

The Army's University Affiliated Research Center

The UTEXAS UARC

Chem-Bio Program Objectives



Dr. Steve Kornguth, Director

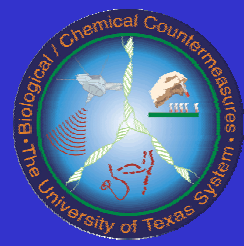
Biological and Chemical (B/C) Countermeasures

Report Documentation Page

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Simultaneous Detection of Multiple Pathogenicity Islands (PI)

Dr. Shelley Payne, UT Austin

Dr. Kerry Oliver, Radix BioSolutions, Ltd.

Dr. James J. Valdes, Edgewood Chemical Biological Command

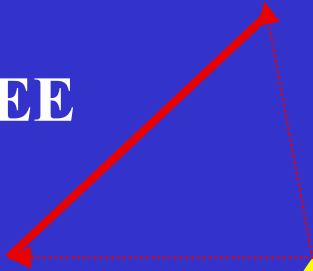
Dr. Steve Kornguth, Dr. Robert C. Chin*, Institute for Advanced Technology, UT Austin

Goal

- Characterize pathogenicity islands, DNA factors present in virulent strains but absent from closely related, avirulent strains of bacteria
- Transition UT pathogenicity island technology to rapid commercial screening platform

E. coli O157:H7

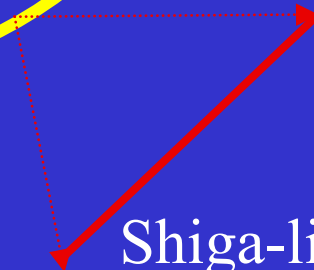
LEE

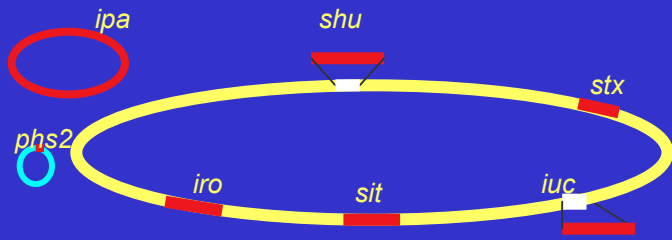


Shu

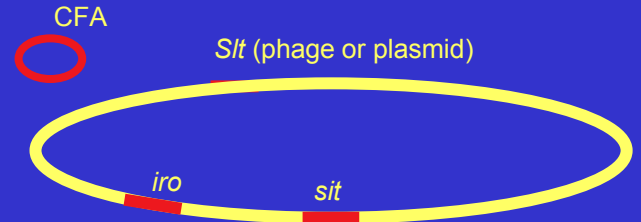


Shiga-like toxin



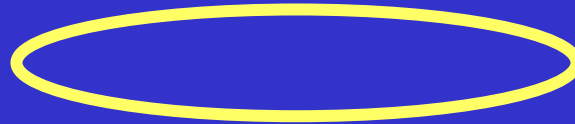


Shigella/EIEC

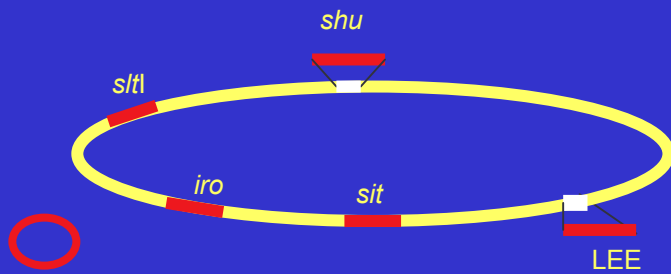


E. coli ETEC

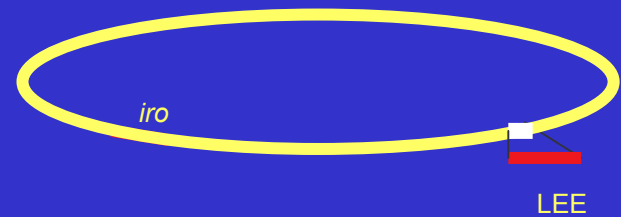
non-pathogenic *E. coli*



E. coli O157:H7



E. coli EPEC



General Characteristics of a rapid screening platform

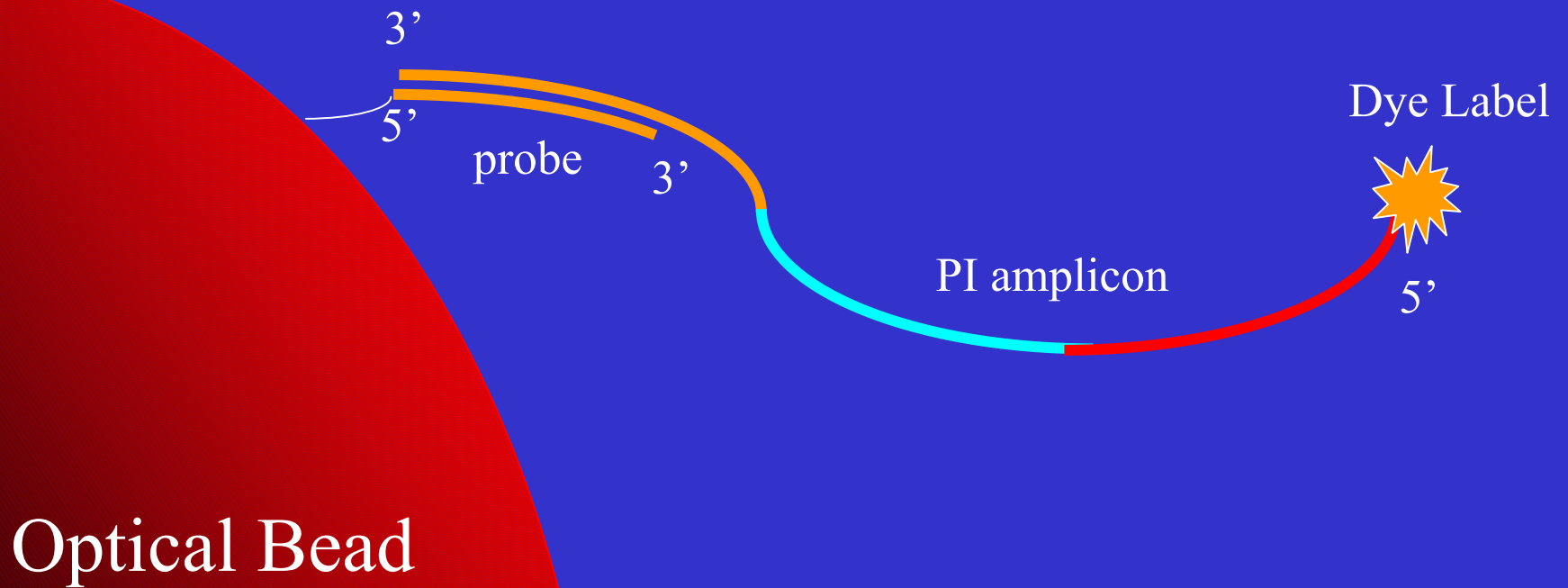
- Flexible
- Reproducible
- Simple
- Stable
- Minimize false positives and negatives

Pathogenicity Island

Multiplexing

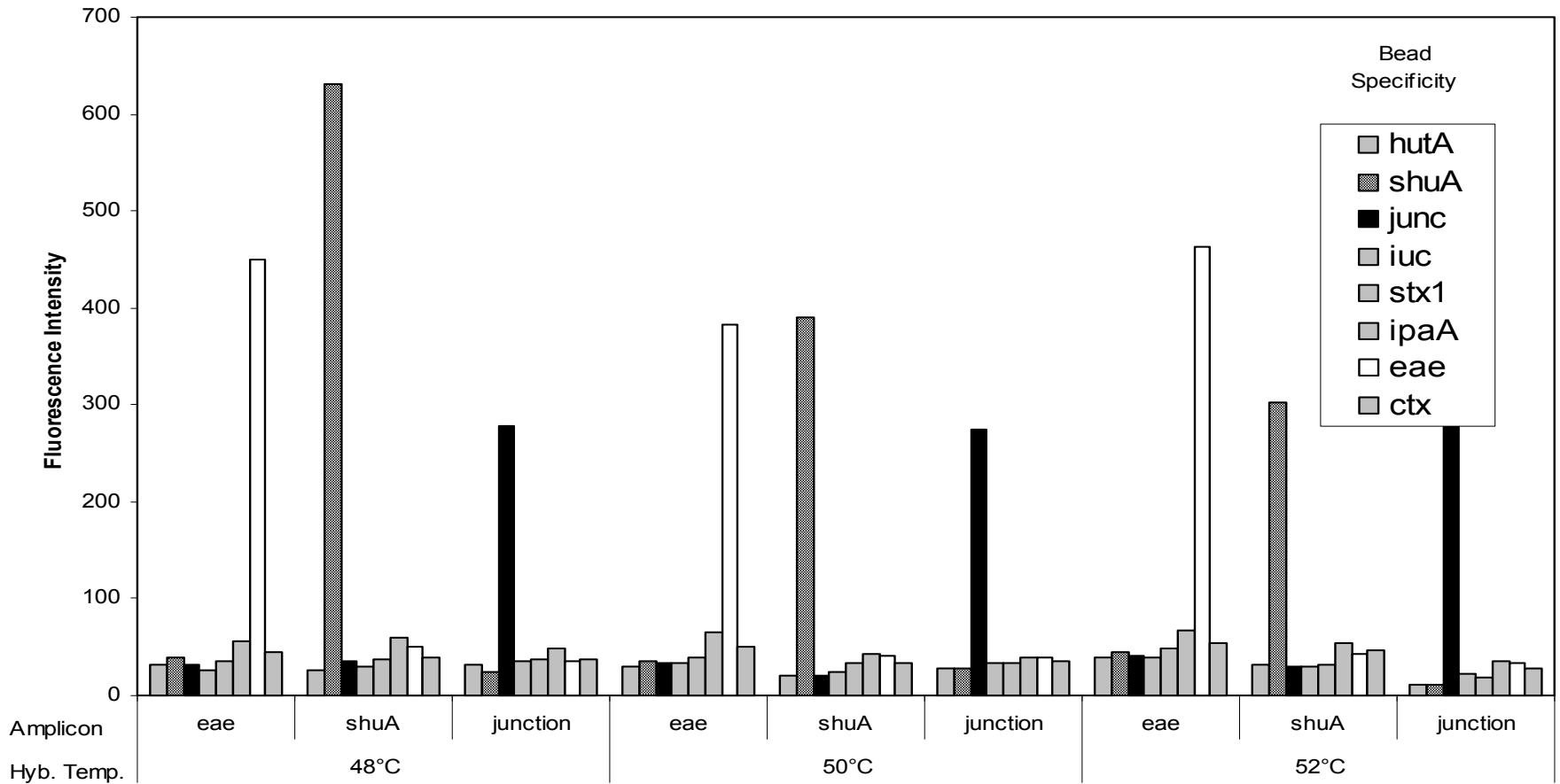
- Identify pathogenic properties rather than specific organisms
- Pattern identification of pathogens
- As new factors can be rapidly added to current stocks
- Multiple sequences for each factor can essentially eliminate false positives and false negatives

Pathogenicity Island Assay Format



IAT/Radix Pathogenicity Island Multiplexing Assay

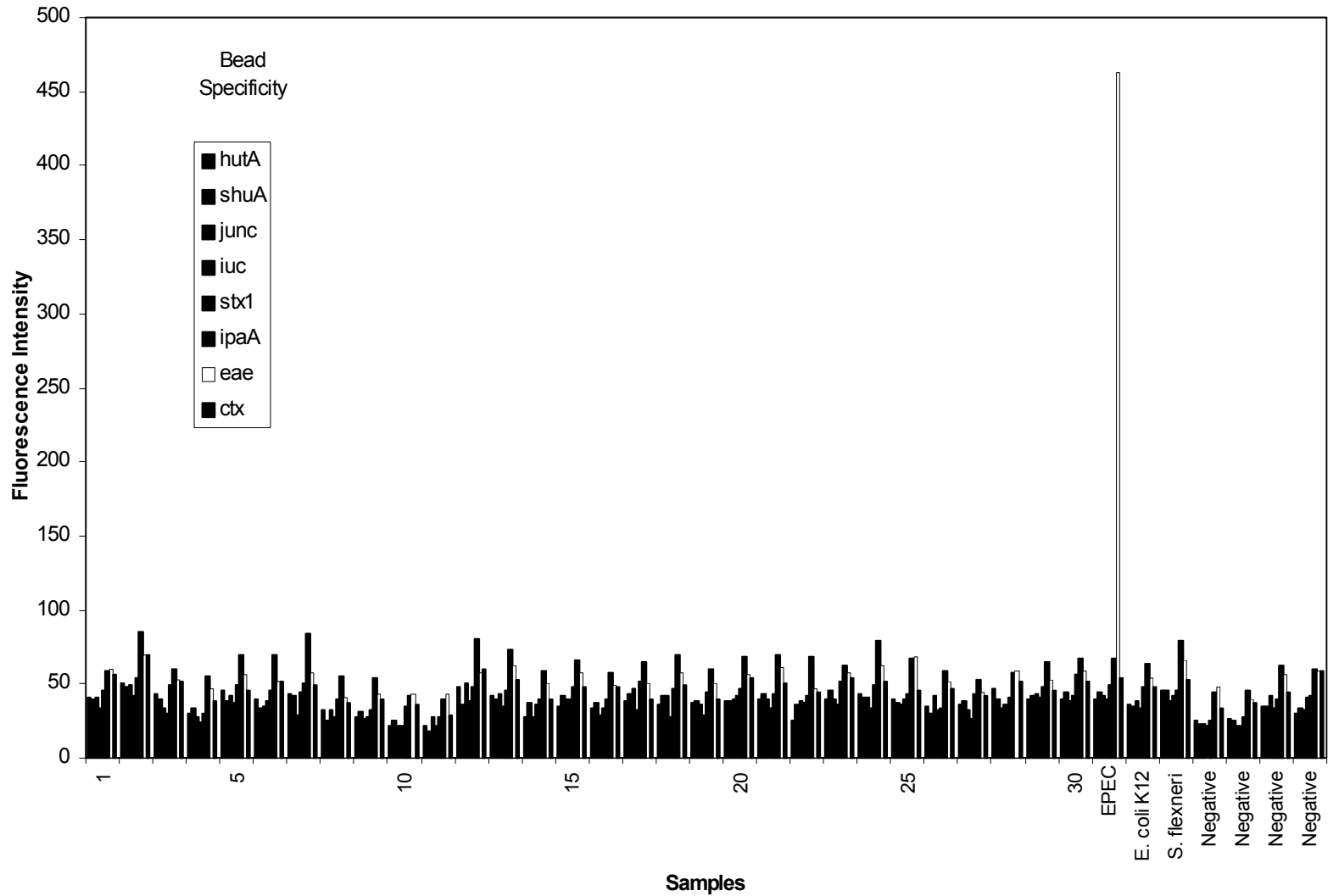
Temperature study



Are Pathogenicity Islands Ubiquitously Distributed in the Environment?

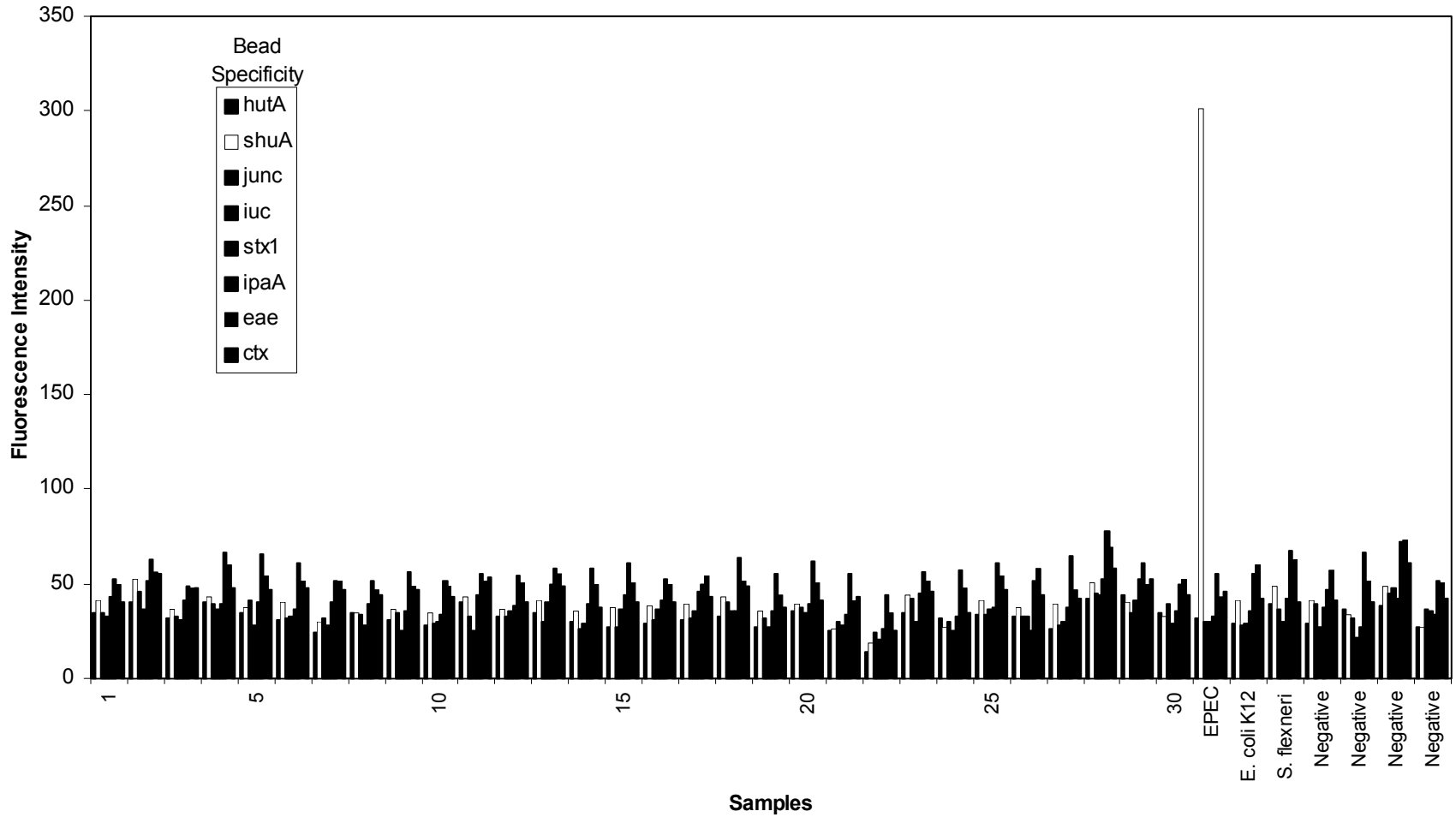
- 30 Dormitory rooms sampled
 - Surface swabbed (3 surfaces per room)
 - Inoculated into broth
- Amplified with 4 different PI primer sets
- Hybridized with 8 different probes at 52°C

30 Dormitory room samples eae amplification

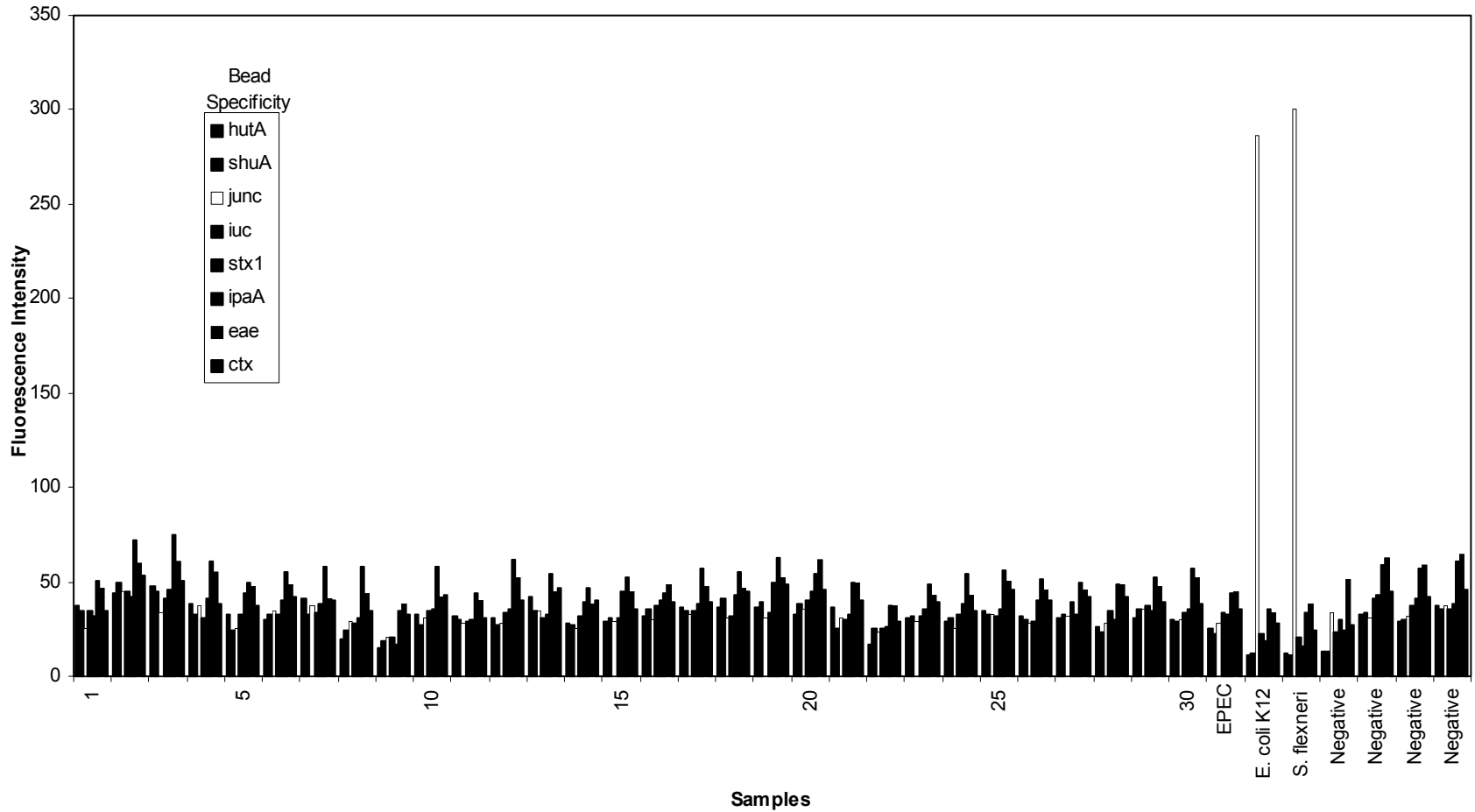


30 Dormitory room samples

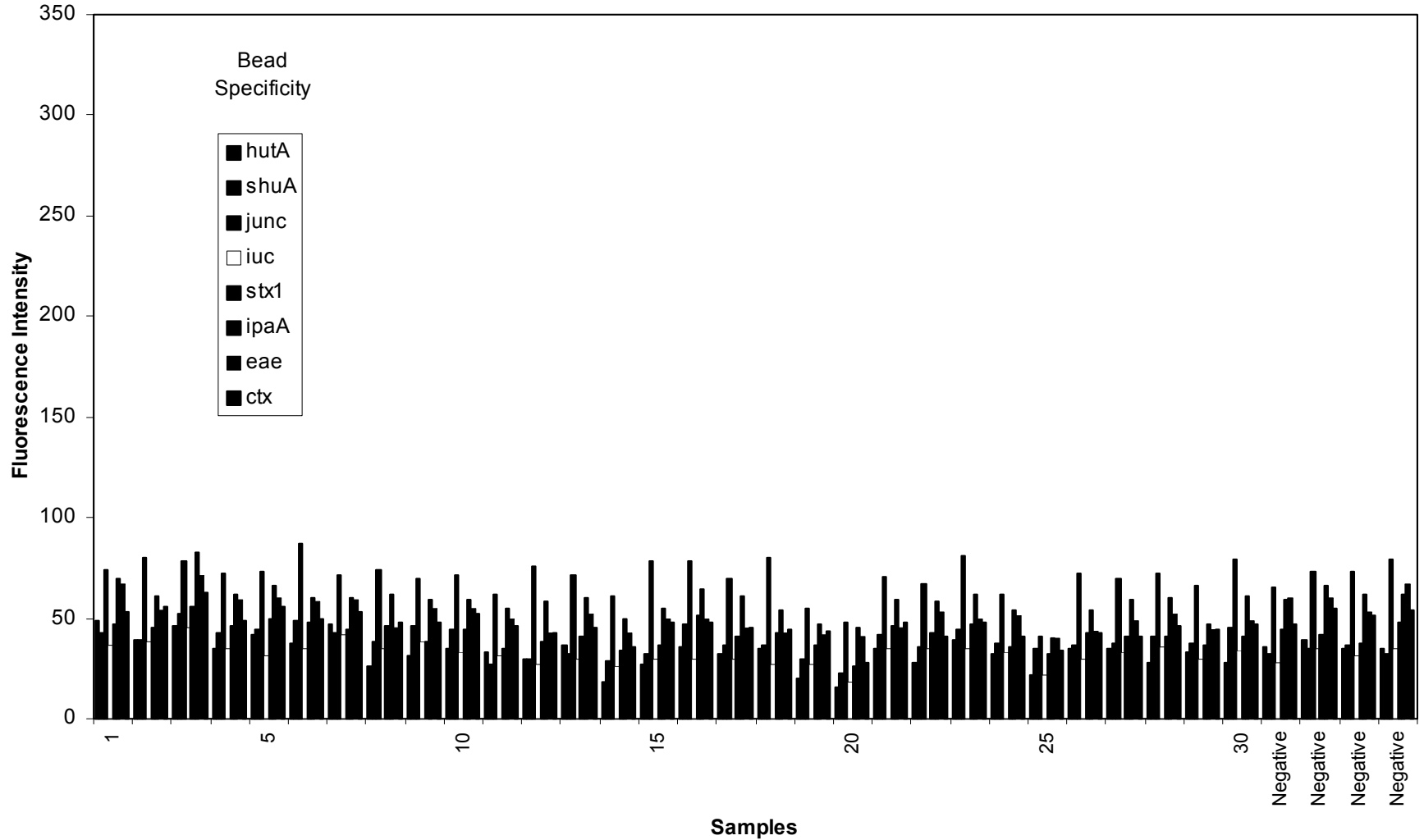
Shu amplification



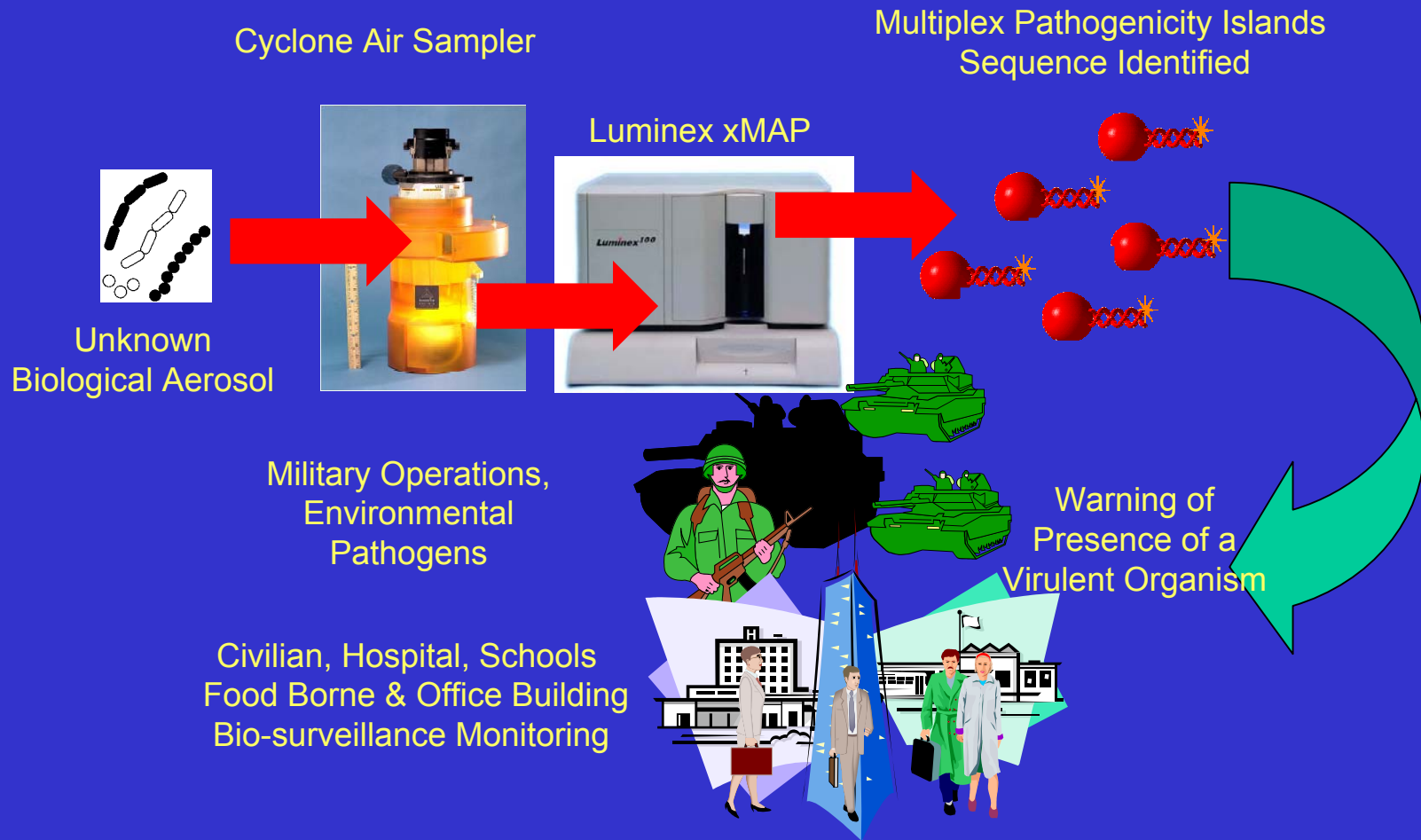
30 Dormitory room samples junction amplification



30 Dormitory room samples iuc amplification



UT-Radix Detection/Identification Platform



Conclusions

- Probes have been designed to hybridize to specific PI sequences
- The PI assay has good sensitivity
- No genomic sequences for pathogens are detected in dormitory environments, therefore pathogens are NOT distributed ubiquitously in such environments

Future Plans

- Optimize probe design to eliminate cross-reactivity thus eliminating false positives and false negatives
- Optimize amplicon design needs to be undertaken to eliminate steric effects in the assay design
- Determine maximum PI amplicons that can be simultaneously screened

THE UNIVERSITY OF TEXAS COMPONENT

