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### 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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**Authors**

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

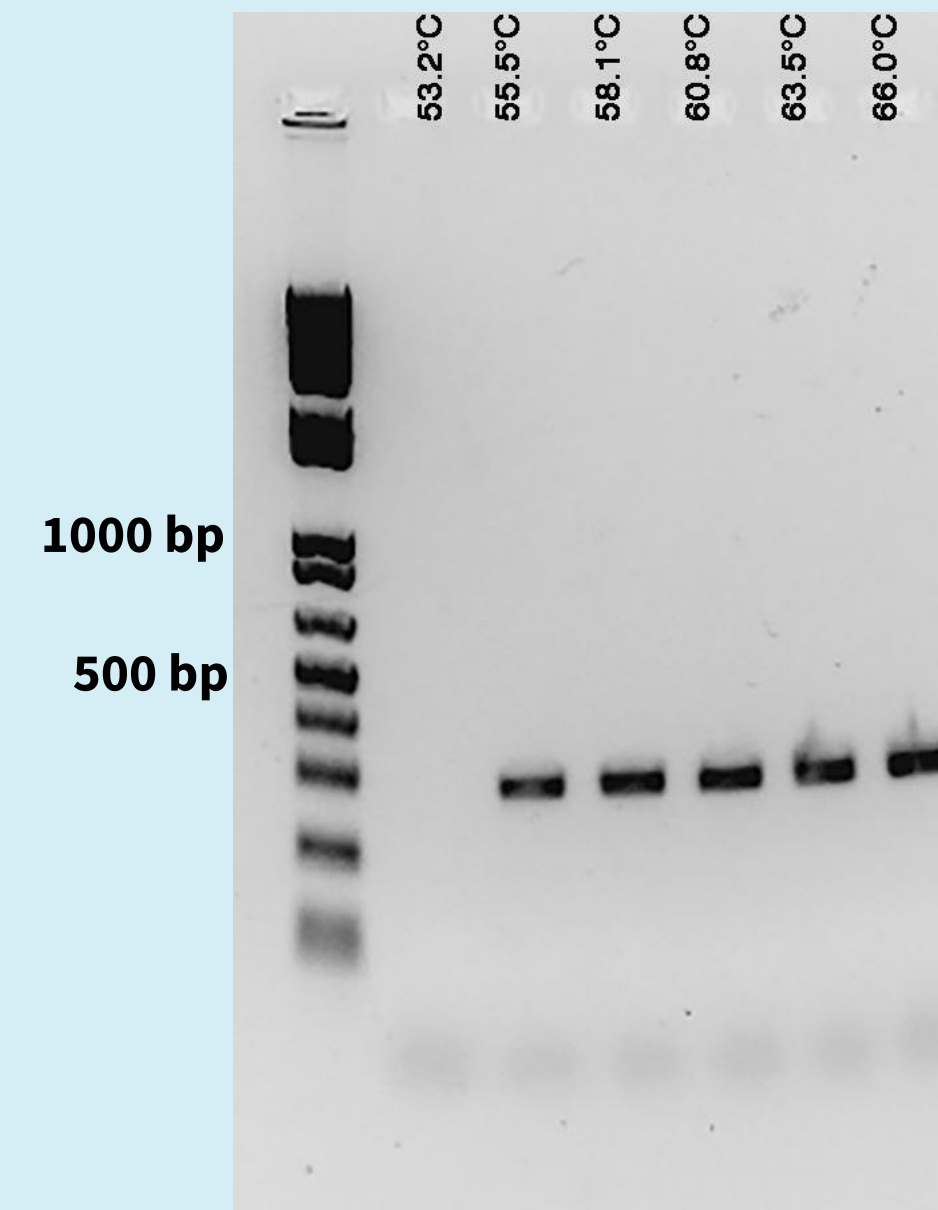


## PROJECT DESCRIPTION

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\(s\)](#) ▼ Next Match

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

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
    
```

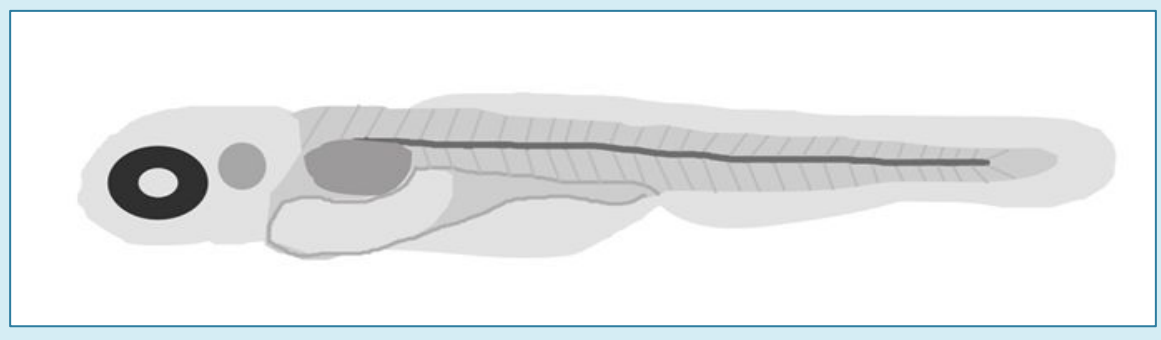
### Fish #2

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 GTCAGAGCTCTCCTCCCGTGGTCTGCTGCTGTCTGTGCTCCGCTATAGGT 214
Sbjct 129 GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT 182
    
```

Nicotine +



Behavior  
Nicotine Seeker

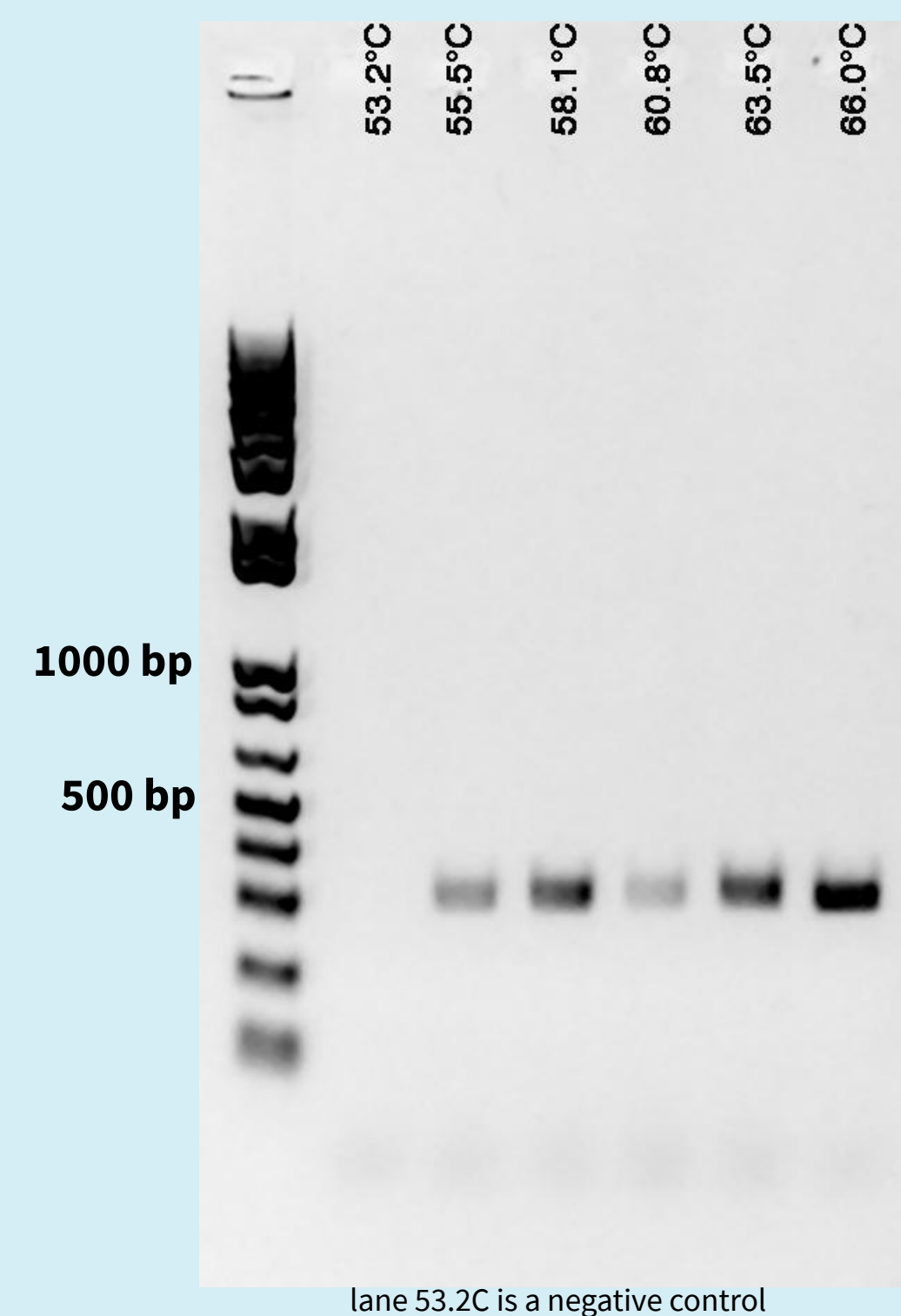
Behavior  
Non-seeker

DNA Isolation,  
PCR &  
Sequencing

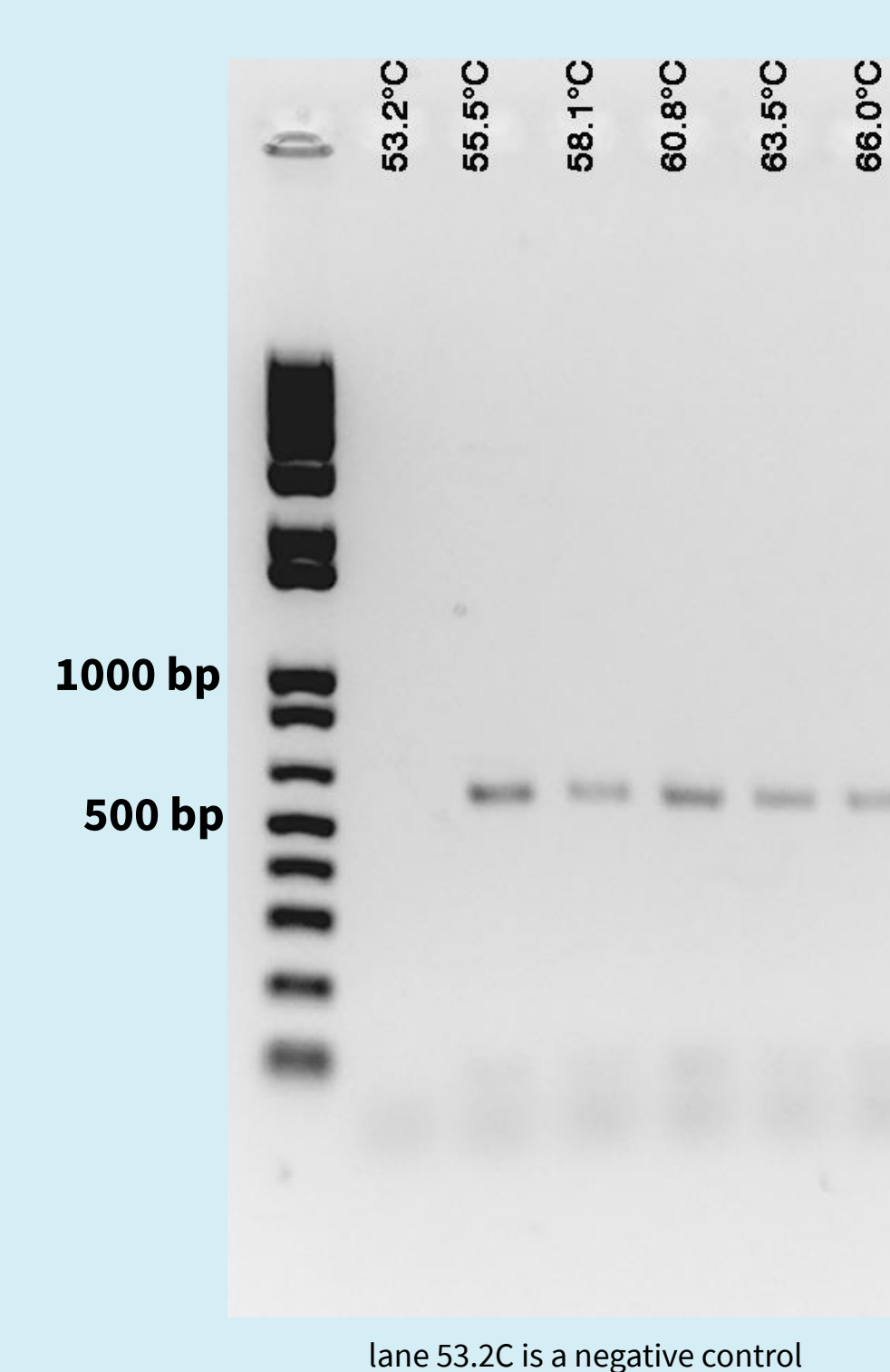


Sequence  
Alignment  
of SNP region

### chrna3 PCR



### cyp2y3 PCR



### nol4lb PCR

