SUPPLEMENTARY INFORMATION

Magneto-DNA nanoparticle system allows target specific bacterial identification

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Supplementary Methods

Probe specificity tests. Before conjugation of probes to beads and MNPs, probe hybridization and specificity to the target nucleic acid were tested. Each set of probes were mixed with the amplified PCR product derived from each bacteria type (molar ratio of target DNA : capture probe : detection probe = 1:3:3), incubated in PBS at 37 ^{o}C for 1 h, and observed retardation of the target nucleic acids by polyacrylamide gel electrophoresis.

Quantification of probe conjugations. The amount of oligonucleotide probes conjugated onto the beads and MNPs were quantified using the Qubit ssDNA quantification kit (Invitrogen). The beads or MNPs after conjugation were added with the Qubit ssDNA reagent and applied to the Qubit 2.0 Fluorometer (Invitrogen). DNA concentrations were calculated based on a calibration curve using Qubit ssDNA standards.

Preparation of sections for transmission electron microscopy. The hybridized samples containing bead-nanoparticle complexes were formed into pellets and treated with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.4 (Electron Microscopy Sciences, Hatfield, PA) for 1 h, and embedded in 2% agarose in PBS. Pellets were then dehydrated through a graded series of ethanol, infiltrated in a 1:1 solution of 100% ethanol and epon resin (Ted Pella, Redding, CA), and embedded in fresh 100% epon. Thin sections were cut on a Leica EM UC7 ultramicrotome, and collected onto formvar-coated grids.

Microscopic observations. For transmission electron microscopy, the hybridized bead-nanoparticle complexes were mounted as a whole onto carbon-mesh coated copper grids (Ted Pella) and observed with JEM 2011 (JEOL USA). The thin section samples were observed with JEM 1011 at 80 kV. For scanning electron microscopy, the bead-nanoparticle complexes were mounted on a Si wafer, dried by an N_2 stream, and observed with a field emission scanning electron microscope (Ultra Plus, Carl Zeiss, Jena, Germany). For atomic force microscopy, samples were mounted on mica substrates (SPI Supplies, V1 grade) and imaged by MFP-3D (Asylum Research, Santa Barbara, CA) equipped with a silicon probe (AC200TS, Asylum research). The samples were scanned in a tapping mode with a scan rate of 0.5 Hz or below.

Real-time PCR. For quantitative real-time PCR, the cDNA derived from bacteria or clinical samples were added with the Fast SYBR Green Master Mix and specific primers used above for conventional PCR. The reaction mixtures were then applied to the 7500 Fast Real-Time PCR System (Life Technologies) and thermal cycling was carried out for 40 cycles at conditions of 95 ^{o}C for 3 sec (denaturation) followed by 60 ^{o}C for 30 sec (annealing/extension). C_T values were obtained using the 7500 Fast software (provided by the manufacturer), and relative amounts of target nucleic acid were calculated based on the C_T values.

Supplementary Table S1: Sequences of probes and primers for bacterial detection.

Target	Capture probe	Detection probe	Amplicon	Forward primer	Reverse primer
Universal*	AAAAAAGGTGTGACC**Y**TGACTCTGT	AGGATGCCCTCCGTCGTCA AAAAAA	CCACACTGG**R**ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC*	CCACACTGG**R**ACTGAGACAC	GAAGATTCCCTACTGCTGCC
Staphylococcus	CTTACACATATGTTCTTCC AAAAAA	AAAAAATAGTTAGCCGTGGCTTTCT	GGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTAC	GGGAAGAACATATGTGTAAGTA	GTAGTTAGCCGTGGCTTTCT
Streptococcus	AAGAACGAGTGTGAGAGTG AAAAAA	AAAAAAGTTAGCCGTCCCTTTCTG	GAAGAACGAGTGTGAGAGTGGAAAGTTCACACTGTGACGGTATCTTACCAGAAAGGGACGGCTAACTA	GAAGAACGAGTGTGAGAGTG	TAGTTAGCCGTCCCTTTCTG
Enterococcus	ACTAACGTCCTTGTTCTTC AAAAAA	AAAAAATAGTTAGCCGTGGCTTTCT	GAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACTAC	GAAGAACAAGGACGTTAGTA	GTAGTTAGCCGTGGCTTTCT
Escherichia	TAACTTTACTCCCTTCCTC AAAAAA	AAAAAAGAGTTAGCCGGTGCTTCT	GGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC	GGAGGAAGGGAGTAAAGTTAAT	GGAGTTAGCCGGTGCTTCT
Pseudomonas	AGCTTACTGCCCTTCCTCC AAAAAA	AAAAAAACGAAGTTAGCCGGTGCTT	GGAGGAAGGGCAGTAAGCTAATATCCTTGCGGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGT	GGAGGAAGGGCAGTAAGCT	ACGAAGTTAGCCGGTGCTTA
Klebsiella	AAAAAAGAGGCAAGGCGATAAGGT	AGAAGCACCGGCTAACTGC AAAAAA	GGAGGCAAGGCGATAAGGTTAATAACCTTGGCGATTGGACGTTACCCGCAGAAGAAGCACCGGCTAACTGCCGT	GGAGGCAAGGCGATAAGGT	ACGGCAGTTAGCCGGTGCTTCT
Enterobacter	AAAAAAGAGGAAGGCGTTAAGGTTA	AAGCACCGGCTAACTCCGAAAAAA	GGAGGAAGGCGTTAAGGTTAATAACCTTAGCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGT	GGAGGAAGGCGTTAAGGTTA	ACGGAGTTAGCCGGTGCTT
Citrobacter	AAAAAAGAGGAAGGTGTTGTGGTTA	AAGCACCGGCTAACTCCGAAAAAA	GGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGT	GGAGGAAGGTGTTGTGGTTA	ACGGAGTTAGCCGGTGCTT
Acinetobacter	CAACTAGAGTAGCCTCCTCAAAAAA	AAAAAAACAGAGTTAGCCGGTGCTT	GGAGGAGGCTACTCTAGTTAATACCTAGGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGT	GGAGGAGGCTACTCTAGTTA	ACAGAGTTAGCCGGTGCTTA
Proteus	TAACCTTATCACCTTCCTCAAAAAA	AAAAAAGAGTTAGCCGGTGCTTCT	GGAGGAAGGTGATAAGGTTAATACCCTTNTCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC	GGAGGAAGGTGATAAGGTTA A	GGAGTTAGCCGGTGCTTCT
Haemophilus	AAAAATCCGATTCTACGCGGTATTAGCGAC	TCATGGCATGCGGCCTTGCGAAAAA	GTCGCTAATACCGCGTAGAATCGGAAGATGAAAGTGCGGGACCGCAAGGCCGCATGCCATGA	GTCGCTAATACCGCGTAGAATCGGA	TCATGGCATGCGGCCTTGCG
Stenotrophomonas	AAAAAAATGCGTAGAGATCAGGAGGAA	AGCTACCTGGACCAACATTGAAAAAA	AATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGA	AATGCGTAGAGATCAGGAGGAA	TCAATGTTGGTCCAGGTAGCT
Lactobacillus	AAAAAAGGAGGCAGCAGTAGGGAATC	ACGCAAGTCTGATGGAGCAAAAAAAA	GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAA	GGAGGCAGCAGTAGGGAATC	TTGCTCCATCAGACTTGCGT

*Bases in bold letters, **R** = A (for Staphylococcus, Escherichia, Pseudomonas, Klebsiella, Enterobacter, Haemophilus, Stenotrophomonas) or G (for Streptococcus, Enterococcus, Acinetobacter, Proteus, Lactobacillus). **Y** = T (for Staphylococcus, Escherichia, Pseudomonas, Klebsiella, Enterobacter, Haemophilus, Stenotrophomonas) or C (for Streptococcus, Enterococcus, Acinetobacter, Proteus, Lactobacillus).

Supplementary Table S2: Quantification of probes conjugated onto beads and MNPs.

Target	#Oligos/particle	
	Bead-capture probe (× 10^3)	MNP-detection probe
Universal I*	461 ± 76	22.8 ± 2.7
Universal II*	354 ± 10	
Staphylococcus	660 ± 67	26.4 ± 0.8
Streptococcus	505 ± 32	25.3 ± 0.8
Enterococcus	465 ± 9	29.6 ± 5.1
Escherichia	339 ± 19	27.8 ± 2.9
Pseudomonas	345 ± 12	18.2 ± 1.9
Klebsiella	382 ± 30	19.6 ± 2.3
Enterobacter	340 ± 9	22.8 ± 2.1
Citrobacter	510 ± 23	23.4 ± 1.8
Acinetobacter	478 ± 40	28.5 ± 2.9
Proteus	775 ± 81	18.5 ± 2.4
Haemophilus	271 ± 24	16.2 ± 1.3
Stenotrophomonas	535 ± 11	24.0 ± 2.5
Lactobacillus	550 ± 63	18.1 ± 3.3

*Single base difference: **Y** = T for Universal I and C for Universal II (See Supplementary Table S1)

Supplementary Table S3. Comparison of methods for bacterial detection

		Magneto-DNA	qPCR	Standard culture
Assay time (sample processing to detection)		< 2 hr	< 2 hr	2~10 days
Cost	**Instrument**	~$200 (μNMR)	$20,000~50,000 (standard qPCR or automated system such as GeneXpert)	$4,000~10,000 (incubator, culture hood)
	Per assay	~$1 (magnetic nanoparticles, beads, probes, polymerase, primers)	~$1 (polymerases, dyes, primers)	~$0.1 (media, plates, chemicals)
POC use		Easily applicable, currently can be made on-chip	Might be applicable in the future	Difficult to apply

Supplemental Figures

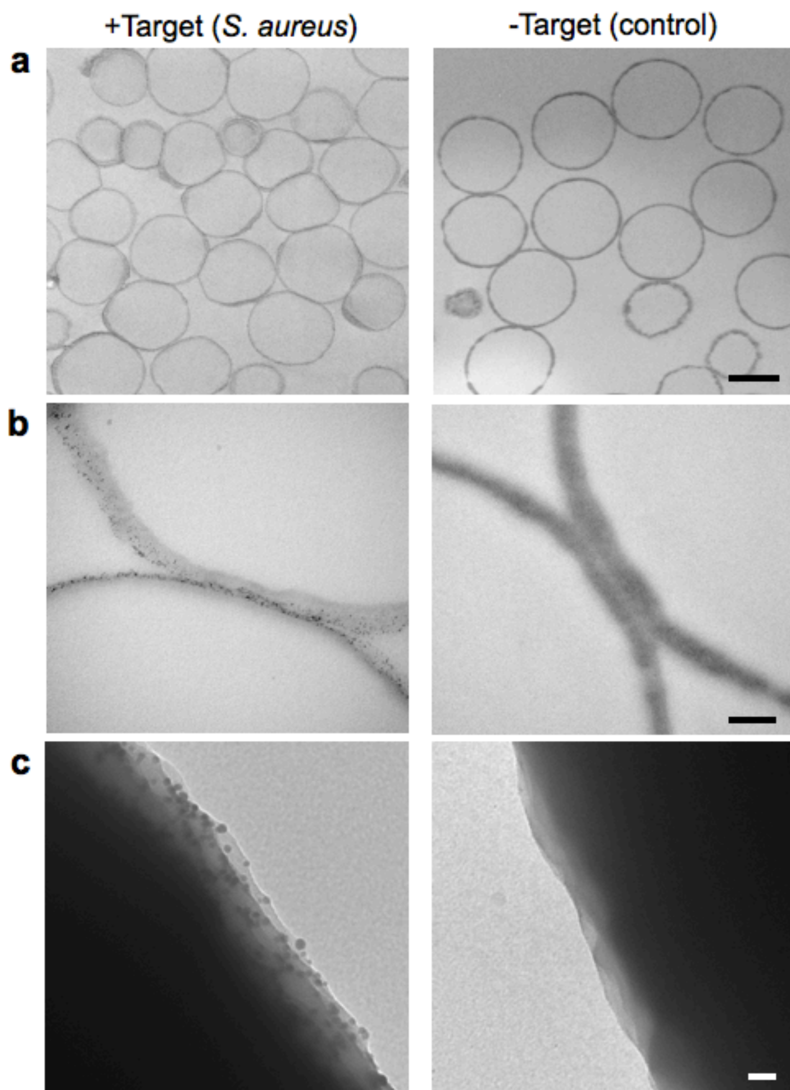

Figure S1: Transmission electron microscopy of magneto-DNA complexes. Hybridization of bead-capture probe and MNP-detection probe was performed in presence (+Target) or absence (-Target) of target DNA. Probes were specific for Staphylococcus and target DNA was obtained from RT-PCR of *S.aureus* 16S rRNA. Samples were observed after sectioning (**a**, low magnification, scale bar 2 µm; **b**, high magnification, scale bar 200 nm) or as whole (**c**, scale bar 20 nm). Note that MNPs were uniformly bound to the bead surface only for samples with target DNA.

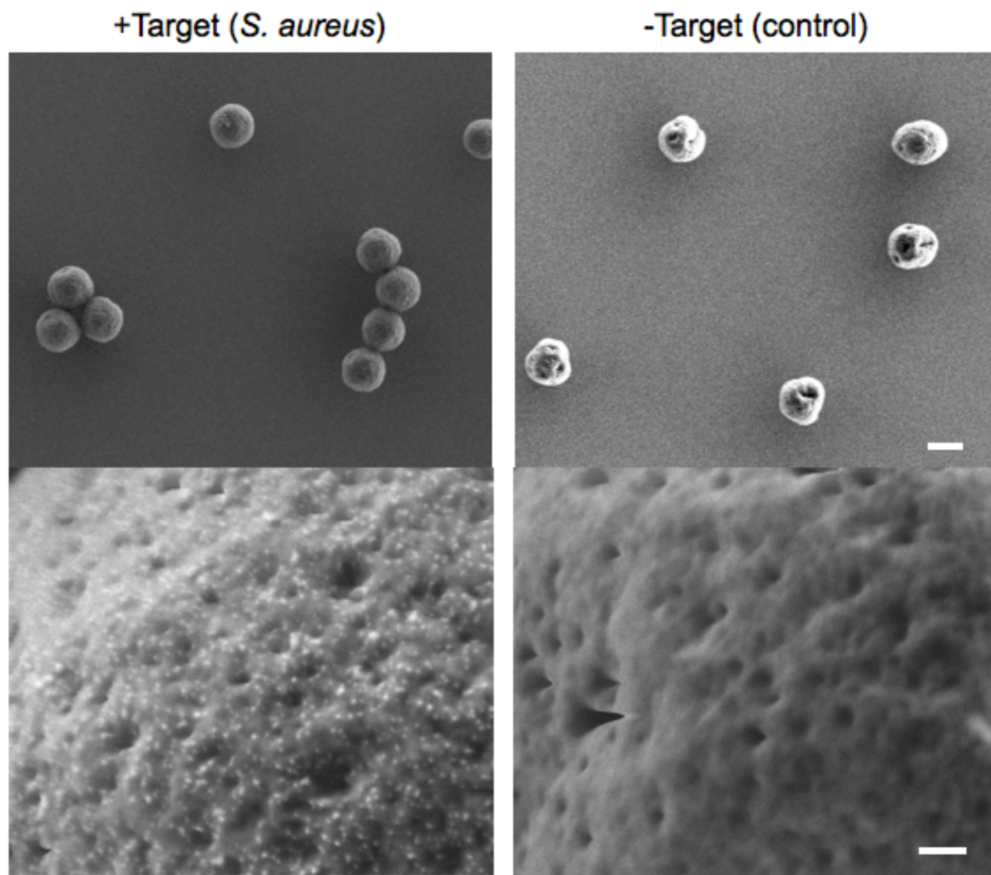

Figure S2: Scanning electron microscopy of magneto-DNA complexes. Hybridization of bead-capture probe and MNP-detection probe was performed in presence (+Target) or absence (-Target) of target DNA. Probes were specific for Staphylococcus and target DNA was obtained from RT-PCR of *S.aureus* 16S rRNA (scale bar: upper row 2 μm, lower row 100 nm). Not that MNPs appear as white dots throughout the bead surface only for samples with target DNA and not the control.

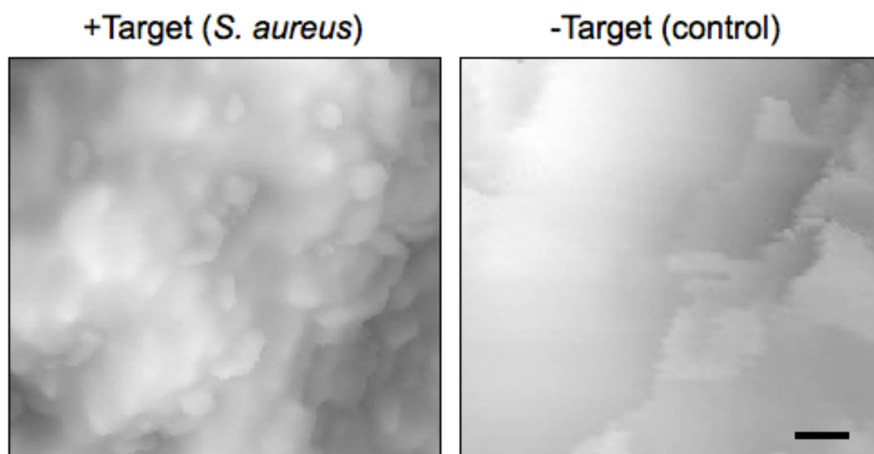

Figure S3: Atomic force microscopy of magneto-DNA complexes. Hybridization of bead-capture probe and MNP-detection probe (specific for Staphylococcus) was performed in presence (+Target) or absence (-Target) of target DNA obtained from RT-PCR of S.aureus 16S rRNA (scale bar, 20 nm). Note the MNPs appear as white bumps projected on the surface of beads only for samples with target DNA and not the control.

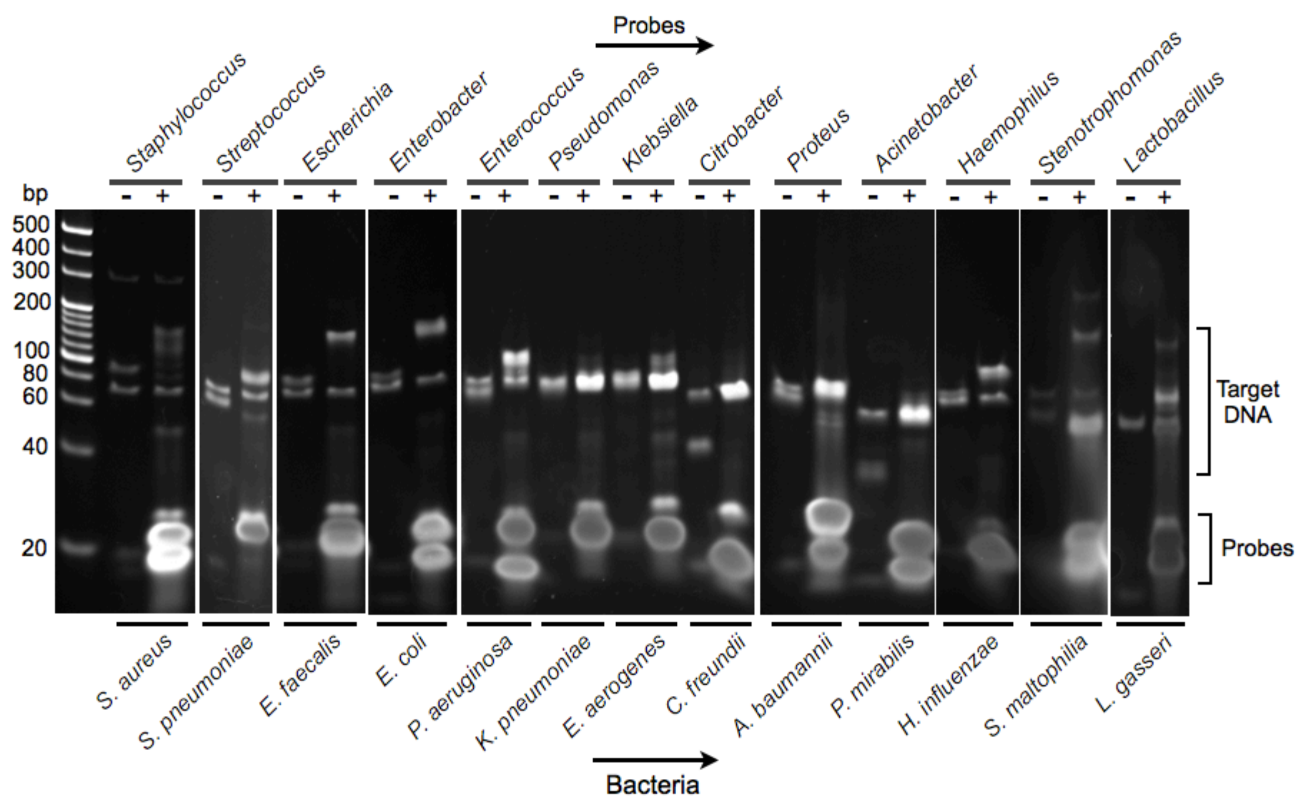

Figure S4: Probe specificity test by gel retardation. Unmodified probes (before conjugation to beads and MNPs) were hybridized with target DNA and polyacrylamide gel electrophoresis was performed. Target DNAs were obtained by RT-PCR of variable 16S rRNA regions from each bacteria type. Note that in absence of probes, target DNAs appear as a heterogenous mixture of double- and single-stranded DNA with sizes of 50~70 bp. Single-stranded DNA usually appears below the double-strand DNA, however in some cases it is shifted above or overlapped with the double-strand due to secondary structure formation. When hybridized with the capture and detection probes, a retardation of target DNA is observed. Incubation of target DNAs with probes for other targets did not show any retardation, demonstrating that probes were specific to each target without off-target binding.

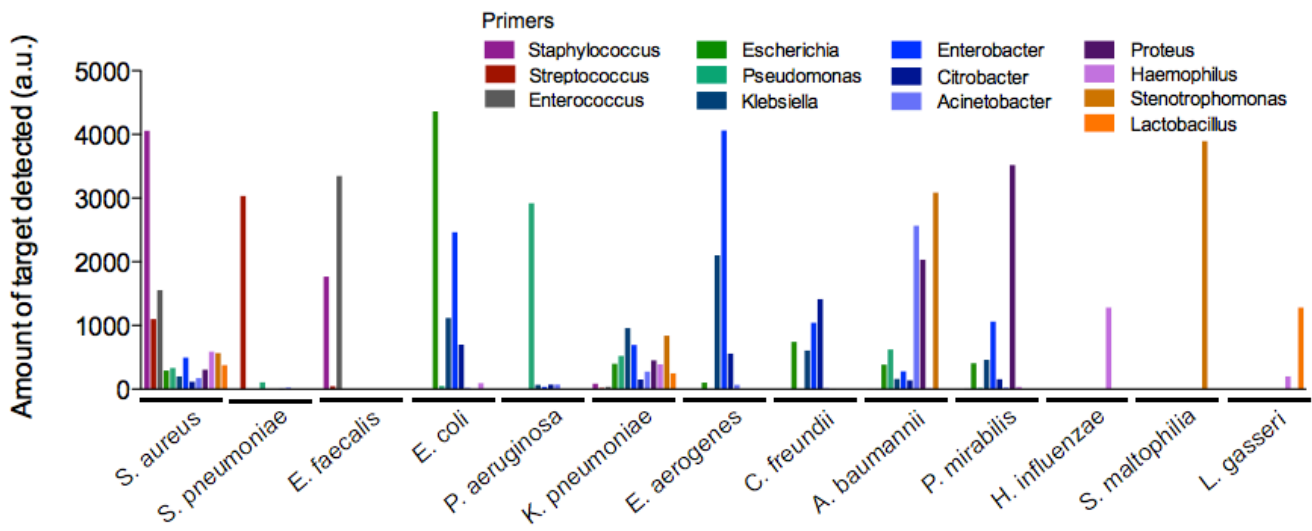

Figure S5: qPCR results for differential detection. Using specific primers as listed in Supplementary Table S1, target cDNAs obtained by reverse transcription of each bacterial 16S rRNA from culture were amplified by the Fast SYBR green assay. Target amounts were obtained from relative quantitation using C_T values.

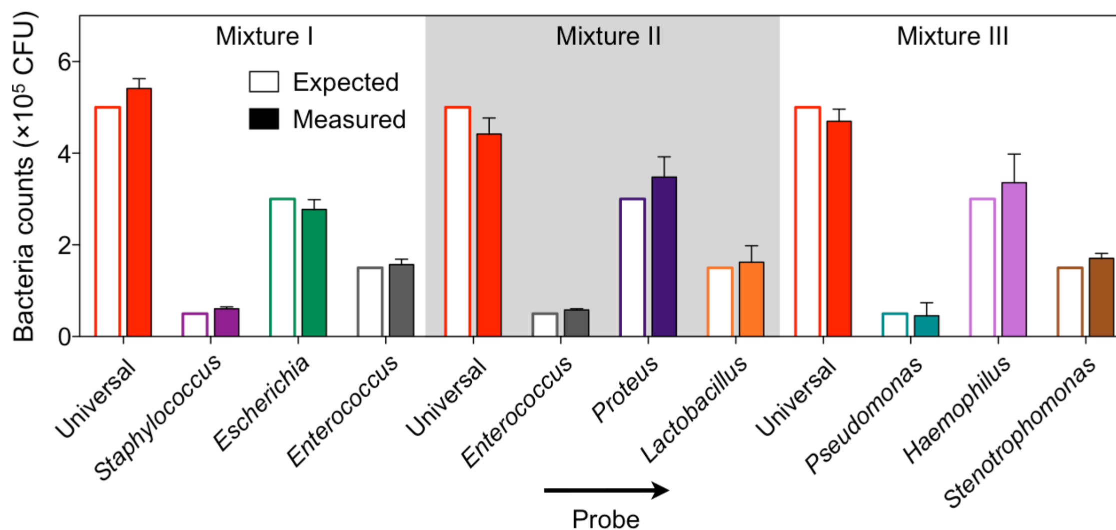

Figure S6: Universal and differential detection of target mixtures by the magneto-DNA assay. Blends of 3 different bacterial DNAs (*S. aureus, E. coli, E. faecalis* for mixture I, *E. faecalis, P. mirabilis, L. gasseri* for mixture II, and *P. aeruginosa, H. influenzae, S. maltophilia* for mixture III) were added into the magneto-DNA nanoparticle system. Equivalent bacterial numbers for each type in a mixture were 10:60:30 and total bacteria number was equivalent to 50,000 CFU. Target DNAs were obtained by RT-PCR of 16S rRNA of each bacterial species. Predicted values were obtained by conversion of normalized ΔR_2 (s^{-1}) values (obtained from Figure 3b and 4c) to bacterial counts, multiplied by blend ratios of the target specific to the added probes. Clear and solid bars indicate expected and measured bacterial counts, respectively.

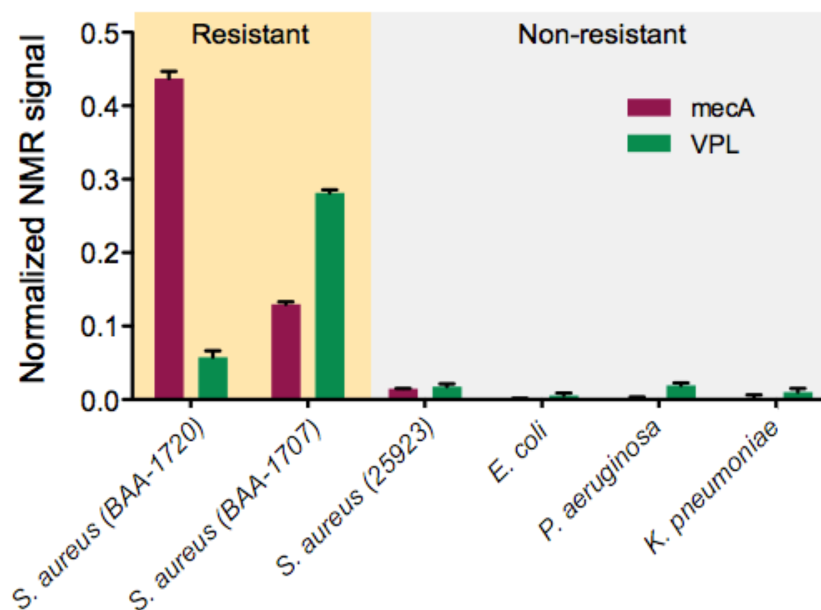

Figure S7. Detection of bacteria with antibiotic resistance using the magneto-DNA assay. Three strains of *S. aureus. E. coli, P. aeruginosa* and *K. pneumoniae* were tested for the resistance genes *mecA* and VPL. *S. aureus* strains BAA-1720 is positive for *mecA* and strain BAA-1707 is positive for PVL and *mecA*; both strains are methicillin resistant (MRSA). The other bacterial strains are negative for the resistance genes. ΔR_2 (s^{-1}) values for *mecA* and PVL detection were normalized to values for 16S rRNA detection of each species.