

High Throughput Method for Biosensor Substrate Screening

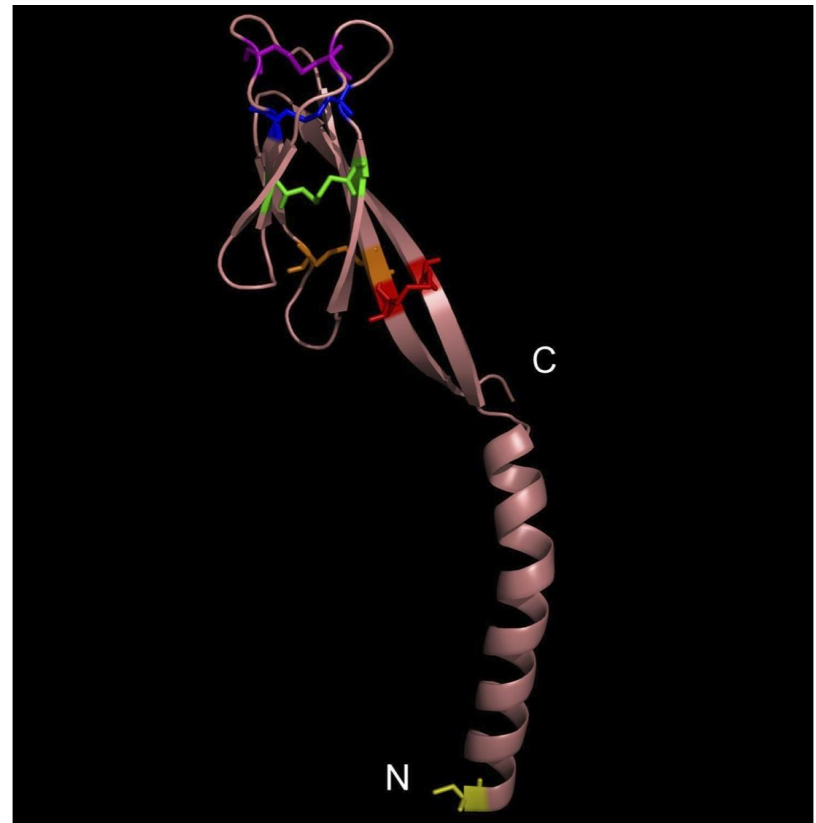
Patrick Caveney
May 7, 2013

Outline

- Background
- Need
- Fusion Proteins
- Proposed Process
- Challenges
- Future Applications

Background

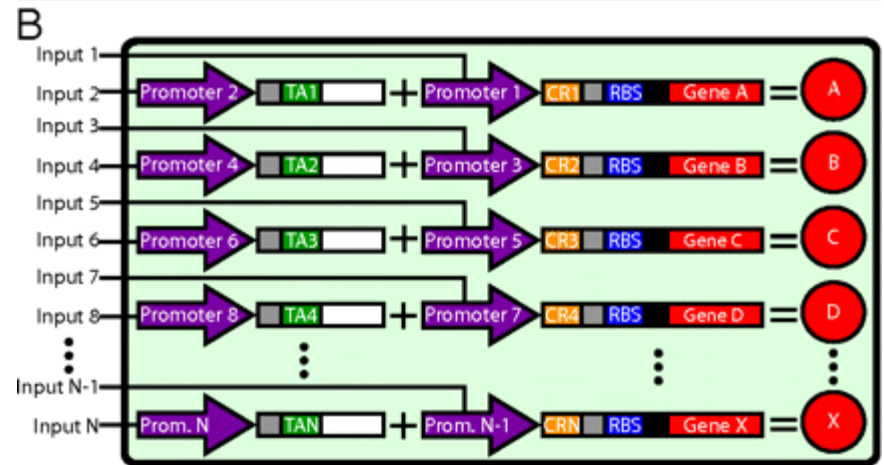
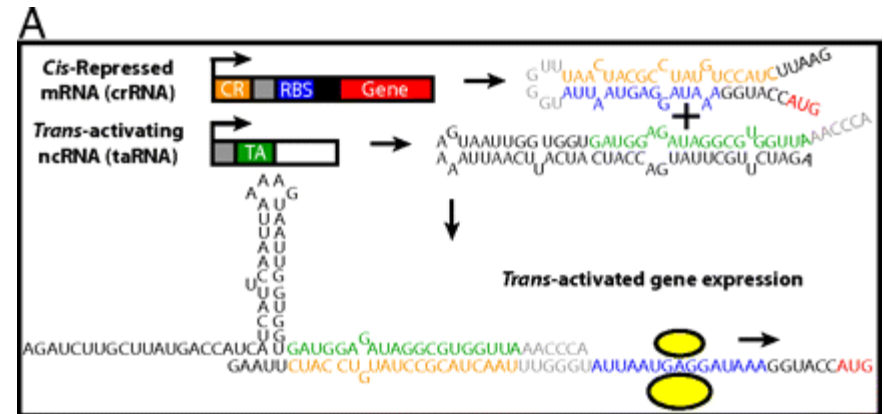
- Individual Domains
- Connected by linker regions



Igor Zhulin. "Whole Genome Reconstruction and Analysis."
Lecture. University of Tennessee. April 28, 2013.

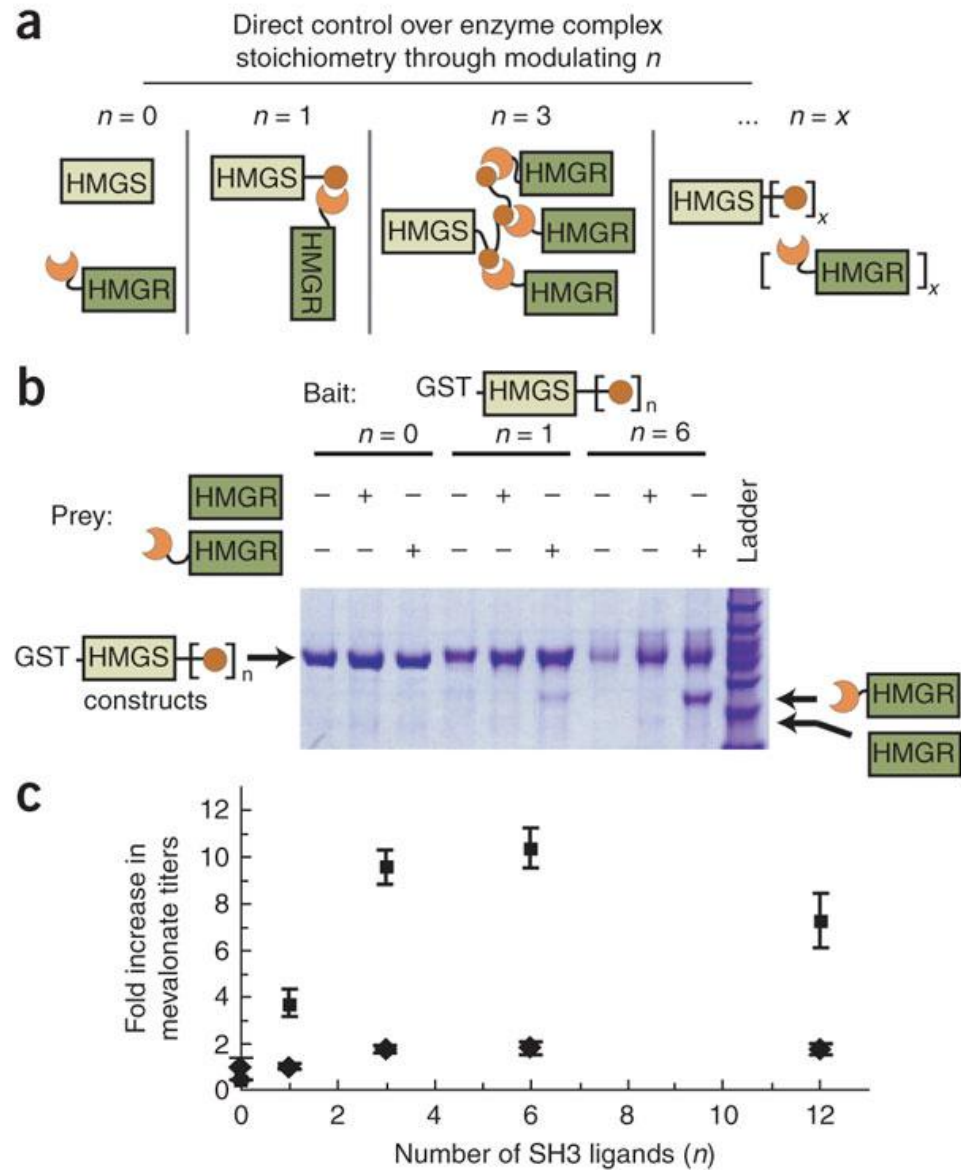
Need

- 40,000 sensors identified
- 100 characterized
- Third largest class of proteins



Fusion Proteins

- Evolution of proteins
- Resolving protein
- Creating protein scaffolds
- Tar/EnvZ

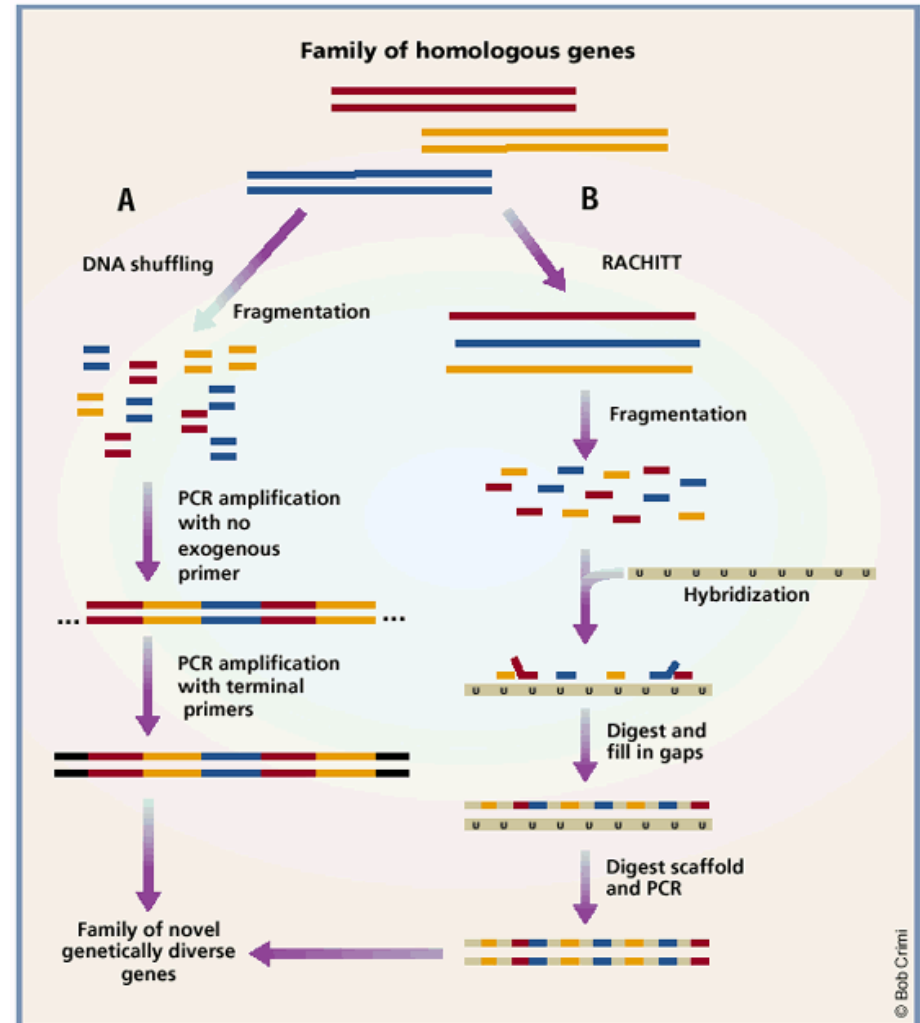


Proposed Process

- RACHITT
- emPCR
- Doping

RACHITT

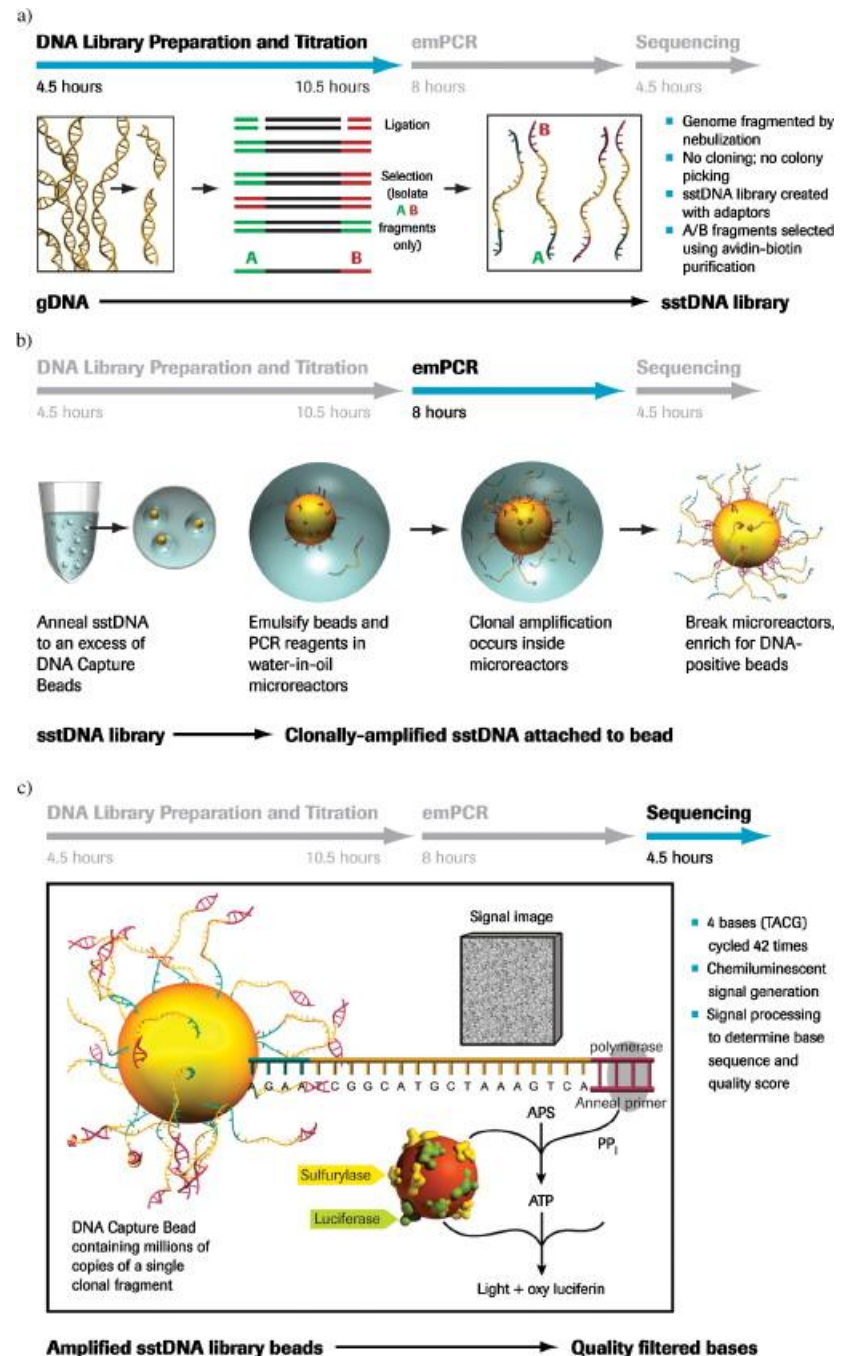
- ssDNA
- Fragmentation
- Hybridization
- Digestion
- PCR



Testing

- Isolate Mutants
- PCR each
- Divide copies
- Add crude lysate
- Dope mutants
- Observe results
- Screen the positive population

Burkhard Ziebolz and Marcus Droege. "Toward a new era in sequencing." *Biotechnology Annual Review*. 13 (2007): 1-26. accessed April 30, 2013. doi: 10.1016/S1387-2656(07)13001-5



Challenges

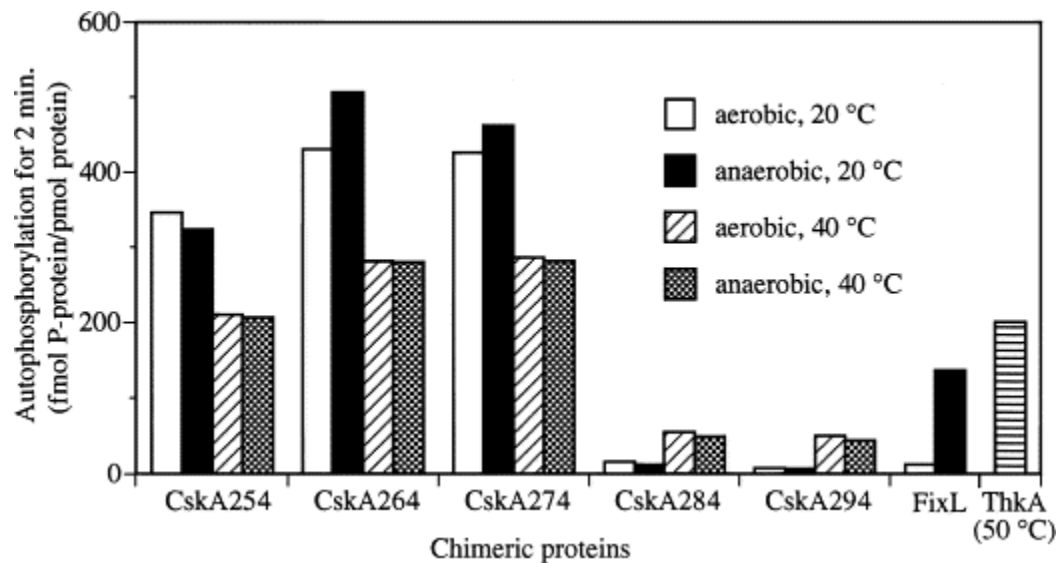
- Fusing Proteins
- Reproducibility
- Standard Module
- Cost
- Number of Tests

Fusing Proteins

- Lack of characterized sequences
- RACHITT
- Protein evolution

Reproducibility

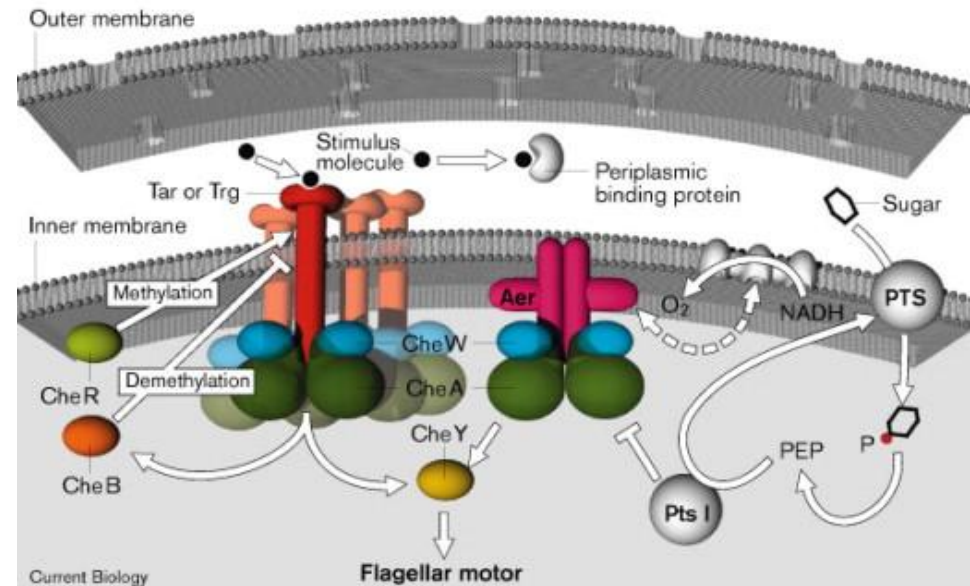
- FixL and O₂ sensors
- Procedure is a screen



Hideyuki Kumita, Seiji Yamada, and Yoshitsugu Shiro. "Chimeric sensory kinases containing O₂ sensor domain of FixL and histidine kinase domain from thermophile." *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*. 1646 (2003): 136-144 accessed April 30, 2013. doi: 10.1016/S1570-9639(02)00555-1

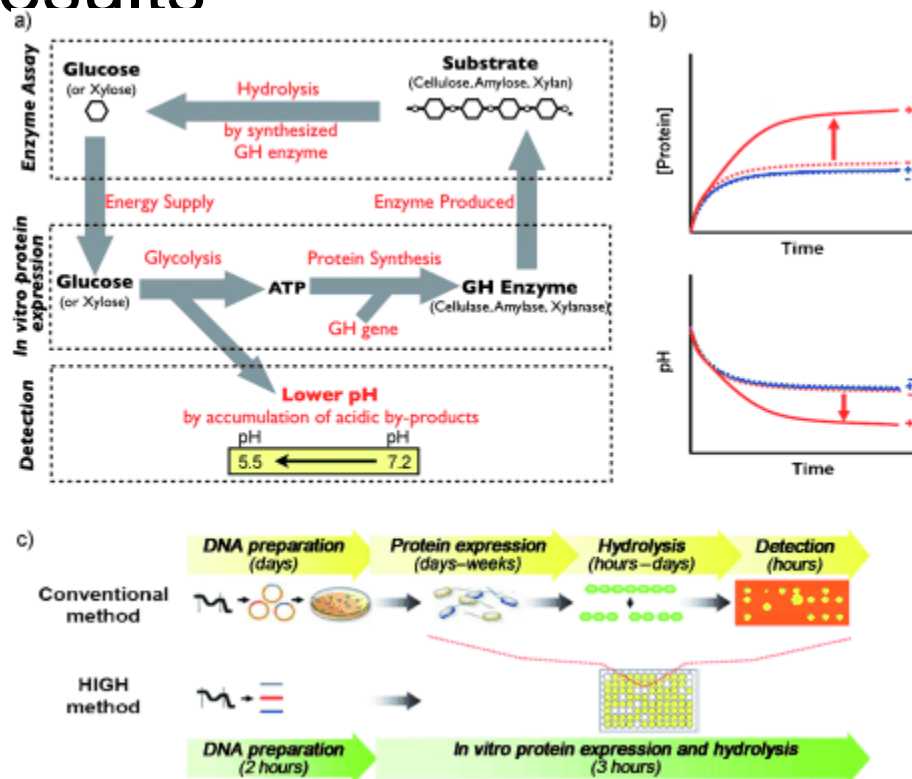
Standard Module

- Transmembrane
 - Sensor domain, EnvZ, OmpR, OmpC, GFP
- Cytoplasmic
 - Sensor domain TodS, TodT, TodX, GFP



Cost

- Cell Free is more expensive
- Faster results



Number of Tests

- Variety of mutants
- Variety of substrates
 - delimited by environment
 - delimited by substrate type
- Mitigated by high throughput

Future Applications

- Characterizing sensors
- Synthetic control
- Bacterial environments a priori

Thank you.

References

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Questions?

Single Genome Sequencing

Suzan Yilmaz and Anup K Singh. "Single cell genome sequencing." *Current Opinion in Biotechnology*. 23 (2012): 437-443 accessed May 6, 2013 doi: 10.1016/j.copbio.2011.11.018

