Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Estimating P-Coverage of Biosynthetic Pathways in DNA Libraries and Screening by Genetic Selection: Biotin Biosynthesis in the Marine Microorganism *Chromohalobacter*

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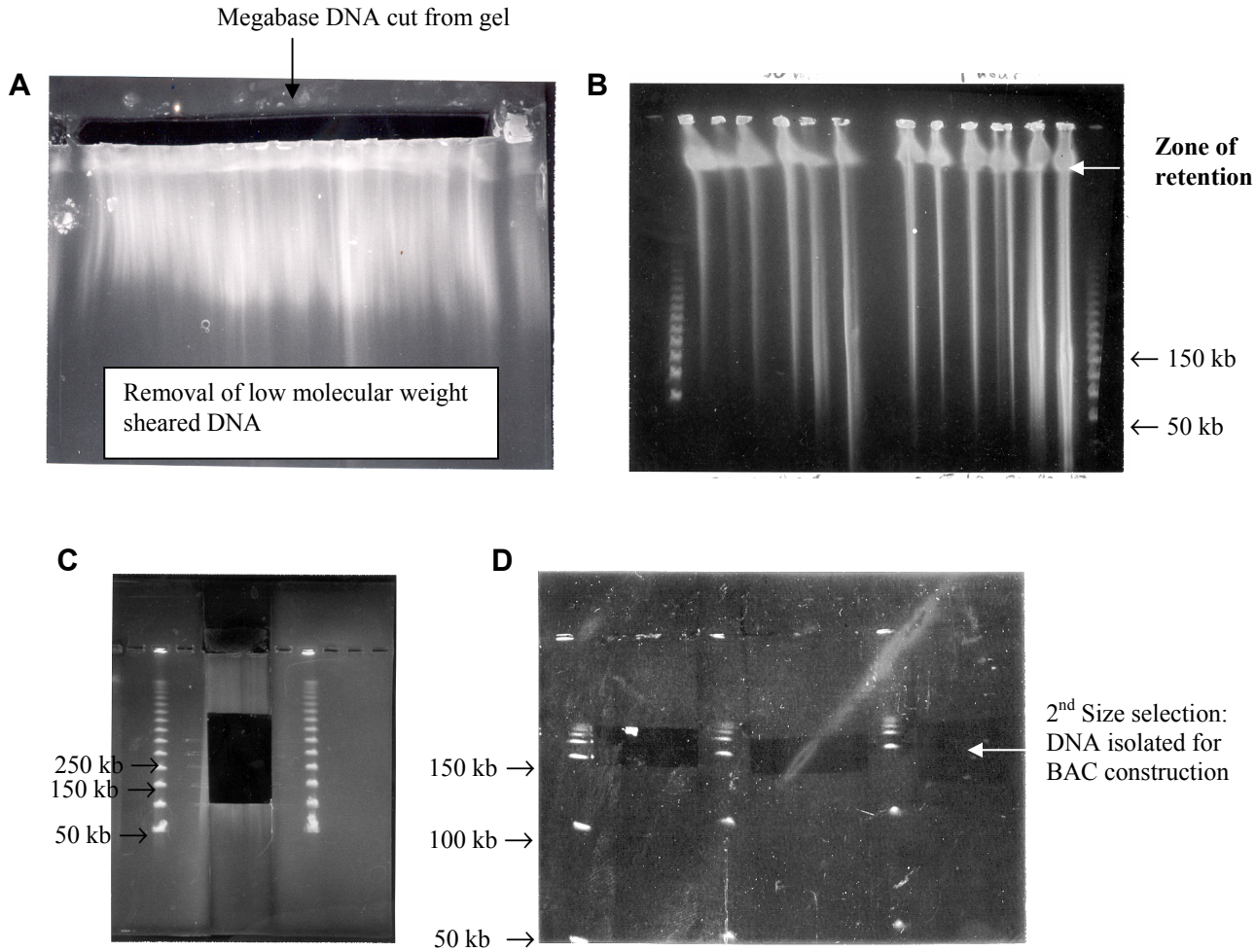
Representative gels on Hon6 library construction



(A) High molecular weight genomic DNA isolated from Hon6. (B) Optimization of restriction enzyme digestion with *Hind* III (C) First size fractionation with *Hind* III cut DNA (D) second size selection to eliminate small fragments with altered CHEF gel conditions

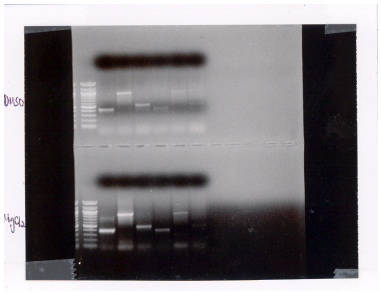
Description of Biotin Degenerate Probe Design

Biotin biosynthetic pathway degenerate probes were designed using multiple sequence alignments to search for regions of high homology. Protein sequences were downloaded from the NCBI website (http://www.ncbi.nlm.nih.gov.). Sequences (10) were chosen for for BioA, 17 sequences for BioB, 16 sequences for BioC, 16 sequences for BioD, 16 sequences for BioF, and 16 sequences for BioH. Sequences were chosen from multiple species and strains, which were identified by BLAST search analysis (http://ncbi.nlm.nih.gov/blst/Blst.cgi). Multiple sequence alignment was performed on the Baylor College of Medicine Multiple sequence alignment tool using Clustal X (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). Highly conserved sequenced were identified and manually reverse-translated into degenerate codons. Sequences were chosen which minimized degeneracy and maximized specificity. Oligonucleotides were ordered from the GTL (Texas A&M University, Gene Technological Lab). These oligonucleotides were used as PCR primers.

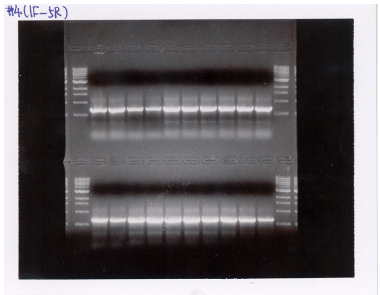
Gel pictures and expected size of PCR products

BioA: 1F-2R 420bp
BioB: 1F-5R 678bp
BioC: 6F-7R 324bp
BioD: 1F-3R 450bp
BioF: 10F-11R 225bp

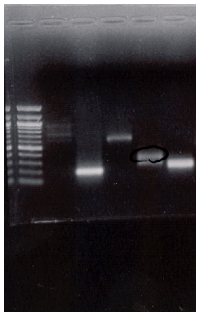
BioA: 420bp (2nd lane)(100bp ladder used)



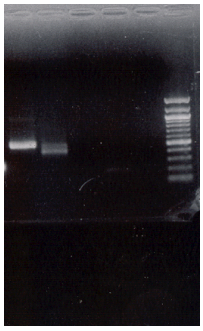
BioB: 678bp (1kb ladder used)



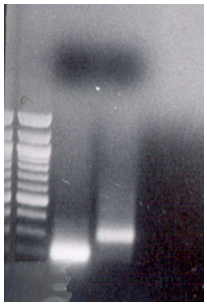
BioC: 324bp (5th lane) (100bp ladder used)



BioD: 450bp (2nd lane)(100bp ladder used)

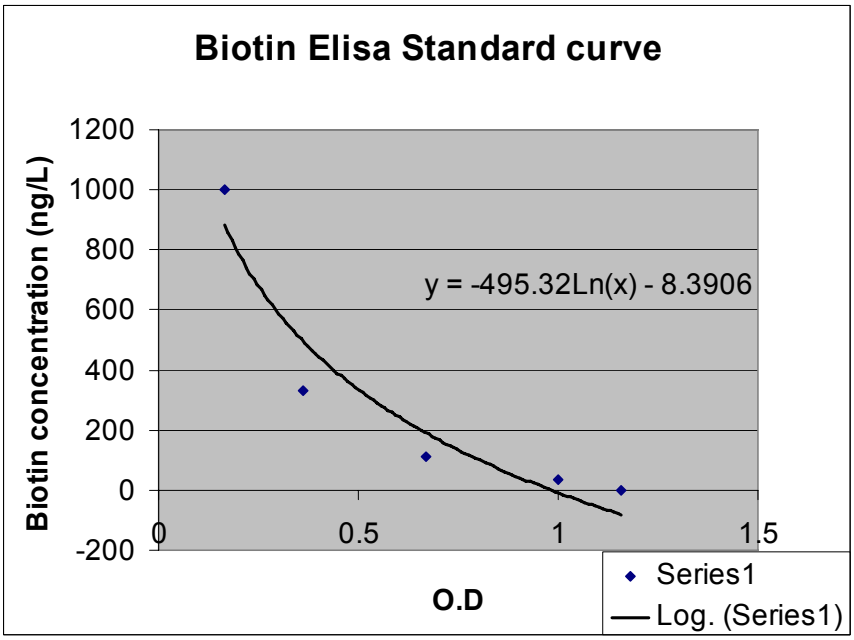


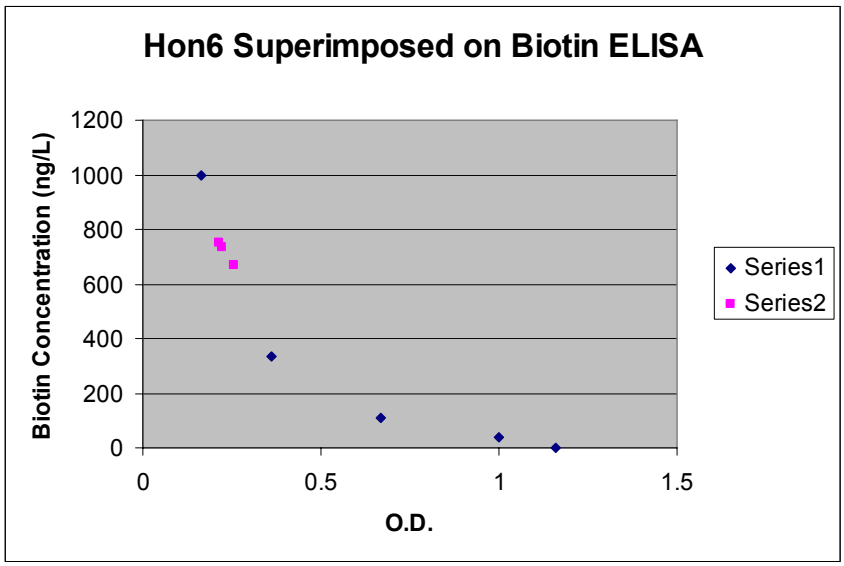
BioF:225bp (2nd lane)(100bp ladder used)



Biotin ELISA Tabulated Results

Standards and samples	Biotin Concentration (ng/L)	O.D.
STD #1	0	1.158
STD #2	37	1.000
STD #3	111	0.668
STD #4	333	0.362
STD #5	1000	0.165
Hon6#2-1	753	0.215
Hon6#2-2	732	0.224
Hon6#2-3	667	0.256





Photograph of the Hon6 strain cultured on a marine agar plate