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Fall 10-2020

Tools for Genotyping of Nicotine-Seeking Zebrafish

Emma Kennedy
DePauw University

Aziza Shemet Pitcher
DePauw University

Sabrina Krause
DePauw University

Autumn McDaniel
DePauw University

Molly Ruggles
DePauw University

See next page for additional authors

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Recommended Citation

Kennedy, Emma; Shemet Pitcher, Aziza; Krause, Sabrina; McDaniel, Autumn; Ruggles, Molly; and Schneider, Henning, "Tools for Genotyping of Nicotine-Seeking Zebrafish" (2020). *Annual Student Research Poster Session*. 35.

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Authors

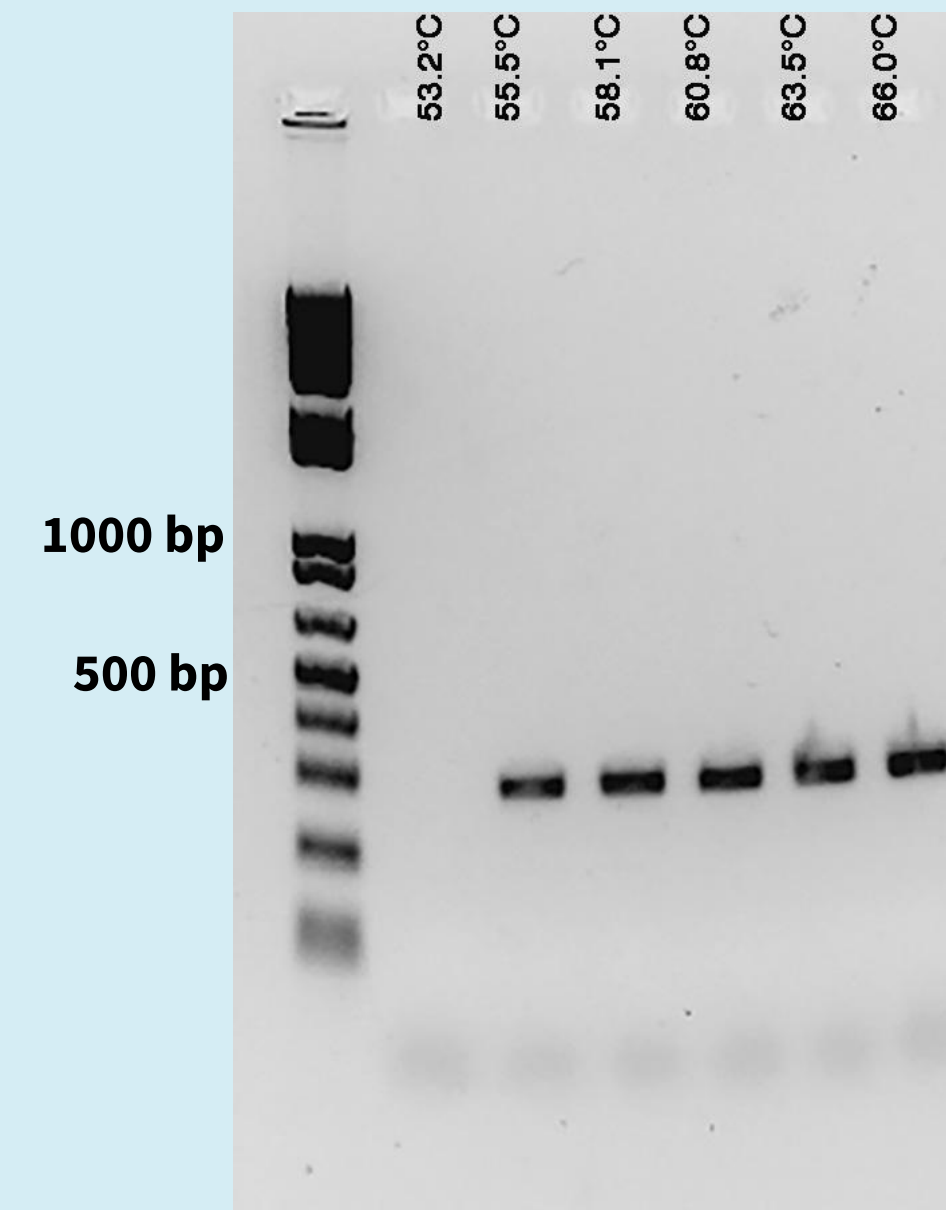
Emma Kennedy, Aziza Shemet Pitcher, Sabrina Krause, Autumn McDaniel, Molly Ruggles, and Henning Schneider

PROJECT DESCRIPTION

Tobacco dependence is the number one cause of preventable diseases in the U.S. (NIH). Certain single nucleotide polymorphisms (SNPs) in genes for acetylcholine receptors (chrna) have been linked to heavy cigarette smoking in humans. Zebrafish represent an excellent model organism for studies of nicotine use behavior because of behavioral, developmental and genetic tools that are available. We developed a protocol for analyzing single nucleotide polymorphisms (SNPs) in genes of individual zebrafish that have been identified as nicotine-seekers or non-seekers in behavioral choice tests. Zebrafish genes for acetylcholine receptors (chrna3 and 5), a transcription factor (nol4lb) and a cytochrome P450 gene (cyp2y3) were studied. Results show that the applied methodology can be used to map SNPs in selected genes in individual zebrafish.

Single Nucleotide Polymorphism in chrna5

chrna5 PCR



Known SNPs in zebrafish chrna5 gene



Sequenced amplicons of zebrafish chrna5

Danio rerio cholinergic receptor, nicotinic, alpha 5 (chrna5), mRNA
 Sequence ID: [NM_001017885.1](#) Length: 1740 Number of Matches: 1 **Fish #1**
[See 1 more title\(s\)](#)

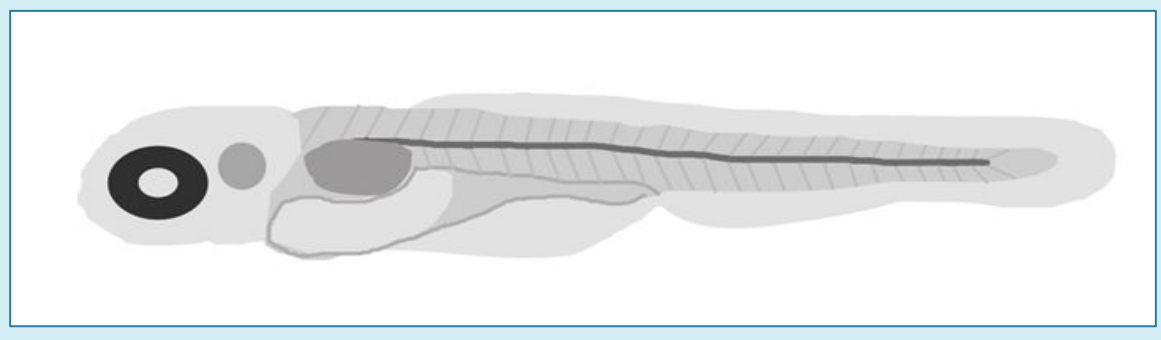
Range 1: 69 to 182 [GenBank](#) [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
206 bits(111)	1e-48	113/114(99%)	0/114(0%)	Plus/Plus
Query 106	GATCTTCTCTGTGGTTTTGCTGCTGCTGCTGATGGATCATGGTAACAGCTCTCAGCTTG	165		
Sbjct 69	GATCTTCTCTGTGGTTTTGCTGCTGCTGCTGAGGGATCATGGTAACAGCTCTCAGCTTG	128		
Query 166	GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT	219		
Sbjct 129	GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT	182		

Fish #2

Score	Expect	Identities	Gaps	Strand
200 bits(108)	6e-47	112/114(98%)	0/114(0%)	Plus/Plus
Query 101	GATCTTCTCTGTGGTTTTGCTGCTGCTGCTGATGGATCATGGTAACAGCTCTCAGCTTG	160		
Sbjct 69	GATCTTCTCTGTGGTTTTGCTGCTGCTGCTGAGGGATCATGGTAACAGCTCTCAGCTTG	128		
Query 161	GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT	214		
Sbjct 129	GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT	182		

Nicotine +



Behavior
Nicotine Seeker

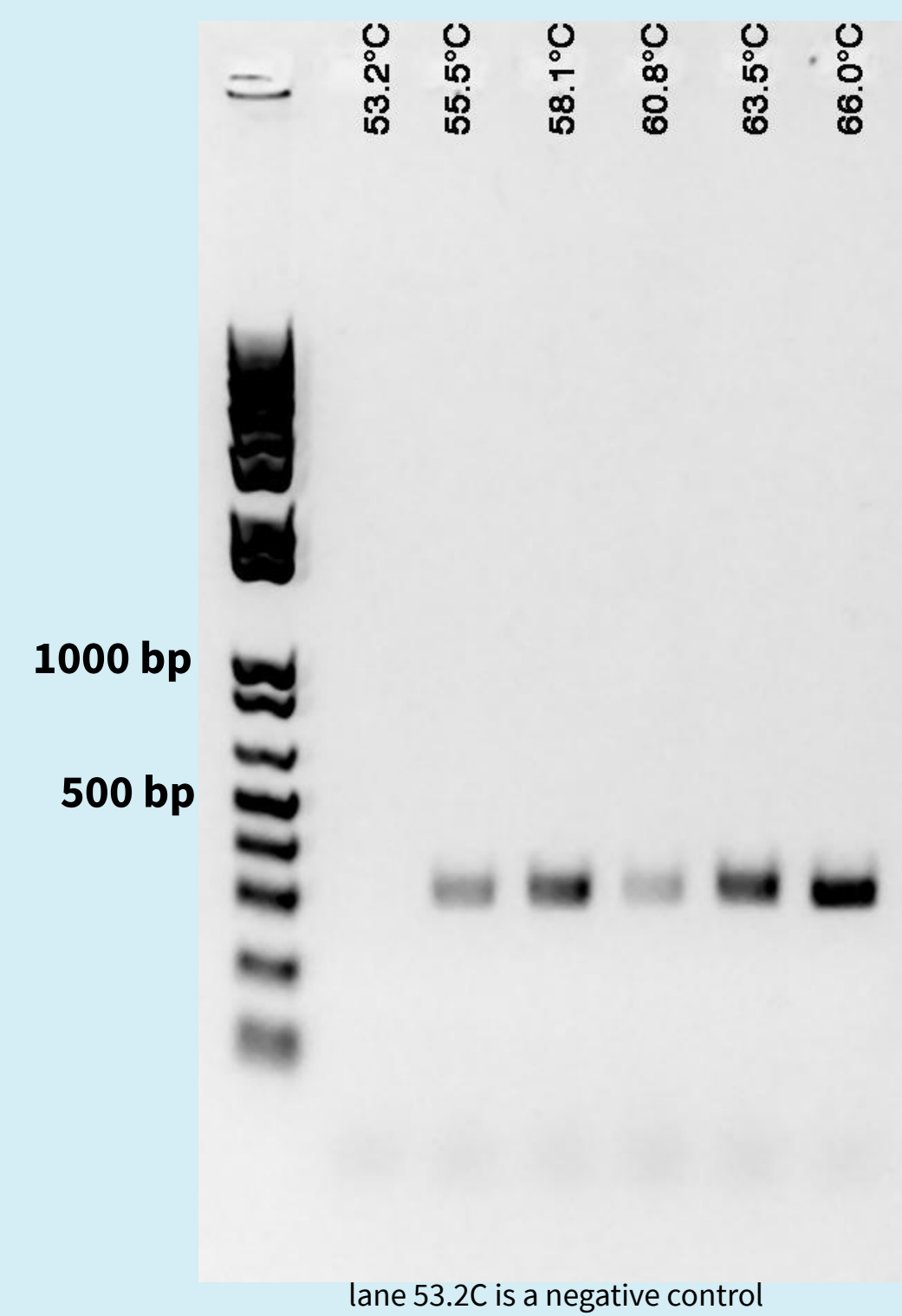
Behavior
Non-seeker

DNA Isolation,
PCR &
Sequencing

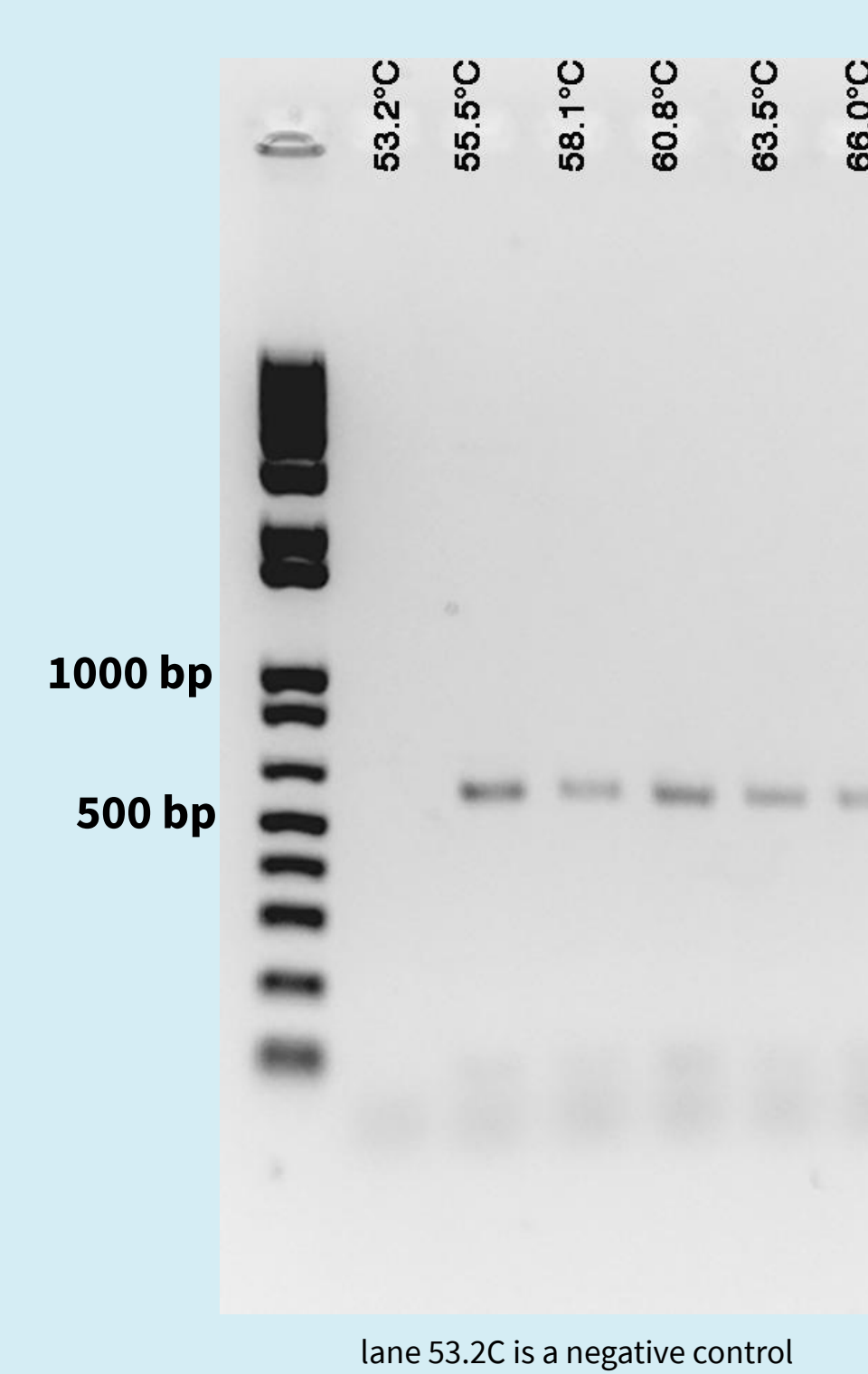


Sequence
Alignment
of SNP region

chrna3 PCR



cyp2y3 PCR



nol4lb PCR

