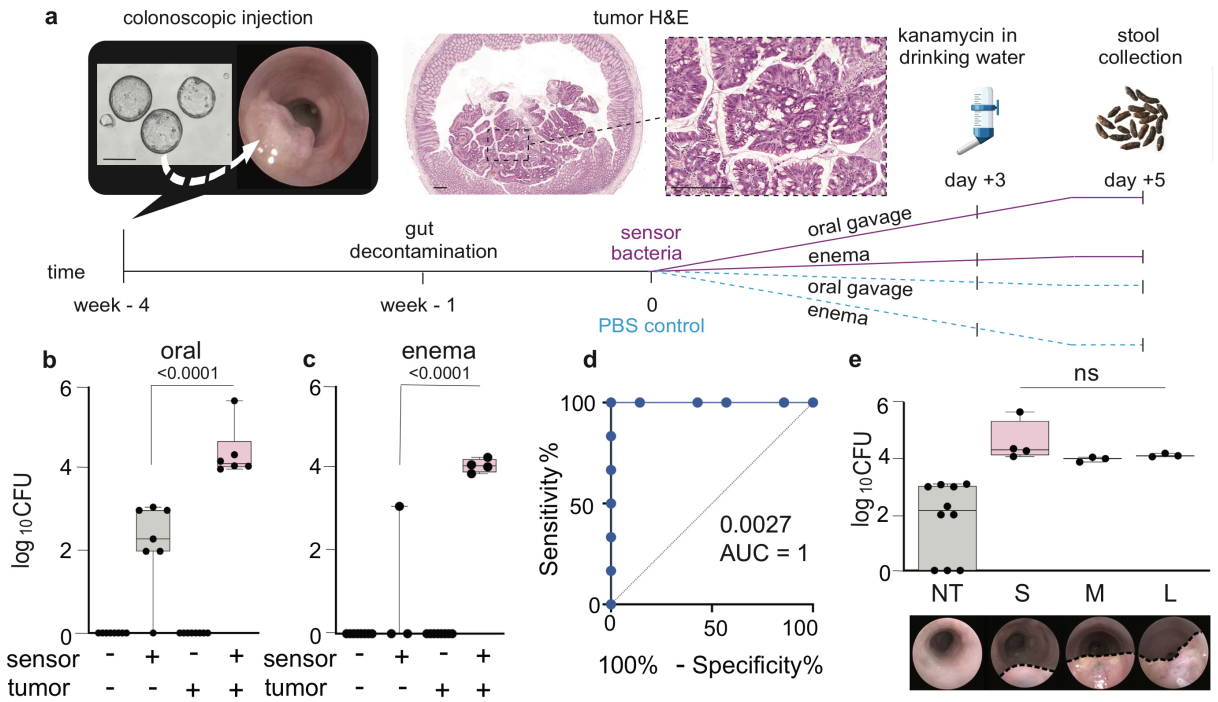

Figure 4. Horizontal gene transfer detected in stool from mice bearing BTRZI-*KRAS-kanR* tumors after oral or rectal dosing of *A. baylyi* sensor bacteria. **a** Schema depicting *in vivo* HGT experiments: generation of BTRZI-*KRAS-kanR* (CRC donor) tumors in mice via colonoscopic injection of CRC donor 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.

References

1. Slomovic, S., Pardee, K. & Collins, J. J. Synthetic biology devices for in vitro and in vivo diagnostics. *Proc National Acad Sci* 112, 14429–14435 (2015).

2. Sedlmayer, F., Aubel, D. & Fussenegger, M. Synthetic gene circuits for the detection, elimination and prevention of disease. *Nat Biomed Eng* 2, 399–415 (2018).

3. Lim, W. A. & June, C. H. The Principles of Engineering Immune Cells to Treat Cancer. *Cell* 168, 724–740 (2017).

4. Riglar, D. T. *et al.* Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nat Biotechnol* 35, 653–658 (2017).

5. Mark, M. *et al.* An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science* 360, 915 (2018).

6. Mao, N., Cubillos-Ruiz, A., Cameron, D. E. & Collins, J. J. Probiotic strains detect and suppress cholera in mice. *Sci Transl Med* 10, eaao2586 (2018).

7. Danino, T. *et al.* Programmable probiotics for detection of cancer in urine. *Sci Transl Med* 7, 289ra84-289ra84 (2015).

8. Dina, K. *et al.* Effect of Oral Capsule– vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *Jama* 318, 1985–1993 (2017).

9. Kotula, J. W. *et al.* Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc National Acad Sci* 111, 4838–4843 (2014).

10. Gardner, T. S., Cantor, C. R. & Collins, J. J. Construction of a genetic toggle switch in Escherichia coli. *Nature* 403, 339–342 (2000).

11. Courbet, A., Endy, D., Renard, E., Molina, F. & Bonnet, J. Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Sci Transl Med* 7, 289ra83 (2015).

12. Chang, M., Joshua & J, R., Rosemary. Natural competence and the evolution of DNA uptake specificity. *J Bacteriol* 196, 1471–1483 (2014).

13. Soucy, S. M., Huang, J. & Gogarten, J. P. Horizontal gene transfer: building the web of life. *Nature Reviews Genetics* 16, 472–482 (2015).

262 14. Robinson, K. M., Sieber, K. B. & Hotopp, J. C. D. A Review of Bacteria-Animal Lateral
263 Gene Transfer May Inform Our Understanding of Diseases like Cancer. *Plos Genet* 9, e1003877
264 (2013).

265 15. Hotopp, J. C. D. Horizontal gene transfer between bacteria and animals. *Trends Genet* 27,
266 157–163 (2011).

267 16. Young, D. M., Parke, D. & Ornston, L. N. Opportunities for genetic investigation afforded
268 by Acinetobacter baylyi, a nutritionally versatile bacterial species that is highly competent for
269 natural transformation. *Annu Rev Microbiol* 59, 519–551 (2005).

270 17. Chen, T.-L. *et al.* Acinetobacter baylyi as a Pathogen for Opportunistic Infection ▿ . *J Clin*
271 *Microbiol* 46, 2938–2944 (2008).

272 18. Nordgård, L. *et al.* Lack of detectable DNA uptake by bacterial gut isolates grown in vitro
273 and by Acinetobacter baylyi colonizing rodents in vivo. *Environmental Biosafety Research* 6,
274 149–160 (2007).

275 19. Vogelstein, B. *et al.* Genetic Alterations during Colorectal-Tumor Development. *New Engl J*
276 *Medicine* 319, 525–532 (1988).

277 20. Simpson, D. J., Dawson, L. F., Fry, J. C., Rogers, H. J. & Day, M. J. Influence of flanking
278 homology and insert size on the transformation frequency of Acinetobacter baylyi BD413.
279 *Environmental Biosafety Research* 6, 55–69 (2007).

280 21. Cooper, R. M., Tsimring, L. & Hasty, J. Inter-species population dynamics enhance
281 microbial horizontal gene transfer and spread of antibiotic resistance. *eLife* 6, 8053 (2017).

282 22. Palmen, R., Vosman, B., Buijsman, P., Breek, C. K. D. & Hellingwerf, K. J. Physiological
283 characterization of natural transformation in Acinetobacter calcoaceticus. *Microbiology*+ 139,
284 295–305 (1993).

285 23. Consortium, T. A. P. G. AACR Project GENIE: Powering Precision Medicine through an
286 International Consortium. *Cancer Discov* 7, 818–831 (2017).

287 24. Priestley, P. *et al.* Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature*
288 575, 210–216 (2019).

289 25. Cooper, R. M. & Hasty, J. One-Day Construction of Multiplex Arrays to Harness Natural
290 CRISPR-Cas Systems. *Acs Synth Biol* 9, 1129–1137 (2020).

291 26. van de Wetering, M. *et al.* Prospective Derivation of a Living Organoid Biobank of
292 Colorectal Cancer Patients. *Cell* 161, 933–945 (2015).

293 27. Lannagan, T. R. M. *et al.* Genetic editing of colonic organoids provides a molecularly
294 distinct and orthotopic preclinical model of serrated carcinogenesis. *Gut* 68, 684–692 (2018).

295 28. Zhong, Y., Xu, F., Wu, J., Schubert, J. & Li, M. M. Application of Next Generation
296 Sequencing in Laboratory Medicine. *Ann Lab Med* 41, 25–43 (2021).

297 29. Iwamoto, M. *et al.* Bacterial enteric infections detected by culture-independent diagnostic
298 tests--FoodNet, United States, 2012-2014. *Mmwr Morbidity Mortal Wkly Rep* 64, 252–7 (2015).

299 30. Shimada, O. *et al.* Detection of Deoxyribonuclease I Along the Secretory Pathway in Paneth
300 Cells of Human Small Intestine. *J Histochem Cytochem* 46, 833–840 (1998).

301 31. Wilcks, A., Hoek, A. H. A. M. van, Joosten, R. G., Jacobsen, B. B. L. & Aarts, H. J. M.
302 Persistence of DNA studied in different ex vivo and in vivo rat models simulating the human gut
303 situation. *Food Chem Toxicol* 42, 493–502 (2004).

304 32. Netherwood, T. *et al.* Assessing the survival of transgenic plant DNA in the human
305 gastrointestinal tract. *Nat Biotechnol* 22, 204–209 (2004).

306 33. Imperiale, T. F. *et al.* Multitarget Stool DNA Testing for Colorectal-Cancer Screening. *New*
307 *England Journal of Medicine* 370, 1287–1297 (2014).

308 34. Zmora, N. *et al.* Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is
309 Associated with Unique Host and Microbiome Features. *Cell* 174, 1388-1405.e21 (2018).

310 35. Tang, Q. *et al.* Current Sampling Methods for Gut Microbiota: A Call for More Precise
311 Devices. *Front Cell Infect Mi* 10, 151 (2020).

312 36. Din, M. O. *et al.* Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* 536, 81–5
313 (2016).

314 37. Sepich-Poore, G. D. *et al.* The microbiome and human cancer. *Science* 371, eabc4552
315 (2021).

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320 **Figure legends**
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342 Fraction of total biosensor cells expressing the indicated CRISPR spacers that were
343 transformed by plasmid donor DNA with wild type (**i**) or mutant G12D (**j**) *KRAS*.
344 Statistics were obtained using two-sample, one-sided t-tests. Data points below detection
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347 **Figure 3: Detection of donor DNA from BTRZI-*KRAS-kan^R* organoids.**
348 Schema depicting *in vitro* co-culture of *A. baylyi* sensor bacteria with BTRZI-*KRAS-*
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350 resistance gene (kan^R). **b**. Recombination with DNA from crude lysates enables growth of
351 *A. baylyi* sensor on kanamycin plates with transformation efficiency of 1.4×10^{-5} (limit of
352 detection 10^{-8}). **c**. Representative images of GFP-tagged *A. baylyi* sensor surrounding
353 parental BTRZI (control) and BTRZI-*KRAS-kan^R* donor organoids at 24h. Scale bar
354 100μm **d**. Co-culture of established CRC BTRZI-*KRAS-kan^R* donor organoids with *A.*
355 *baylyi* sensor enables growth of *A. baylyi* sensor on kanamycin plates with transformation
356 efficiency 3.8×10^{-7} (limit of detection 10^{-9}). In **b, d,** n = 5 independent experiments each

357 with 5 technical replicates, one sample t-test on transformed data was used for statistical
358 analysis with *P* values as indicated.
359
360 **Figure 4. Horizontal gene transfer detected in stool from mice bearing BTRZI-**
361 ***KRAS-kan^R* tumors after oral or rectal dosing of *A. baylyi* sensor bacteria. a**,
362 Schema depicting *in vivo* HGT experiments: generation of BTRZI-*KRAS-kanR* (CRC
363 donor) tumors in mice, administration of PBS control or sensor *A. baylyi* and stool
364 collection. Scale bars 200μm. **b**, oral or **c**, rectal delivery of *A. baylyi* sensor to mice
365 bearing CRC donor tumors results in kanamycin resistant *A. baylyi* sensor in stool via
366 HGT. Average CFU per stool from 2-4 stools per mouse grown on Kanamycin selection
367 plates is shown, n=3-8 mice/group. **d**, ROC curve analysis of HGT CFU following oral
368 gavage. **e**, HGT CFU rate in stool was not affected by donor tumor size in recipient mice,
369 as determined by colonoscopic scoring (S small, M medium, L large). In **b**,**c**,**e**, one-way
370 Anova with Tukey's post-hoc on log10 transformed data was used for statistical analysis
371 with P values shown in the corresponding panels. Limit of detection 80 CFUs.
372
373

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.

419 **Creation of BTRZI CRC donor organoids**

420 BTRZI (Braf^{V600E};Tgfbr2$^{\Delta/\Delta}$;Rnf43 $^{\Delta/\Delta}$ /Znf43 $^{\Delta/\Delta}$;p16 Ink4a $^{\Delta/\Delta}$) organoids were generated
421 using CRISPR-Cas9 engineering (Lannagan et al, 2019 Gut) and grown in 50 µl domes of
422 GFR-Matrigel (Corning,; 356231) in organoid media: Advanced Dulbecco's modified Eagle
423 medium/F12 (Life Technologies) supplemented with 1x
424 gentamicin/antimycotic/antibiotic (Life Technologies), 10mM HEPES (Gibco), 2 mM
425 GlutaMAX (Gibco), 1x B27 (Life Technologies; 12504-044), 1x N2 (Life Technologies;
426 17502048), 50 ng/ml mouse recombinant EGF (Peprotech; 315-09), 10 ng/ml human
427 recombinant TGF-β1 (Peprotech; 100-21). Following each split, organoids were cultured
428 in 10 µM Y-27632 (MedChemExpress; HY-10583), 3 µM iPSC (Calbiochem; 420220), 3
429 µM GSK-3 inhibitor (XVI, Calbiochem; 361559) for the first 3 days.

430 To create BTRZI CRC donor organoids, lentiviral expression plasmid pD2119-FLuc2
431 KRasG12D donor was co-transfected with viral packaging vectors, psPAX2 (Addgene;
432 plasmid; 12260) and MD2G (Addgene; plasmid; 12259), into HEK293T cells. At 48 and
433 72 h after transfection, viral supernatants were harvested, filtered through a 0.45-µm
434 filter, and concentrated using Amicon Ultra Centrifugal Filters (Merck Millipore;
435 UFC910024). Concentrated lentivirus particles were used for transduction. The viral
436 supernatant generated was used to transduce BTRZI organoids by spinoculation. Briefly,
437 organoids were dissociated to single cells using TrypLE. 1x10^5 single cells were mixed with
438 250 µl organoid media; 10 µM Y-27632; 250 µl concentrated viral supernatant and 4 µg/ml
439 polybrene (Sigma,; H9268) in a 48 well tray before centrifugation at 600 xg for 90 minutes
440 at 32 °C. Meanwhile, 120 µl 50:50 ADMEM:Matrigel mixture was added to a cold 24-well
441 tray before centrifugation of this bottom matrigel layer for 40 minutes at 200xg at room
442 temperature, followed by solidifying the Matrigel by incubating at 37 °C for 30 minutes.
443 After spinoculation, cells were scraped from the well and plated on top of the Matrigel
444 monolayer with organoid media. The following day, the media was removed and the upper
445 layer of Matrigel was set over the organoids by adding 120 µl 50:50 ADMEM:Matrigel
446 and allowing to set for 30 minutes before adding organoid media. 48 hours after
447 transduction, BTRZI donor organoids were selected with 8 µg/ml puromycin for 1 week,
448 then maintained in organoid media with 4 µg/ml puromycin.

449

450 **Organoid lysate mixed with *A. baylyi* sensor bacteria**

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

457 10 µg/ml Chloramphenicol to OD_{600} 0.35 before mixing with organoid lysate at a 1:1 ratio
458 and grow overnight on LB agar plates at 30 °C. All bacteria was scraped into 200 µl
459 LB/20% glycerol before spotting 5x 5 µl spots onto kanamycin and chloramphenicol plates
460 and grown overnight at 37 °C. Colonies were counted and the dilution factor was
461 accounted for to calculate CFU per ml. Rate of HGT was calculated by dividing the CFU
462 per ml of transformants (Kanamycin plates) by the CFU per of total *A. baylyi*
463 (chloramphenicol plates) for 5 independent experiments.
464
465 **Coculture organoids with *A. baylyi* sensor bacteria**
466 For co-culture experiments, 24-well trays were coated with Matrigel monolayers. Briefly,
467 200 µl 50:50 ADMEM:Matrigel mixture was added to a cold 24-well tray and centrifuged
468 for 40 minutes at 200xg at room temperature, followed by a 30 minute incubation at 37
469 °C to solidify matrigel. BTRZI (parental) and BTRZI donor organoids were dissociated
470 into small clusters using TrypLE and grown for 5 days on a Matrigel monolayer in
471 organoid media without antibiotics before 50 µl OD_{600} 0.35 *A. baylyi* sensor was added to
472 each well. After 24 hours, organoids were photographed then collected and grown
473 overnight on LB agar plates at 30 °C. All bacteria was scraped into 200 µl LB/20%
474 glycerol before spotting 5x 5 µl spots onto kanamycin and chloramphenicol plates and
475 grown overnight at 37 °C. Colonies were counted and the dilution factor was accounted
476 for to calculate CFU per ml. Rate of HGT was calculated by dividing the CFU per ml of
477 transformants (kanamycin plates) by the CFU per ml of total *A. baylyi* (chloramphenicol
478 plates) for 5 independent experiments.
479
480 ***A. baylyi* colonisation trial**
481 This study was approved by the SAHMRI Animal Ethics committee (SAM20.036).
482 NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice (male and female, 10-13 weeks old) were
483 obtained from the SAHMRI Bioresources facility and housed under pathogen-free
484 conditions. NSG mice were administered with antibiotics (2.7mM Ampicillin, Sigma;
485 A1066 and 0.55mM Neomycin, Sigma; N1876) in drinking water a week prior to oral
486 gavage/enema. *A. baylyi*-mCherry/KanR was grown in liquid culture with 50 µg/ml
487 kanamycin to OD_{600} 0.3. *A. baylyi* was washed with PBS before 3 mice received 10^{10} *A.*
488 *baylyi* via oral gavage, 3 mice received 10^{10} *A. baylyi* via enema and 2 control mice received
489 PBS (1x enema and 1x oral gavage). Oral gavage was administered using a 20G curved
490 feeding needle at a volume of 200 µl per mouse. Enema was performed as per previous
491 publication. Briefly, mice were anaesthetised with isofluorane and colon flushed with 1 ml
492 of room temperature sterile PBS to clear the colon cavity of any remaining stool. A P200
493 pipette tip coated with warm water was then inserted parallel into the lumen to deliver
494 50 mL of bacteria into the colon over the course of 30 seconds. After infusion, the anal

495 verge was sealed with Vetbond Tissue Adhesive (3M; 1469SB) to prevent luminal contents
496 from being immediately excreted. Animals were maintained on anaesthesia for 5 minutes,
497 and then allowed to recover on heat mat and anal canal inspected 6 hours after the
498 procedure to make sure that the adhesive has been degraded. Stool was collected for 2
499 weeks in 250 µl PBS/20% glycerol, vortexed and stored at -80 °C. Stool slurry (50 µl)
500 was plated onto a LB agar plate and grown overnight at 37 ºC. All bacteria was scraped
501 into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted onto kanamycin plates.
502 Colonies were counted and dilutions were factored to calculate CFU *A. baylyi* per stool.
503
504 **Horizontal gene transfer *in vivo***
505 BTRZI donor organoids were isolated from Matrigel and dissociated into small clusters
506 using TrypLE. The cell clusters (equivalent to ~150 organoids per injection) were
507 washed three times with cold PBS containing 10 µM Y-27632 and then resuspended in 20
508 µl 10% GFR matrigel 1:1000 india ink, 10 µM Y-27632 in PBS and orthotopically injected
509 into the mucosa of the proximal and distal colon of anaesthetised 10-13 week old NSG
510 mice (150 organoids per injection), as previously described (Lannagan et al, 2019 Gut).
511 Briefly, a customised needle (Hamilton Inc. part number 7803-05, removable needle, 33
512 gauge, 12 inches long, point 4, 12 degree bevel) was used. In each mouse up to 2 injections
513 of 20µl were performed. CRC donor tumor growth was monitored by colonoscopy for 4
514 weeks and the videos were viewed offline using QuickTime Player for analysis.
515 Colonoscopy was performed using a Karl Storz Image 1 Camera System comprised of:
516 Image1 HDTV HUB CCU; Cold Light Fountain LED Nova 150 light source; Full HD
517 Image1 3 Chip H3-Z Camera Head; Hopkins Telescope, 1.9mm, 0 degrees. A sealed luer
518 lock was placed on the working channel of the telescope sheath to ensure minimal air
519 leakage (Coherent Scientific, # 14034-40). Tumor growth of the largest tumor visualised
520 was scored as previously described using the Becker Scale (Rex et al, 2012 Am J
521 Gastroenterol). Mice were administered antibiotics (2.7mM Ampicillin, Sigma; A1066 and
522 0.55mM Neomycin, Sigma; N1876) in drinking water a week prior to oral gavage/enema.
523 *A. baylyi* sensor was grown in liquid culture with 10 µg/ml Chloramphenicol to OD_{600} 0.3.
524 *A. baylyi* sensor was washed with PBS before 13 mice received 10^{10} *A. baylyi* sensor via
525 oral gavage (7 mice without tumors and 6 mice with CRC donor tumors), 7 mice received
526 10^{10} *A. baylyi* sensor via enema (3 mice without tumors and 4 mice with CRC donor
527 tumors). Three days after *A. baylyi* administration, mice received 10 mg/L kanamycin in
528 their drinking water, except 2 mice from the oral gavage *A. baylyi* sensor, CRC donor
529 tumor cohort, 5 mice from the oral gavage *A. baylyi* sensor, no tumor cohort and 2 mice
530 from the enema *A. baylyi* sensor, no tumor cohort . Stool was collected 5 days after *A.*
531 *baylyi* administration into 250 µl PBS/20% glycerol, vortexed and stored at -80 °C. Stool
532 slurry (50 µl) was plated onto a LB agar plate and grown overnight at 37 °C. All bacteria

533 was scraped into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted onto
534 chloramphenicol and kanamycin plates. Colonies were counted and dilutions were factored
535 to calculate CFU *A. baylyi* per stool.
536
537 **Sequencing gDNA from bacterial colonies grown on kanamycin plates**
538 *A. baylyi* transformants were individually picked from kanamycin plates and grown in
539 liquid culture LB supplemented with 25 µg/ml Kanamycin. gDNA was extracted using
540 purelink genomic DNA minikit (Invitrogen; K182001). Genomic regions of interest were
541 amplified using Primestar Max DNA polymerase (Takara, # R045A) and primers
542 HGTpcrF: CAAAATCGGCTCCGTCGATACTA;
543 HGTpcrR: TAGCATCACCTTCACCCTC;
544 Kan seqF: AAAGATACGGAAGGAATGTCTCC;
545 Kan seqR: CGGCCGTCTAAGCTATTCGT. Sanger sequencing was conducted by
546 AGRF using the same primers.
547
548 **DNase treatment of stool**
549 Stool slurry (25 µl) was mixed with 2.5 µl 10x DNase 1 buffer with or without 1 µl DNase
550 1 (2.7 U/µl) using RNase-free DNase 1 kit (Qiagen,; 79254). Samples were incubated at
551 37 °C for 30 minutes then the mixture was plated onto LB agar plates and grown overnight
552 at 37 °C. A control to assess DNase 1 activity was set up simultaneously with 25 µl stool
553 (from mouse with no tumor); 1 µl 100 ng/µl KRasG12D donor plasmid DNA (2 ng/ul
554 final concentration); 2.5 µl 10x DNase 1 buffer with or without 1 µl DNase 1 (2.7 U/µl),
555 which was incubated at 37 °C for 30 minutes. Following DNase 1 treatment, controls were
556 mixed with 25 µl of *A. baylyi* sensor liquid culture (OD_{600} 0.35) and incubated at 37 °C 2
557 hrs before the mixture was plated onto LB agar plates and grown overnight at 37 °C. All
558 bacteria was scraped into 200 µl LB/20% glycerol. 5x 5µl serial dilutions were spotted
559 onto kanamycin plates. Colonies were counted and dilutions were factored to calculate
560 CFU *A. baylyi* per stool.
561

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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.

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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

599 the donor DNA, the 2 stop codons are repaired, and the biosensors acquire kanamycin
600 resistance.
601
602 **Extended Data Figure 4: Sensor detection of donor DNA from BTRZI CRC**
603 **organoids.** *A. baylyi* sensor bacteria are constitutively chloramphenicol resistant, hence
604 c*hlorR* CFUs provide a read-out of total *A. baylyi* present. In contrast, kanamycin
605 resistant sensor bacteria rely on incorporation of donor DNA from CRC organoids to
606 correct the defective *kan* gene and enable growth on kanamycin selection plates. **a**
607 Recombination with lysate from CRC donor organoids enables growth of *A. baylyi* sensor
608 on kanamycin plates. Shown here with representative plates and CFU analysis. **b** After
609 co-culturing established CRC donor organoids with *A. baylyi* sensor, recombination with
610 donor DNA from CRC donor organoids enables growth of *A. baylyi* sensor on kanamycin
611 plates. Shown here with representative images and CFU analysis. Scale bars 200 µm. **a,**
612 **b,** Fig 3 contains the same data as shown here but presented as HGT rate (kanamycin
613 resistant CFU *A.baylyi* per ml/chloramphenicol CFU *A.baylyi* per ml*),* n = 5 independent
614 experiments each with 5 technical replicates. **c** Representative Sanger sequencing
615 chromatograms of PCR amplicon covering the region of the *kan* gene containing
616 informative SNPs, to highlight the difference in sequence in gDNA isolated from parental
617 *A. baylyi* sensor bacteria compared to *A.baylyi* colonies isolated from kanamycin plates
618 following mixing with donor organoid lysates or viable organoids.
619
620 **Extended Data Figure 5: *A. baylyi* is detected in stool for 2 weeks after oral**
621 **gavage or enema. a,** Schematic illustrating the experimental pipeline of colonisation
622 trial, n=6 mice administered *A.baylyi* mCherry-*kanR* bacteria (constitutively kanamycin
623 resistant), n=2 PBS control mice. Representative bright-field and fluorescent image of
624 *A.baylyi* mCherry-*kanR* CFU from stool. **b,** *A. baylyi* mCherry-*kanR* is detected in stool
625 from mice. Data points represent the average CFU per stool grown on kanamycin
626 selection plates from 1-3 stools/mouse, with results from each mouse (84.2a, 84.2b, 84.2c,
627 84.2g) plotted separately.
628
629 **Extended Data Figure 6: High sensitivity digital droplet PCR (ddPCR)**
630 **detection of CRC mutation (*BrafV600E*) in stool DNA isolated from tumour**
631 **bearing animals (n=3-4 mice/group). a,** Representative images of ddPCR data. **b,**
632 CRC mutation (*BrafV600E)* positive droplets as a % of total droplets. Analysis of no
633 template negative control samples and stool DNA samples from non-tumour bearing
634 animals was used to determine the sensitivity threshold of the assay. Positive control
635 samples contain 10% *BrafV600E* gDNA spiked into stool DNA sample from non-tumour

636 bearing animal. NT, no tumour; Ts, small tumour; Tm, medium tumour; Tl, large
637 tumour; NTC, no template PCR negative control.
638
639 **Extended Data Figure 7: Efficient horizontal gene transfer detected in stool**
640 **from tumor bearing mice requires both engineered biosensor bacteria, as**
641 **opposed to parental *A. baylyi*, and tumor**. Average CFU per stool from 2-4 stools
642 per mouse grown on kanamycin selection plates is shown, n=4-10 mice/group. Combined
643 data for oral and rectal dosing of biosensors. One-way Anova with Tukey's post-hoc on
644 log10 transformed data was used for statistical analysis with P values shown in the
645 corresponding panel.
646
647 **Extended Data Figure 8: *A. baylyi* sensor in stool from mice bearing BTRZI**
648 **CRC donor tumors become kanamycin resistant via HGT. a,** Representative
649 Sanger sequencing chromatograms of PCR amplicon covering the region of the *kan* gene
650 containing informative SNPs, to highlight the difference in sequence in parental *A. baylyi*
651 sensor bacteria (defective *kanR*) in comparison to colonies isolated from kanamycin plates
652 from stool of tumor bearing mice administered *A. baylyi* sensor bacteria (corrected *kanR*
653 oral and rectal). **b** Representative CFU plates.
654
655 **Extended Data Figure 9: DNase treatment of stool homogenates to remove**
656 **unincorporated donor DNA prior to CFU analysis did not alter number of *A.***
657 ***baylyi* sensor bacteria that were kanamycin resistant.** Stools from mice bearing
658 BTRZI donor tumors and administered *A. baylyi* sensor bacteria were incubated with
659 and without DNase before CFU analysis on kanamycin plates. 1-4 stools from 5 mice
660 were analysed. No statistical difference was evident between the number of kanamycin
661 resistant colonies from CRC donor tumor stools treated with or without DNase. As a
662 positive control for DNase treatment efficacy, stool from a non-tumour bearing mouse
663 was mixed with *A. baylyi* sensor and CRC donor plasmid, then treated with or without
664 DNase. In this case, kanamycin resistant colonies were only evident in the absence of
665 DNase. This suggests that the HGT evident in stool from the experimental animals likely
666 occurred *in vivo* or in stools, but prior to CFU plating. Paired t-test was used for
667 statistical analysis.
668
669 **Extended Data Movie 1:** *A. baylyi* biosensors taking up plasmid donor DNA.
670 *A. baylyi* were grown overnight, washed into fresh LB, mixed with saturating pLenti-
671 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.