# A B-Cell Based Sensor for Rapid Identification of Pathogens: Supporting Online Material, *Science* Online

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## Materials and Methods

M12g3R cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ in RPMI 1640 supplemented with 10% fetal bovine serum, 1-mM sodium pyruvate, 2-mM L-glutamine, 100-μM nonessential amino acids, 50-μM 2-mercaptoethanol, 50-μg/ml streptomycin, 250-ng/mL Fungizone, and 50-U/mL penicillin (Life Technologies). Cells were transfected with pCMV.AEQ.IRES.NEO *(S1)* via electroporation (270 V, 950 μF) and selected in 1-mg/mL G418 for 2 weeks. Antibiotic-resistant cells were incubated in growth medium with 10 μM coelenterazine (Molecular Probes) for 2 hrs in the dark at room temperature, washed twice and resuspended in growth medium. The cells were screened for photon emission in response to anti-murine IgM $F(ab')_2$ in a luminometer.

The light chain expression vector, VKExpress, contains the constant region for the human kappa gene downstream of a multiple cloning site (MCS), under control of the human elongation factor-1α (EF-1α) promoter *(S2)*. The heavy chain vector was generated by modifying pDisplay (Invitrogen), retaining the cytomegalovirus (CMV) promoter and leader sequence, but replacing the

platelet-derived growth factor receptor transmembrane domain with the gene for the membrane-bound constant region of murine IgM *(S3)* and removing both tags on either side of the MCS. The genomic sequence of the membrane-bound constant region of the murine IgM, C$\mu$M, was amplified by PCR using primers that contained EcoR I and Not I sites (5$'$ and 3$'$, respectively). The insert, prepared with a blunted EcoR I site and digested with Not I, was cloned into pDisplay-hygro with blunted Bsm I and digested with Not I. The neomycin-resistance gene was replaced with a hygromycin-resistance gene (hygro$^R$, obtained from pcDNA3.1 Hygro, Invitrogen) by adding Cla I and BstB I restriction sites to the 5$'$ and 3$'$ ends of the hygro$^R$ gene, respectively, by PCR, and cloning the new antibiotic-resistance gene into those sites in pDisplay. The appropriate restriction sites are added to the antibody variable regions using PCR, and the sequence of all PCR products is confirmed before cloning into the expression constructs, as described below. Despite the functional hygromycin resistance on the heavy chain vector, we obtained few colonies under hygromycin selection, and those colonies were not responsive to antigen.

RNA was extracted with Trizol reagent (Life Technologies), according to the manufacturer's recommendations, and first strand synthesis was performed using the Retroscript kit (Ambion). PCR was accomplished using sets of primers designed to anneal to the leader sequences or the framework reagions at the 5$'$ end, and the constant or framework regions at the 3$'$ end *(S4, S5)*. Cloning of the variable regions into the expression vectors proceeded as follows. ApaL I and BamH I restriction sites were added to the 5$'$ and 3$'$ ends of the light chain variable regions by PCR, and cloned into VKExpress as described *(S2)*. The heavy chain variable regions ($V_H$) were cloned into pDisplay-C$\mu$M in a two-step process to eliminate the HA and myc tags. First, overlap extension PCR was used to fuse the $V_H$ to the first 300 base pairs (bp) of C$\mu$M and add a Bgl II restriction site to the 5$'$ end. The insert was digested with Bgl II, which also cuts at bp 293 of the constant region, and cloned into pDis-C$\mu$M digested with the same enzyme. A second overlap extension product fused the $V_H$ to the Ig$\kappa$ leader sequence, which was cloned into the Kpn I and Bgl II sites. We have subsequently modified this cloning process by producing a pDisplay-C$\mu$M

vector with a Bgl II site immediately following the leader to allow for a single cloning step that eliminates both tags.

Cells were prepared for the luminescence assay by incubation at a concentration of $5 \times 10^5$ cells/mL in growth medium with the addition of 2% DMSO. After 20-24 hrs, cells were incubated in the dark at room temperature for 2 hours in assay medium [$CO_2$-Independent medium, 10% fetal bovine serum, 50-μg/ml streptomycin, 50-U/ml penicillin, and 250 ng/mL amphotericin B (Life Technologies)] with 50-μM coelenterazine (Molecular Probes, Eugene, OR). The cells were then washed twice, resuspended in assay medium at a final concentration of $5 \times 10^5$ cells/mL in 1.5 mL microcentrifuge tubes, and left to rotate overnight at room temperature.

Vaccinia virus (VR1508) and anti-E. coli O157:H7 hybridoma (HB-10452) were obtained from the ATCC.

## Supporting Figures

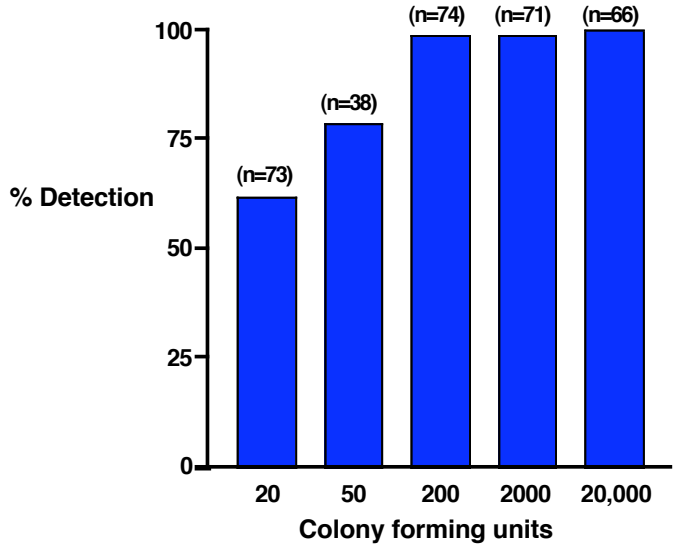


**Fig. S1.** Sensitivity of the B-cell line specific for *Y. pestis*. B cells specific for *Y. pestis* were assayed as described above over a period of several months. Positive responses were defined as those where the signal-to-background ratio was ≥3, and the peak occurred between 15 and 30□s after the cell-delivery centrifugation step. The number of trials performed at each concentration is shown as (n).

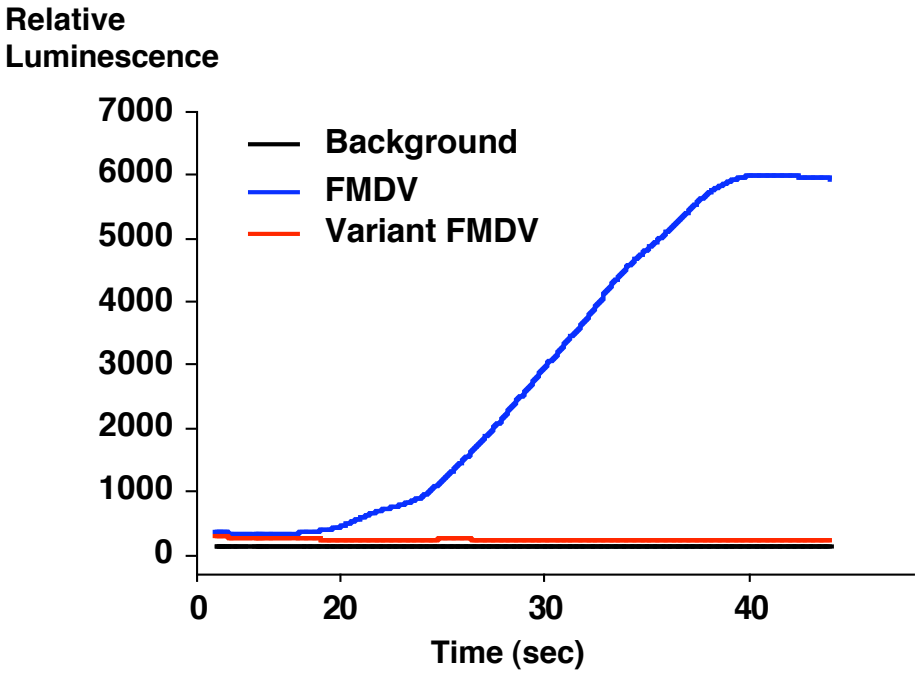


**Figure S2.** B cells specific for the A12 strain of foot-and-mouth disease virus (FMDV). B cells expressing an A12-specific recombinant antibody *(S6)* were prepared as described in Methods and Materials but without DMSO pre-treatment. 25 μl of cells give a strong response when mixed with wt A12 FMDV (5 μl of pRMC35 strain at $1.4 \times 10^8$ pfu/ml) *(S7)*. However, no light is detected after the addition of an equivalent amount of an A12 variant strain (10 μl of B2PD.3, at $7.5 \times 10^7$ pfu/ml) *(S8)* that differs by three amino acids, a change that disrupts the antibody-epitope interaction.

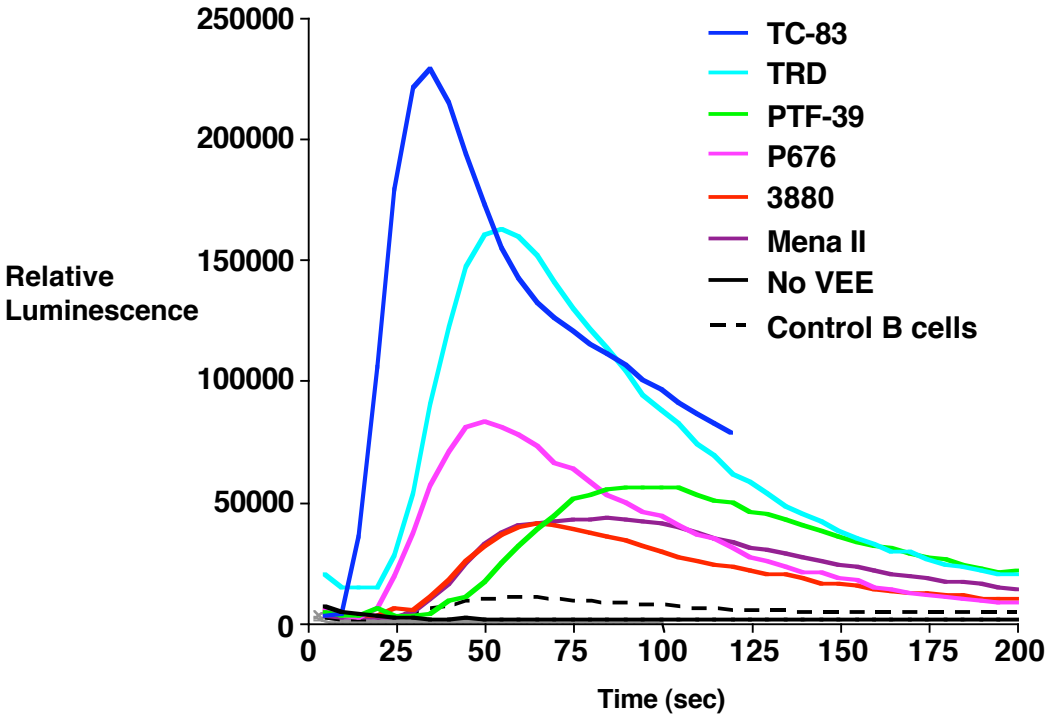


**Figure S3.** B cells that respond to at least six different strains of Venezuelan equine encephalitis (VEE) virus. 20 □l of B cells expressing a recombinant antibody derived from monoclonal 1A3A-9 *(S9)* were prepared and assayed as described in Fig. S1, but without the 1 min concentration step. The B-cell line exhibits specificity similar to that of the parent monoclonal antibody, reacting with VEE strains representing subtypes IA (TC-83, TRD), IB (PTF-39), IC (P676), ID (3880), and IE (Mena II). We have also produced a B-cell line from hybridoma 1A4D-1, which recognizes all but the Mena II strain listed above. Inactivated antigens were derived from either tissue culture (PTF-39, P676, 3880, Mena II) or suckling mouse brain (TC-83, TRD).

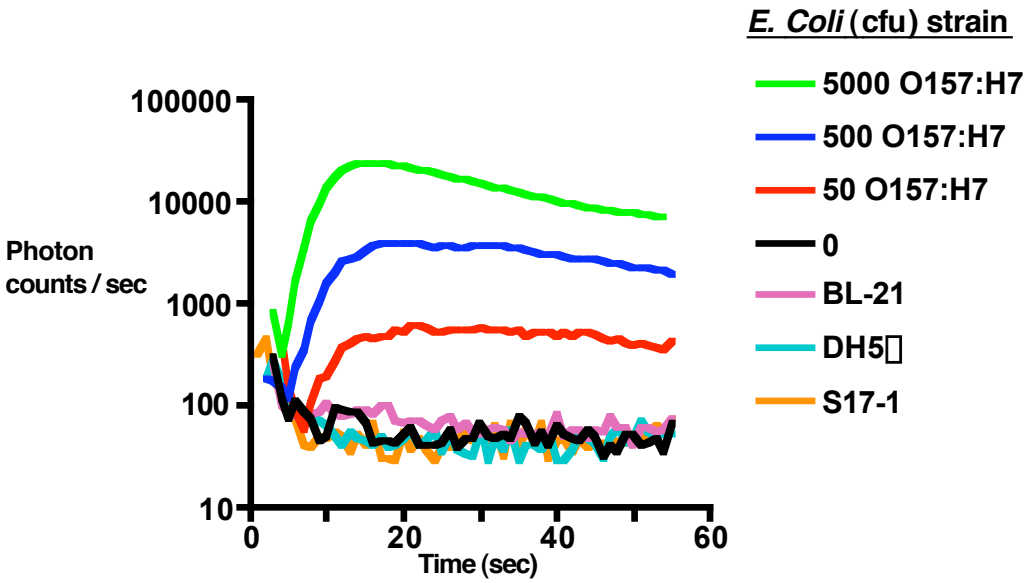


**Figure S4.** Specificity of B cells that respond to *E. coli* O157:H7. B cells that respond to *E. coli* O157:H7 were assayed as described in Fig. S1 with the indicated amounts of *E. coli* O157:H7, or an excess of the non-pathogenic strains of *E. coli*, BL-21, DH5, and S17-1. The cells give a strong response to as little as 50 cfu of pathogenic *E. coli* O157:H7, but no response to any of the non-pathogenic strains of *E. coli*.

**References**

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