Using Q-PCR and Host-Specific *Bacteroides* 16S rRNA Molecular Markers to Quantify Sources of Fecal Contamination in a Reclaimed Water Irrigation Pond

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USDA-ARS, US Arid-Land Agricultural Research Center
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Agricultural Research Service

Pacana Park

Maricopa, Arizona: Population growth from < 2,000 in 1999 to 33,000 in 2007

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Maricopa, Arizona: Population growth from < 2,000 in 1999 to 33,000 in 2007

Pacana Park: 19-acre recreational area

Irrigation is 100% tertiary-treated municipal effluent

Pond: stocked for recreational fishing (Tilapia, catfish)

Monitoring: twice monthly since September 2006

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Fecal Bacteria Over 12-Month Period

Sampling Period	*E. coli* (CFU 100 mL^{-1})	
	Pond	Irrigation
Fall 2006	NM	NM
Winter 2007	17	7
Spring 2007	18	9
Summer 2007	12	9

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Fecal Bacteria Over 12-Month Period

Sampling Period	*E. coli* (CFU 100 mL^{-1})		*Salmonella* sp. (CFU 100 mL^{-1})	
	Pond	Irrigation	Pond	Irrigation
Fall 2006	NM	NM	2.5×10^4	2.1×10^4
Winter 2007	17	7	1.7×10^4	0.9×10^4
Spring 2007	18	9	1.5×10^4	1.7×10^4
Summer 2007	12	9	2.1×10^4	1.3×10^6

Source Tracking Using Host-Specific *Bacteroides* 16s rRNA Molecular Markers

Genus *Bacteroides*: exclusively found in feces, rumens, and other cavities of humans and other animals

16s rRNA genetic markers have been applied to evaluate host-specific fecal pollution

Layton et al. (2006) *Applied and Environmental Microbiology*, 72: 4214-4224

AllBac and HuBac: Taq-man probe assay

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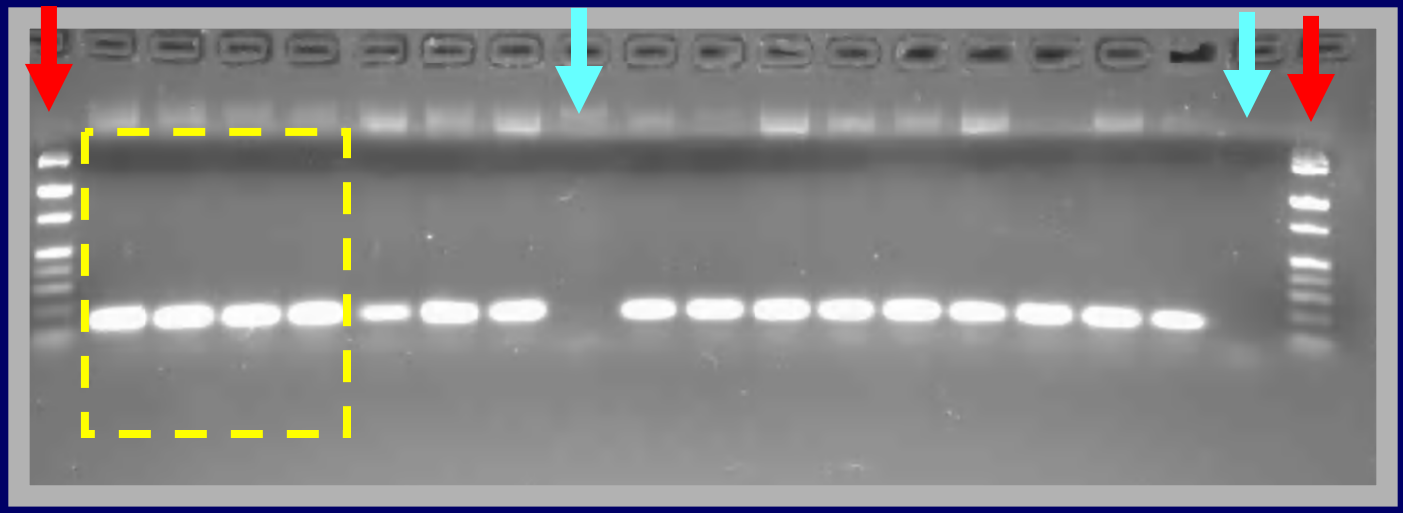
Results: AllBac Primers



Expected size: 106 bp

Tested vs: Human, Fish, Dog, Bird, Bovine

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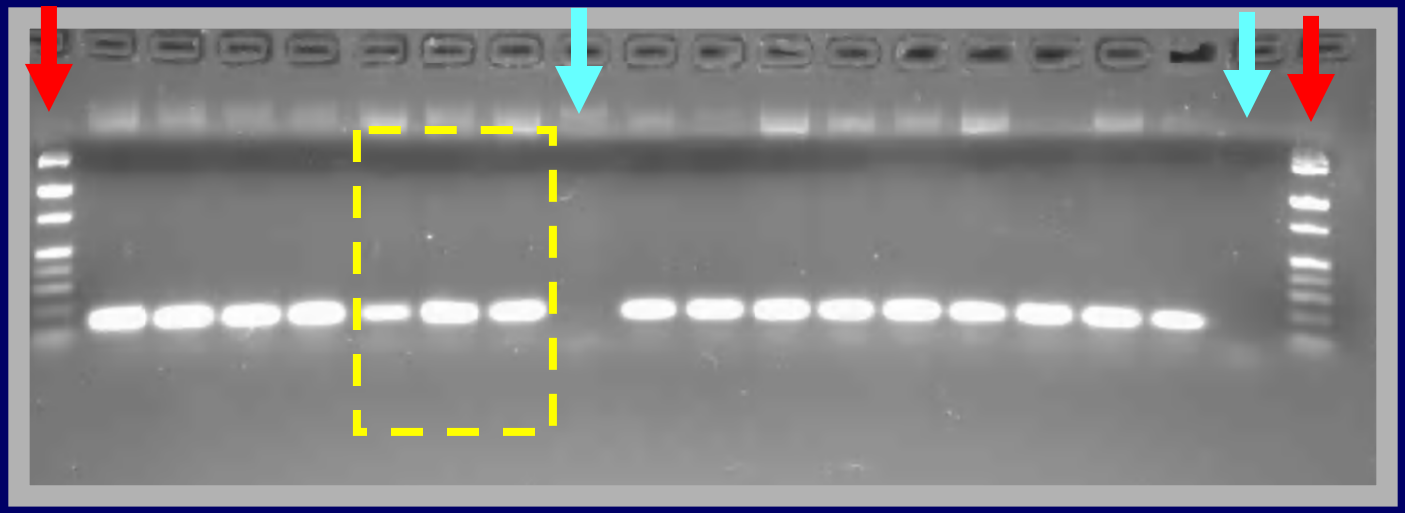
Results: AllBac Primers



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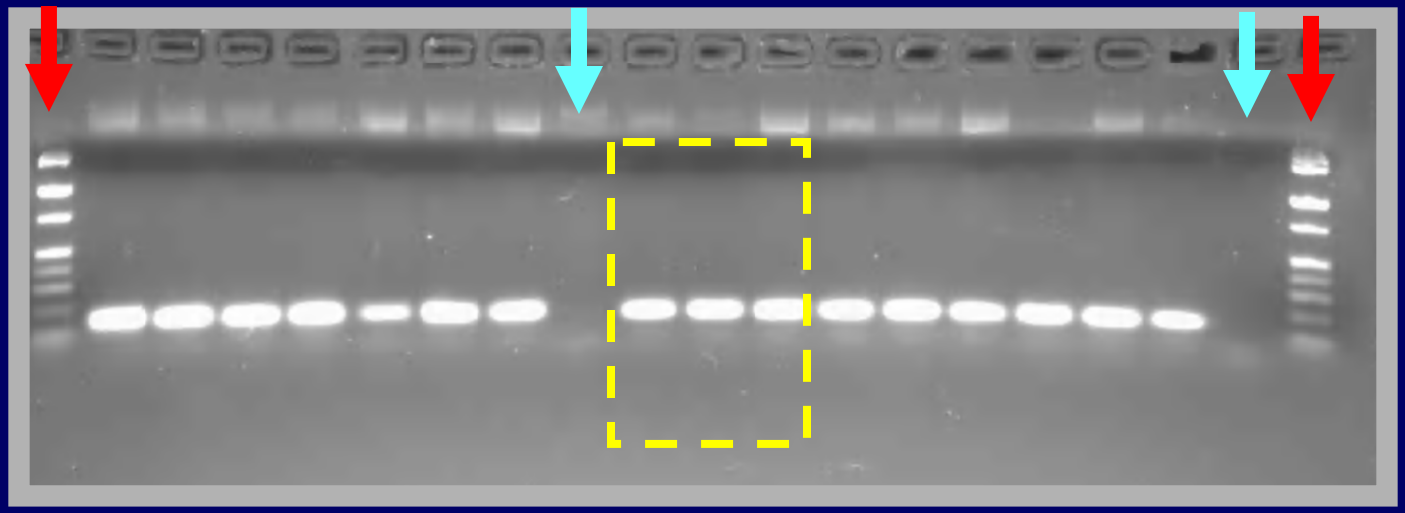

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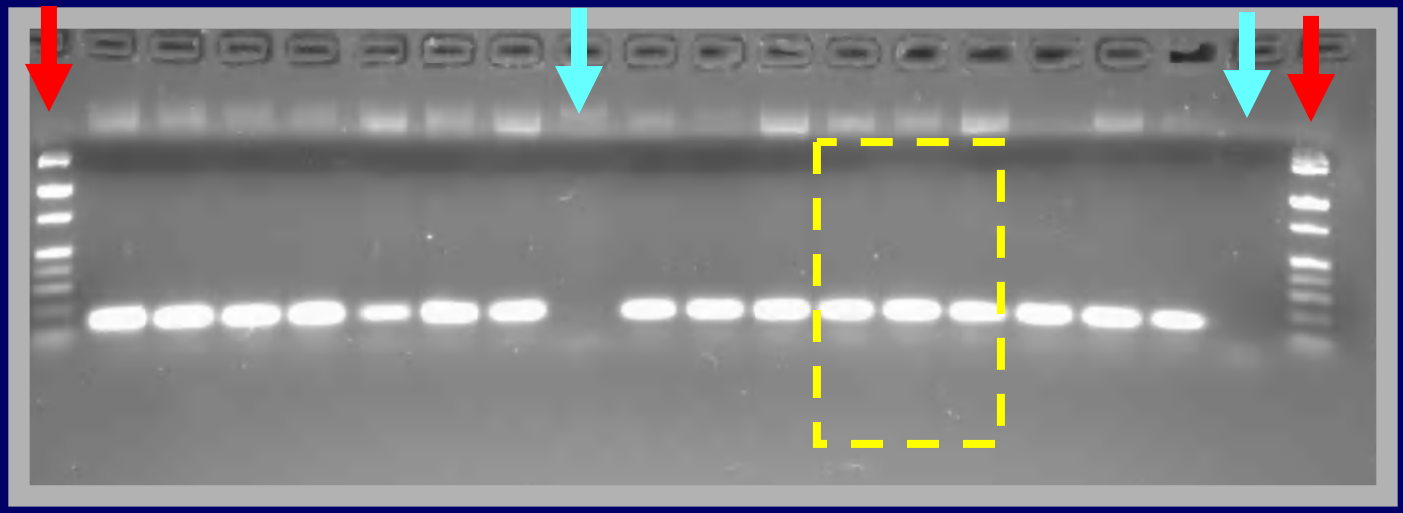

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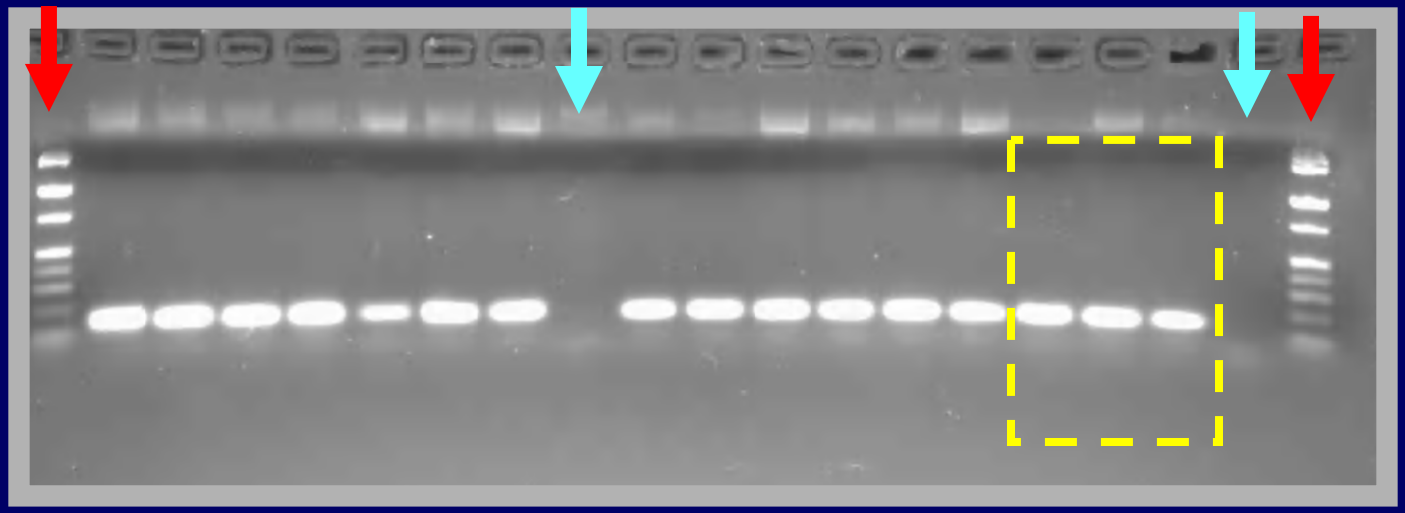

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Standard Curve with each potential source:
$r^2 > 0.995$, slope -0.30 to -0.33

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All Bacteroides, Spring 2006

Sampling Date	Probe Targets 100 mL^{-1}	
	Pond	Irrigation
March 01	3.0×10^6	9.6×10^5
March 29	1.5×10^7	1.5×10^6
April 26	8.7×10^6	1.1×10^6
May 27	4.5×10^5	< LD
June 20	1.5×10^6	4.2×10^5

Limit of detection: 5.8×10^4 targets 100 mL^{-1}

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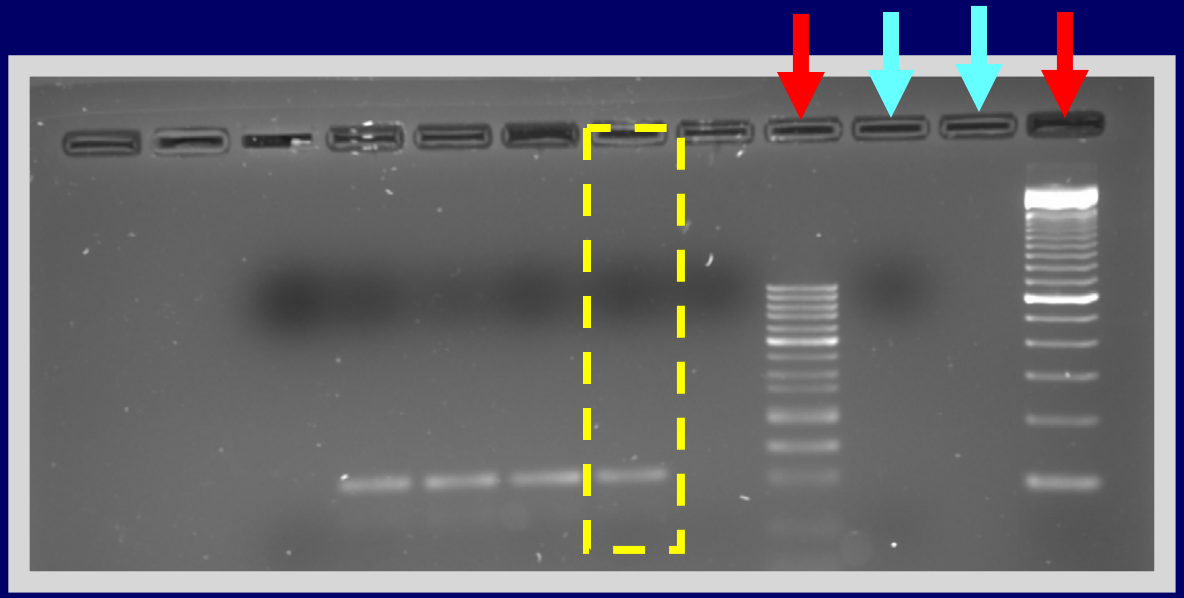
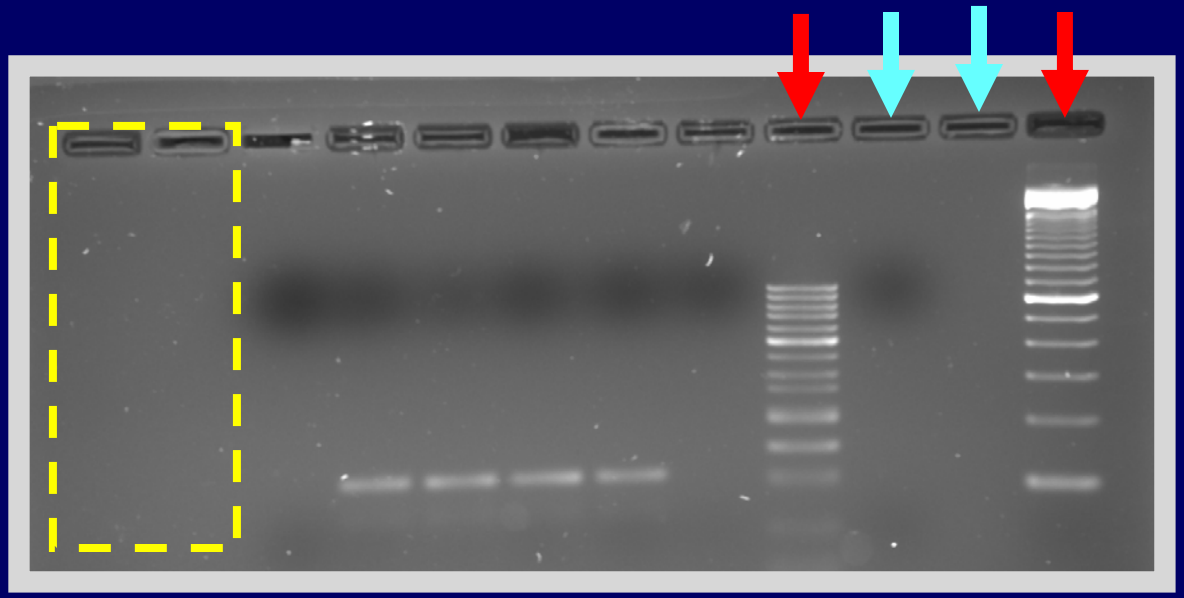
Results: HuBac Primers



Expected size: 116 bp

Tested: Human, Bird, Dog, Fish

Results: HuBac Primers



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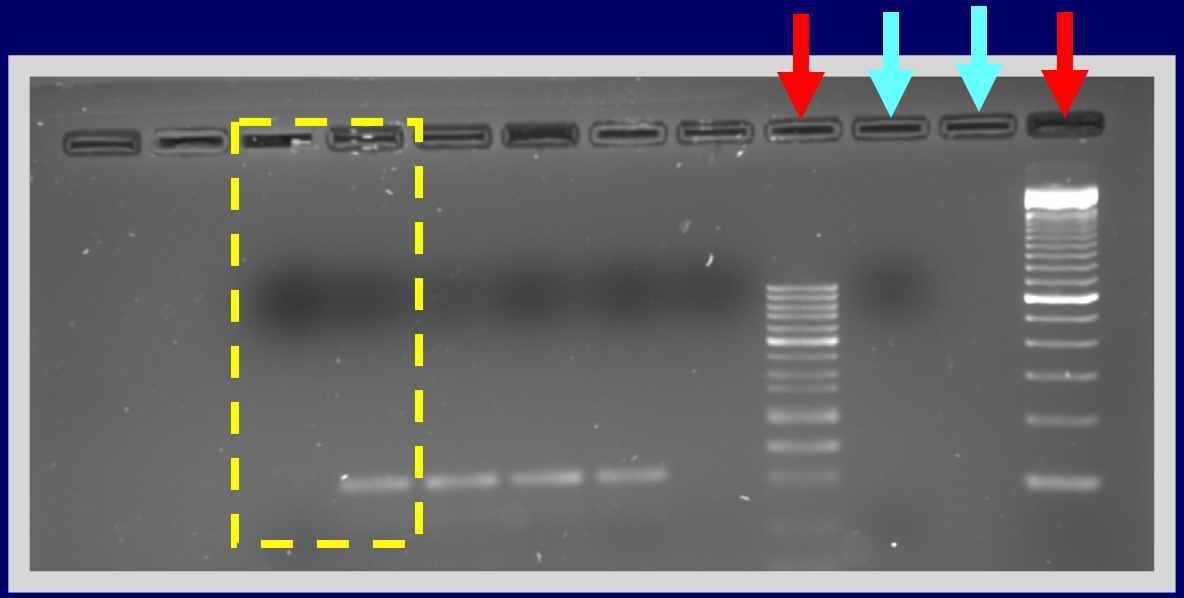

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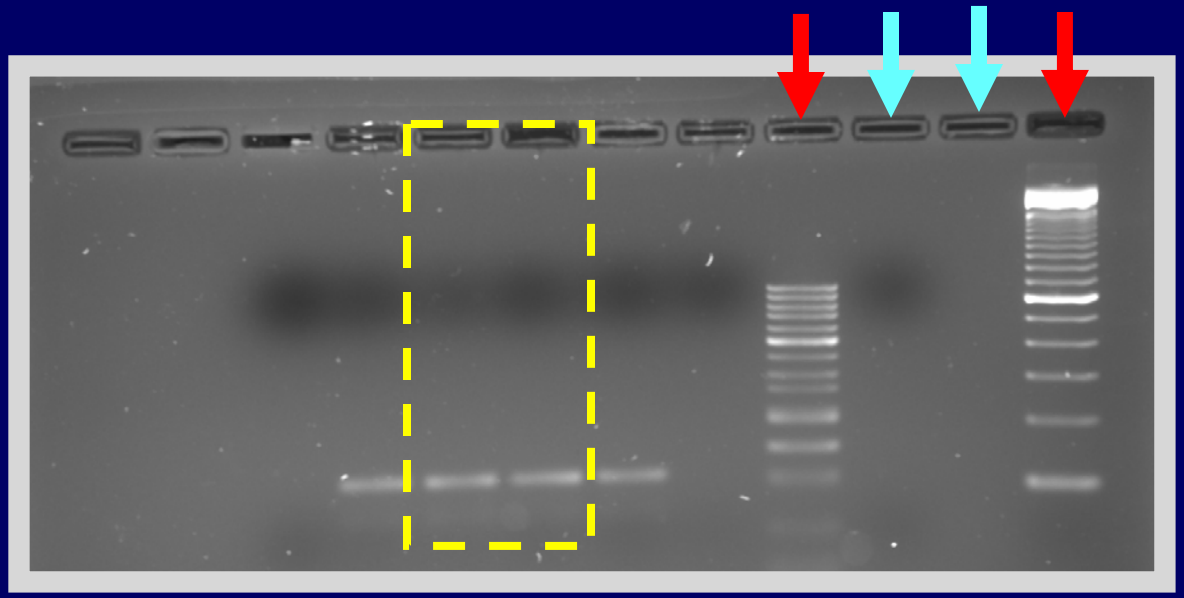

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Human vs. Fish (Tilapia): Cloning and DNA sequencing

Exact matches of HuBac primer and probe to human and fish fecal DNA

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Q-PCR results with HuBac?

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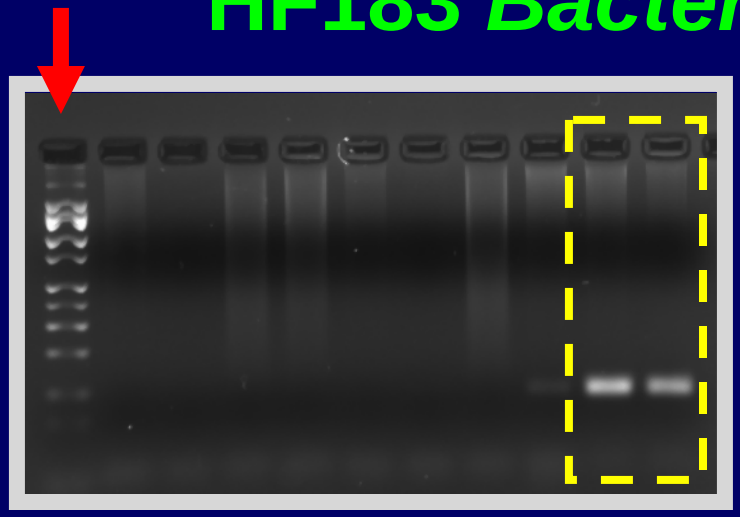
SYBR Green Assay: Human-specific HF183 *Bacteroides* 16S rRNA*



Amplicon: 82 bp

Tested: Human, Bird, Bovine, Dog, Fish

***Seurinck et al. (2005)**
Environmental Microbiology
7(2):249-259

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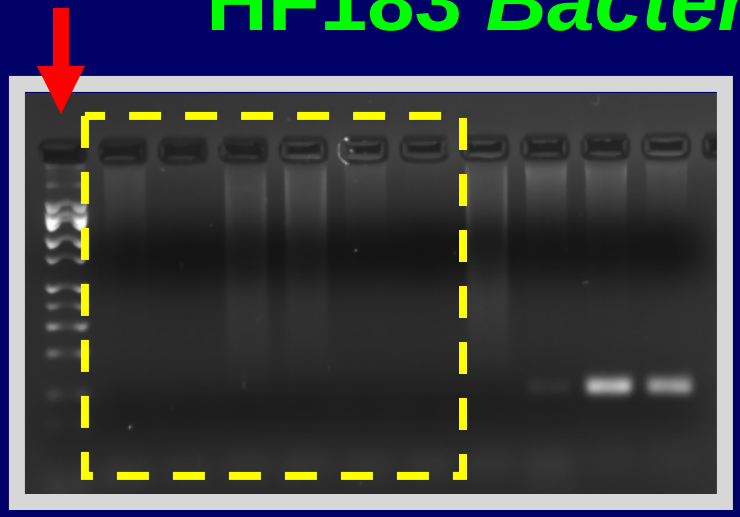

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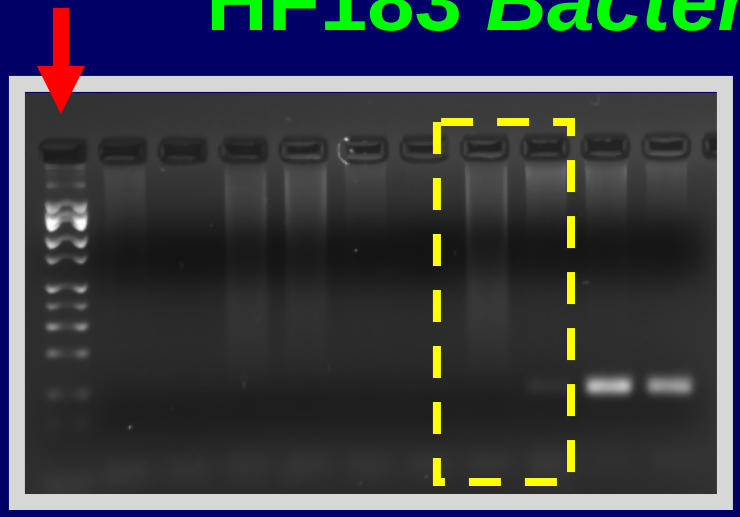

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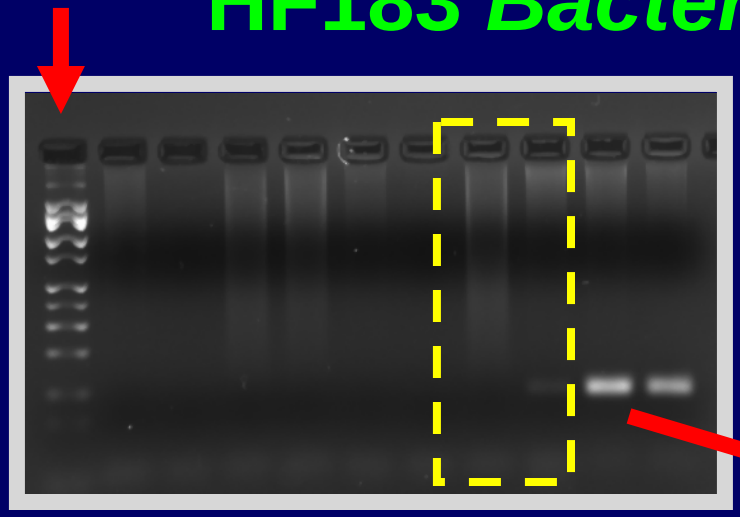

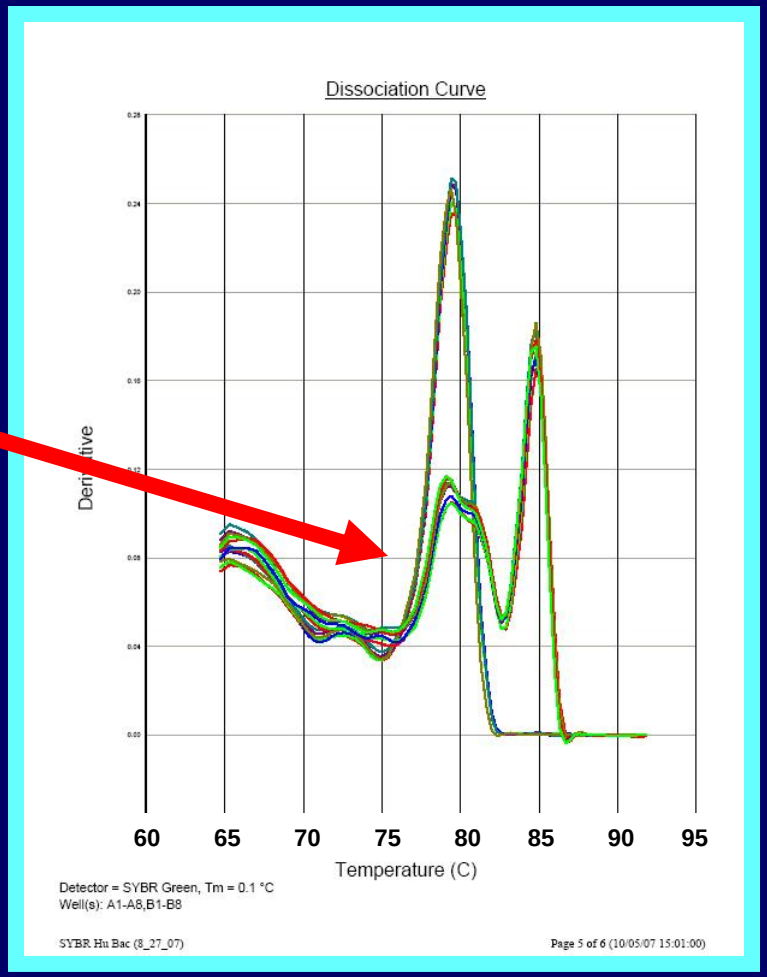

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Melting Temp (Human): 78.4º C

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Human Bacteroides, Spring 2006

Sampling Date	*Bacteroides* Markers 100 mL^{-1}	
	Pond	Irrigation
March 01	3.4×10^2	2.1×10^2
March 29	1.0×10^3	3.4×10^1
April 26	8.8×10^1	6.6×10^2
May 27	3.1×10^2	ND
June 20	9.8×10^1	ND

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Human Bacteroides, Spring 2006

Sampling Date	*Bacteroides* Markers 100 mL^{-1}	
	Pond	Irrigation
March 01	3.4×10^2	2.1×10^2
March 29	1.0×10^3	3.4×10^1
April 26	8.8×10^1	6.6×10^2
May 27	3.1×10^2	ND
June 20	6.8×10^1	ND

Limit of detection: 2.2×10^6 markers 100 mL^{-1}

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What have we learned from the Pacana Park source tracking study?

An excellent example of the extreme importance of testing all known possible fecal inputs for non-target amplification in Q-PCR source tracking studies

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Limit of detection for Taq-Man probe PCR = 2 orders of magnitude lower than SYBR green real-time PCR

Solution: fish-specific primers and probe*

* Kabiri-badr et al., American Society for Microbiology General Meeting, Boston, MA, June 1-5, 2008

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