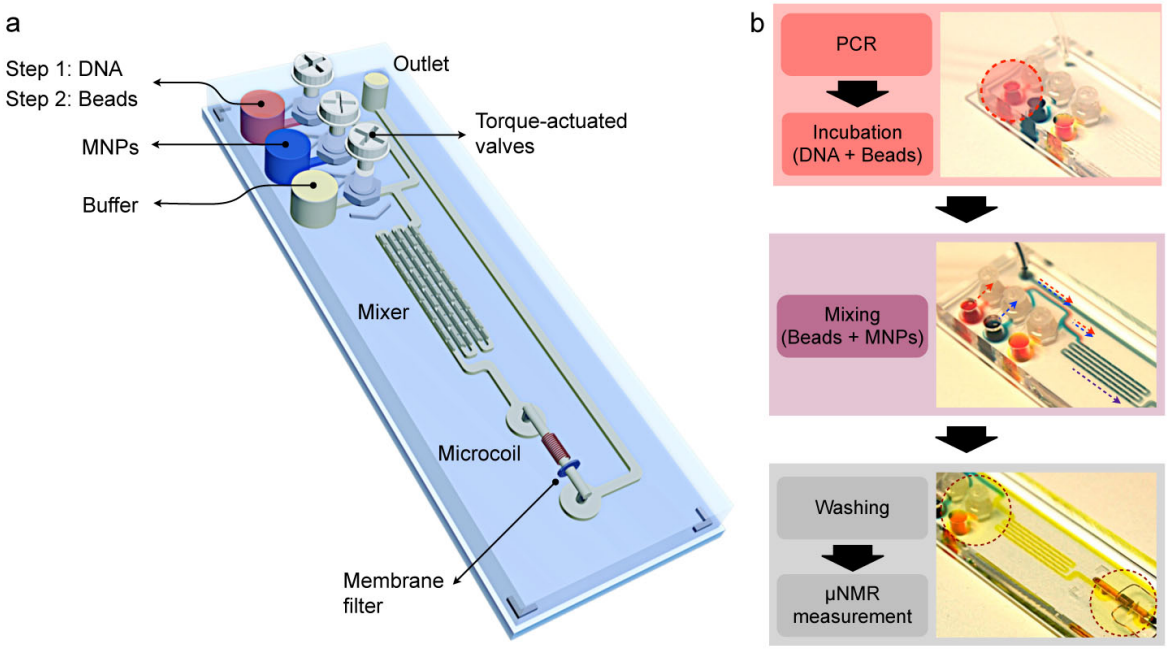
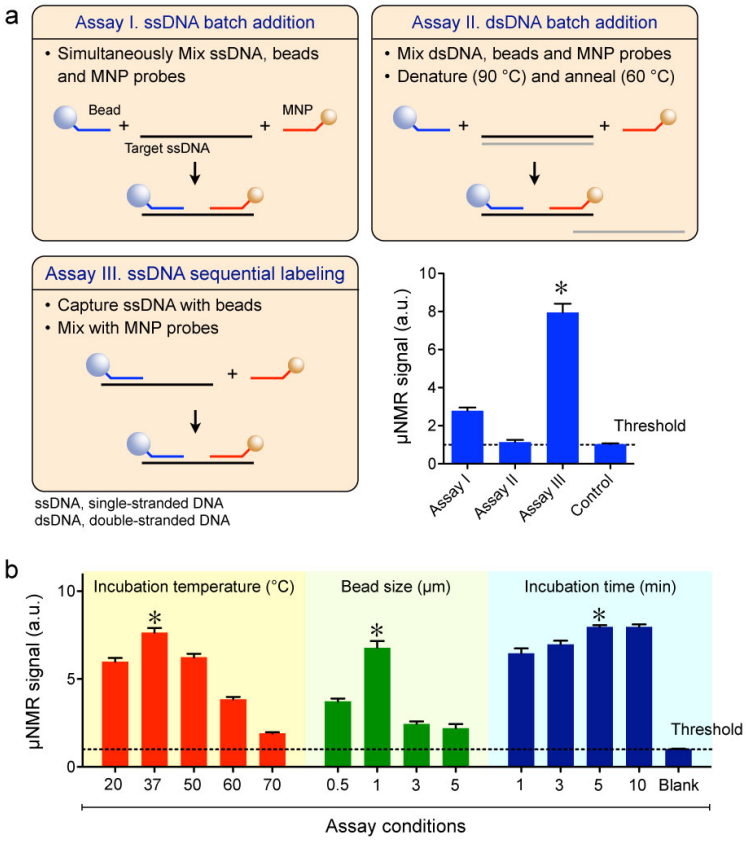
Supplementary Figures

Figure S1. Design and operation of the microfluidic cartridge.



(**a**) The device has three inlets for DNA and capture beads, MNP probes and washing buffer. Each inlet is gated by the torque-actuated valves. (**b**) Assay flow on the chip. (**i**) Genomic DNA is loaded into the chamber and amplified by PCR. Subsequently, capture beads are introduced into the same chamber and incubated. (**ii**) Two valves are opened to flow bead-DNA mixture and MNPs into the mixing chamber. MNP-labeled beads are eventually retained by the membrane filter and concentrated inside the microcoil. (**iii**) Washing buffer is injected to remove excess, unbound MNPs, and μNMR measurement is performed.

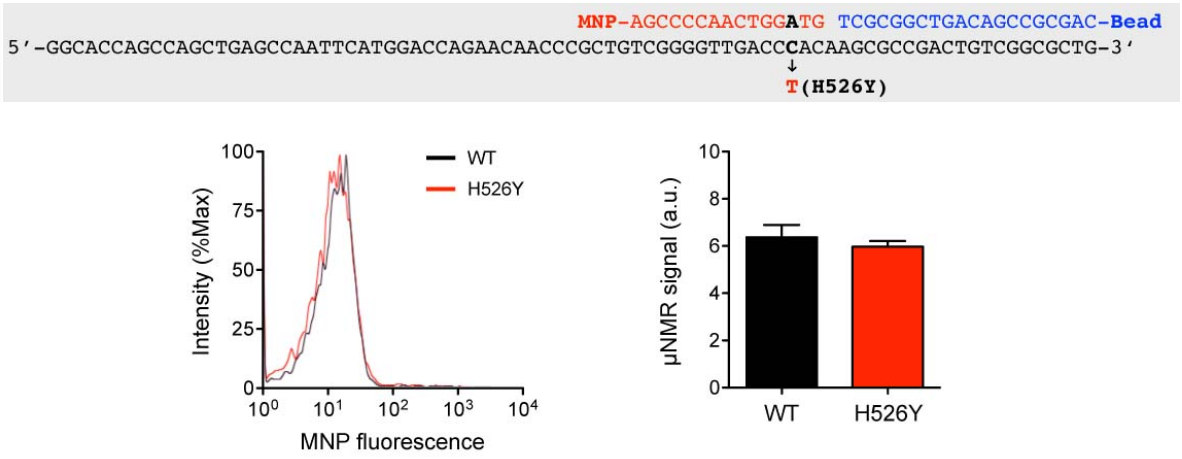
Figure S2. Optimization of the magnetic barcoding assay.



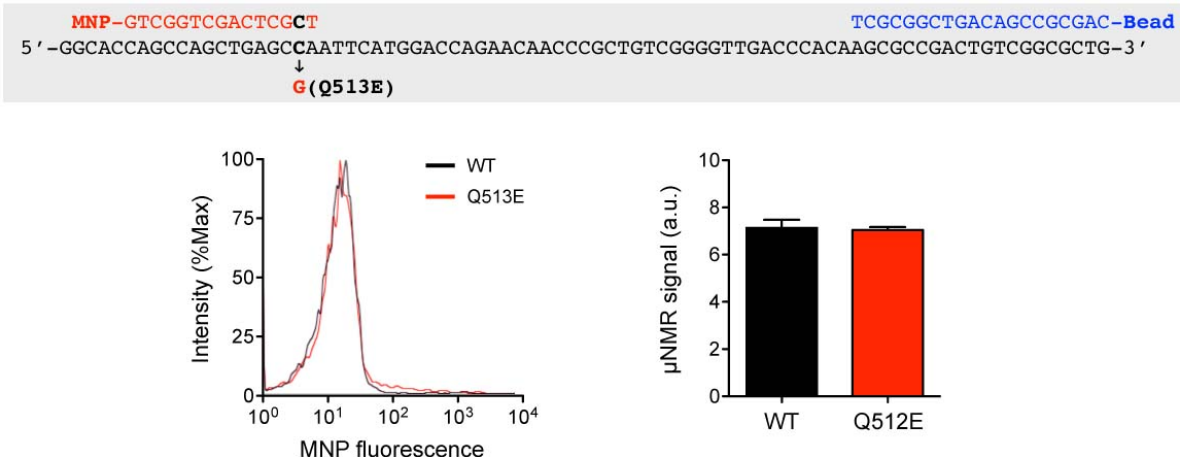
(**a**) The blue lines correspond to the oligonucleotide strands conjugated to the capture beads. The red lines correspond to the oligonucleotide strands conjugated to the magnetic nanoprobes (MNPs). The black lines correspond to either the target single-stranded (ssDNA) or double-stranded DNA (dsDNA). In Assay I: ssDNA batch addition, ssDNA and labeling agents were simultaneously mixed. In Assay II: dsDNA batch labeling, dsDNA and labeling agents were mixed together, and underwent denaturing/annealing processes. In Assay III: ssDNA sequential labeling, the target ssDNA were first captured by the beads and then magnetically labeled. The sequential labeling process showed the highest signal-to-noise ratio in the magnetic measurements, and ultimately led to the use of asymmetric PCR for the production of ssDNA. (**b**) Effects of temperature, bead size, and incubation time (n = 3). (**i**) For the optimization of incubation temperature, 1 μm beads were used for capturing and labeling 92-nt *fadE15* ssDNA. At 37 °C, the oligonucleotide hybridization was most efficient with minimal nonspecific binding. At temperatures over 70 °C, higher nonspecific binding of MNPs resulted in a lower signal-to-noise ratio. (**ii**) Capture beads ranging from 0.5 to 5 μm in diameter were tested in the magnetic barcoding assay using the device. The 1 μm beads provided the highest signal-to-noise ratio due to the large surface area and low nonspecific binding. (**iii**) The magnetic barcoding assay for detecting 92-nt *fadE15* amplicons showed a strong magnetic signal within 1 minute of labeling at 37 °C. The error bars in **a** and **b** represent the standard deviation of three replicates.

Figure S3. **Magnetic detection of single-nucleotide polymorphisms**.

a

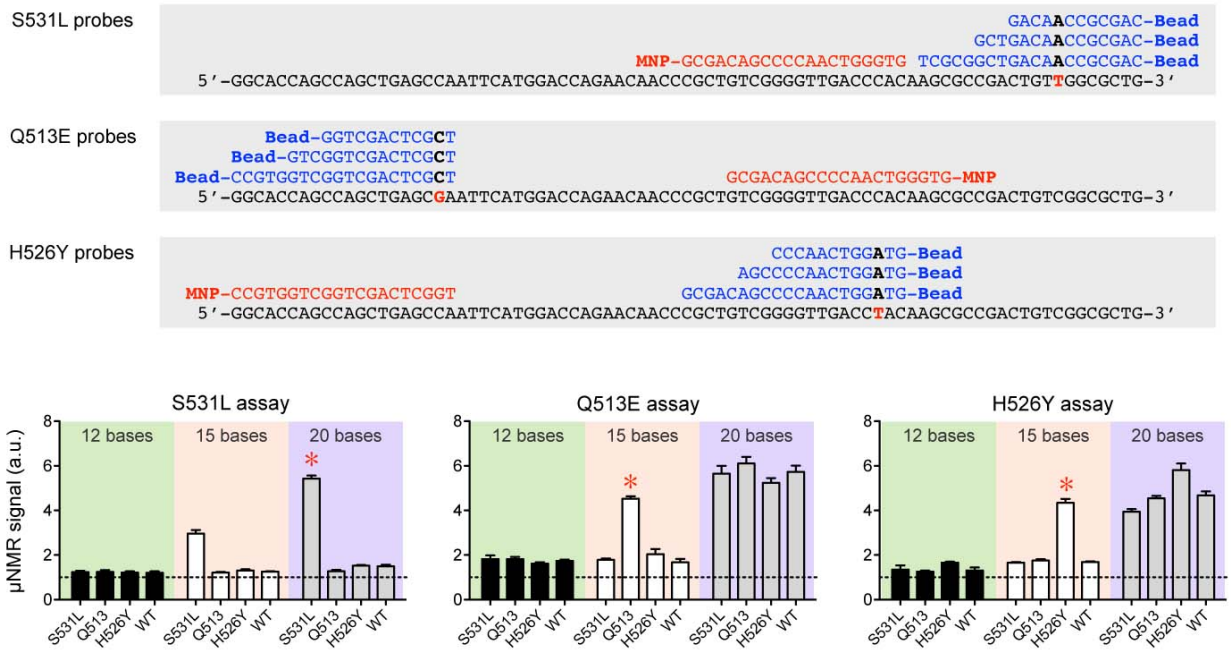


b



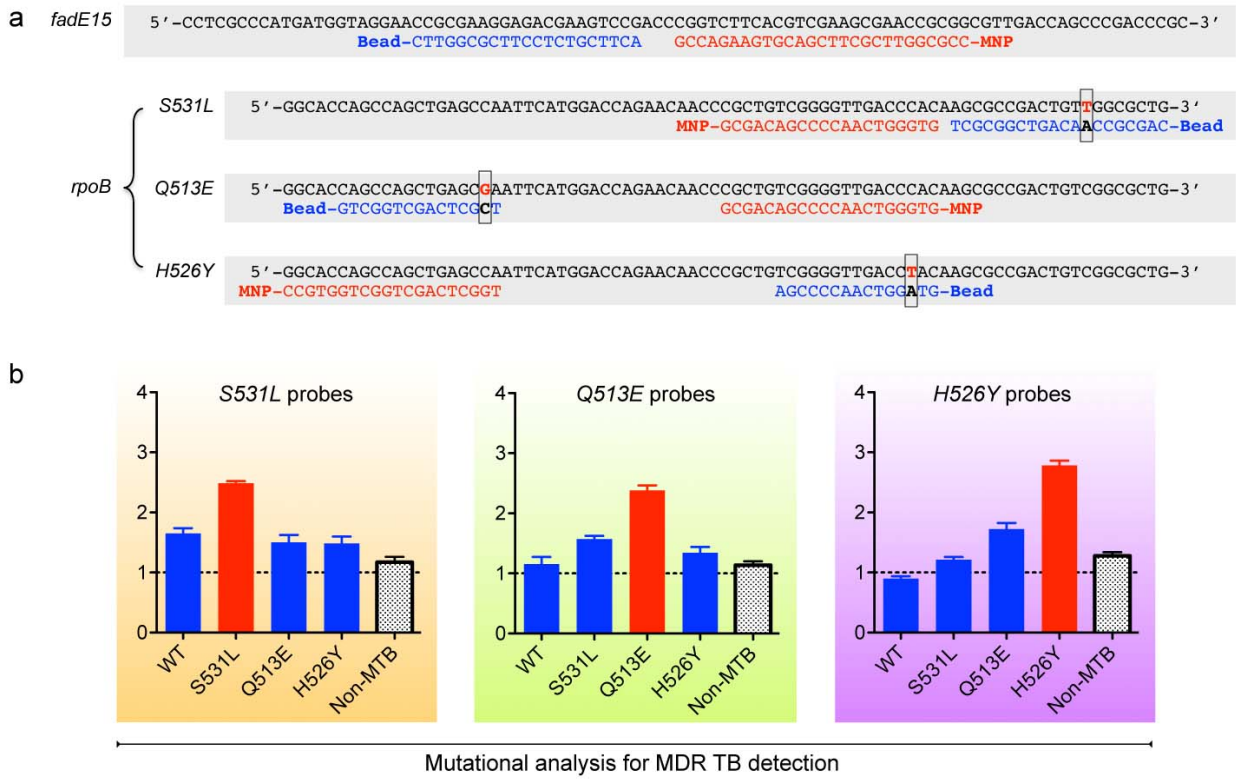
The oligonucleotides conjugated to the MNPs were designed to be fully complementary to either (**a**) H526Y, or (**b**) Q513E mutant ssDNA (n = 3). Unlike the results shown in **Fig. 4a**, fluorescence and magnetic readouts failed to show appreciable differences between wild-type and mutant alleles. The large number of oligonucleotides on the MNPs resulted in multivalent binding, which contributed to the higher affinity. The error bars represent the standard deviation of three replicates.

Figure S4. **Optimization of magnetic probes for the detection of single-nucleotide polymorphisms**.



The design of capture oligonucleotides depended on the base length for detecting single-nucleotide polymorphism in *rpoB* mutant strands (n = 3). The oligonucleotide length of the capture strands was decreased from 20-nt to 12-nt. The 15-nt capture strands were most efficient for distinguishing Q513E and H526Y mutant strands, whereas the 20-nt capture oligonucleotides were most efficient for distinguishing mutation in S531L mutant strand. The optimal probe designs, labeled with asterisks, were used to analyze the RIF-resistant strains. The error bars represent the standard deviation of three replicates.

Figure S5. MTB detection and mutational analyses.

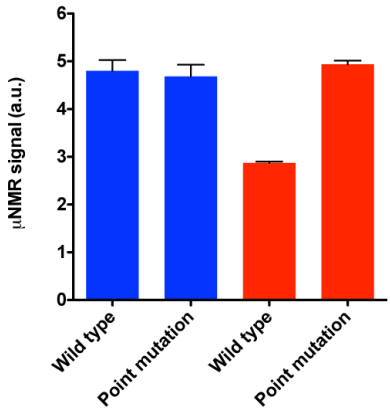


(**a**) Probes for MTB detection. (**b**) Magnetic profiling of single-nucleotide polymorphisms (n = 3). RIF-resistant strains (Q513E C-to-G mutation, H526Y C-to-T mutation, S531L C-to-T mutations) were analyzed and compared to the wild-type (WT) strain. Non-MTB mixed bacteria was used as the control. The *fadE15* probes were used for universal MTB detection, and the *rpoB* probes were used to screen the single-point mutation for RIF-resistance. The error bars represent the standard deviation of three replicates.

Figure S6. Magnetic barcode assay for identifying mutation in epidermal growth factor receptor (EGFR).

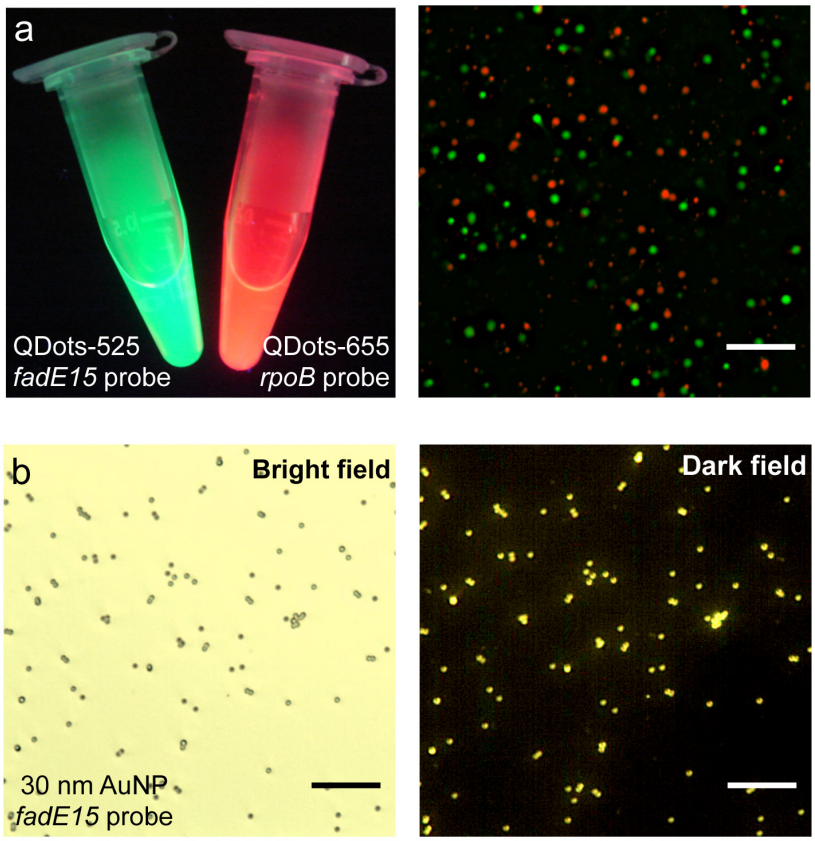
```
          MNP-GTTCTAGTGTCTAAAACCCGCCCGGTTTGACGACCC-Bead
5'-CCGCAGCATGTCAAGATCACAGATTTTGGGCGGGCCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGA-3'
                                  ↓
                                  T(Exon 21 point mutation)
```

```
          MNP-GTTCTAGTGTCTAAAACCCGACCGGTTTGACGACC-Bead
5'-CCGCAGCATGTCAAGATCACAGATTTTGGGCGGGCCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGA-3'
                                  ↓
                                  T(Exon 21 point mutation)
```



By using the two sets of probes shown above, the single-nucleotide polymorphism on EGFR exon 21 can be detected by the magnetic barcode assay (n = 3). When the capture beads were complementary to the wild-type allele, the magnetic readouts were indiscernible between the two alleles. When the capture DNA sequence was modified to fully match the point mutation in exon 21, the magnetic barcode assay could selectively detect the target mutation. The error bars represent the standard deviation of three replicates.

Figure S7. General barcoding strategy for nucleic acid detection.



Using quantum dot and gold conjugates as labeling probes, the barcoded beads could be detected via luminescent and plasmonic readouts. (**a**) Amine-modified green-fluorescent (525 nm) and red-fluorescent quantum dots (655 nm) were conjugated with oligonucleotides designed to bind to *fadE15* and *rpoB* amplicons, respectively. Fluorescence methods such as microscopy were used to simultaneously detect both amplicons. (**b**) 30 nm gold colloids were conjugated with oligonucleotides designed to bind to *fadE15* amplicons. Plasmonic methods such as dark-field microscopy were used to detect the amplicons. Scale bars, 10 μm.

Supplementary Methods

Chemicals. Dimethylformamide, dimethyl sulfoxide, sodium hydroxide, hydrochloric acid, and sodium bicarbonate were purchased from Sigma-Aldrich. Bovine serum albumin (BSA), 10X phosphate buffered saline (PBS) liquid concentrate, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS, 10% solution), Triton X-100, sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), and dithiothreitol (DTT) were purchased from Thermo Fisher Scientific. VivoTag 680 (VT680) was purchased from PerkinElmer. Fluorescein isothiocyanate (FITC), Alexa Fluor 568 (AF568), and TaqMan Gene Expression Master Mix (Part Number 4369016) were purchased from Life Technologies. Streptavidin-coated polystyrene particles (0.4-0.6 μm, 1.0-1.4 μm, 3.0-3.4 μm, 5.0-5.9 μm) were purchased from Spherotech. Fetal bovine serum (FBS) was purchased from ISC BioExpress.

List of oligonucleotides. All oligonucleotides were purchased from Integrated DNA Technologies. The list of oligonucleotides used in the experiments are summarized below.

• Capture and probe strands

fadE15 Capture: 5'-ACTTCGTCTCCTTCGCGGTTCAAAAA-Biotin-3'
fadE15 MNP: 5'-Thiol-AAAAACCGCGGTTCGCTTCGACGTGAAGACCG-3'
fadE15 Amplify: 5'-Thiol-AAAAACGGTCTTCACGTCGAAGCGAACCGCGG-3'

rpoB Capture-1: 5'-TGGCTCAGCTGGCTGGTGCCAAAAA-Biotin-3'
Q513E Capture-20nt: 5'-T**C**GCTCAGCTGGCTGGTGCCAAAAA-Biotin-3'
Q513E Capture-15nt: 5'-T**C**GCTCAGCTGGCTGAAAAA-Biotin-3'
Q513E Capture-12nt: 5'-T**C**GCTCAGCTGGAAAAA-Biotin-3'
Q513E MNP: 5'-GTGGGTCAACCCCGACAGCG-AAAAA-Thiol-3'

rpoB Capture-2: 5'-Biotin-AAAAAGTGGGTCAACCCCGACAGCG-3'
H526Y Capture-20nt: 5'-Biotin-AAAAAGT**A**GGTCAACCCCGACAGCG-3'
H526Y Capture-15nt: 5'-Biotin-AAAAAGT**A**GGTCAACCCCGA-3'
H526Y Capture-12nt: 5'-Biotin-AAAAAGT**A**GGTCAACCC-3'
H526Y MNP: 5'-TGGCTCAGCTGGCTGGTGCC-AAAAA-Thiol-3'

rpoB Capture-3: 5'-Biotin-AAAAACAGCGCCGACAGTCGGCGCTT-3'
S531L Capture-20nt: 5'-Biotin-AAAAACAGCGCC**A**ACAGTCGGCGCTT-3'
S531L Capture-15nt: 5'-Biotin-AAAAACAGCGCC**A**ACAGTCG-3'
S531L Capture-12nt: 5'-Biotin-AAAAACAGCGCC**A**ACAG-3'
S531L MNP: 5'-GTGGGTCAACCCCGACAGCG-AAAAA-Thiol-3'

• Target strands and primers

fadE15 Forward:
CCTCGCCCATGATGGTAGGAACCGCGAAGGAGACGAAGTCCGACCCGGTCTTCACGTCGA
AGCGAACCGCGGCGTTGACCAGCCCGACCCGC

fadE15 Reverse:
GCGGGTCGGGCTGGTCAACGCCGCGGTTCGCTTCGACGTGAAGACCGGGTCGGACTTCG
TCTCCTTCGCGGTTCCTACCATCATGGGCGAGG

fadE15 Primer-1: GCGGGTCGGGCTGGTCAAC

fadE15 Primer-2: CCTCGCCCATGATGGTAGGAAC

Prime Time Assay probe from IDT for real-time polymerase chain reaction (PCR):
/56-FAM/TCGGACTTC**/ZEN/**GTCTCCTTC**/3IABkFQ/**

• rpoB Sequences
rpoB Primer-1: CGCCGCGATCAAGGAGTTCT
rpoB Primer-2: TCACGTGACAGACCGCCGGG
rpoB Wild-type:
GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCAC
AAGCGCCGACTGTCGGCGCTG-3`
rpoB Q513E Mutant:
GGCACCAGCCAGCTGAGC**G**AATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCAC
AAGCGCCGACTGTCGGCGCTG-3`
rpoB H526Y Mutant:
GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACC**T**AC
AAGCGCCGACTGTCGGCGCTG-3`
rpoB S531L Mutant:
GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCAC
AAGCGCCGACTGT**T**GGCGCTG-3`

Real-time (RT) PCR standard. Genomic DNA was isolated from heat-killed MTB. Bacteria pellet was resuspended in 0.5 mL 1X TE buffer. To this solution, 0.25 mL of Phenol:Chloroform:Isoamyl Alcohol (PCIA, Sigma Aldrich) at 70 °C was added to the sample along with glass beads. The sample was mechanically stressed as described above. To this sample, another 0.25 mL of PCIA was added and incubated at room temperature for 30 min. After incubation period, another 0.5 mL of 1X TE buffer was added and incubated on a rocker for 1 hr. The sample was then centrifuged at 20,000X g for 10 min. The aqueous layer was collected and transferred to a new tube. To this fraction, an equal volume of isopropanol, 1/10th volume sodium acetate (NaOAc, 3 M) and 1 μL of Ambion GlycoBlue (Invitrogen, 15 mg/mL) was added and allowed to incubate at -20 °C overnight. Next day, the sample was pelleted at 20,000X g for 10 min and the supernatant was decanted. The sample was washed with 300 μL 70% ethanol, centrifuged, and resuspended in RNAse-free water. To establish genomic DNA concentration standard, initial genomic DNA concentration was determined using NanoDrop 2000 (Thermo Fisher Scientific). Number of genomic copies was estimated using mass calculation, assuming molecular weight of 660 per base-pair and MTB genome size of 4411529. Serial dilution of genomic DNA sample was performed and RT-PCR standard was established (**Fig. 3b**). Separate genomic DNA dilution was performed to contain 1, 10, 25, 100, 1000 genome in 10 μL nuclease-free water. To confirm proper dilution, Ct values of the samples were compared to the standard. The sample dilution was used for μNMR measurements. RT-PCR reactions were performed in triplicate with each reaction containing 10 μL of genomic DNA, 1.1 μL of primer/probe (1 primer: 2 probe) and 11 μL TaqMan Universal Master Mix (Invitrogen). For PCR amplification, an activation step (50 °C for 2 min and 95 °C for 10 min) was followed by 40 cycles of: 95 °C for 15 sec, 60 °C for 60 sec and 72 °C for 15 sec.

Synthesis of luminescent and plasmonic nanoprobes. For the luminescent nanoprobes, green fluorescent Qdot® 525-ITK and red fluorescent Qdot® 655-ITK amino-quantum dots (Life

Technologies) were used for the *fadE15* assay and the *rpoB* assay, respectively. In 0.5 mL PBS solution, 50 μL (8 μM) solution of Qdots were mixed with 0.18 mg sulfo-SMCC for 3 hours at room temperature. The Qdots were subsequently purified using membrane filtration and Sephadex G-50 with PBS as the eluent buffer. The purified maleimide-activated Qdots were eventually mixed with 20 nmol of the reduced thiol-modified oligonucleotides in 2 mL PBS solution (pH 7.2) and the reaction proceeded overnight at room temperature. After this incubation, the conjugates were purified using membrane filtration and Sephadex G-100 with PBS as the eluent buffer. For the plasmonic nanoprobes, 2 mL gold colloids (30 nm, British BioCell International) were mixed with 10 nmol reduced thiol-modified oligonucleotides (*fadE15* probe) and agitated overnight at room temperature. 10X PBS and 10% SDS were then added to the solution at 10% and 1%, respectively, of the overall volume, and agitated overnight at room temperature. The salt concentration was increased slowly to 0.3 M by adding 2 M NaCl in 1X PBS over 3 time periods. After the overnight incubation, the conjugates were purified and dissolved in PBS.

Supplementary Note 1

Cost summary. The costs for the magnetic barcode assay are summarized below:
1) One time cost of the DNA extraction device: $300
2) Disposable devices and reagents: $2.50 per assay
3) One time cost for the reader-containing magnet, electronic circuit, and thermocycler: $4,000
In summary, the one time cost of the device is $4300 in its current configuration, which can likely be scaled down to $200, and the cost of disposables for each assay is < $3 with further optimization likely to be < $1. In comparison, the Cepheid GeneXpert has a subsidized cost of $17,000 for the system and $10 for a single test cartridge.[42]

Supplementary Reference

42. Dorman, S. E. et al. Performance characteristics of the Cepheid Xpert MTB/RIF test in a tuberculosis prevalence survey. *PLoS One* **7**, e43307 (2012).