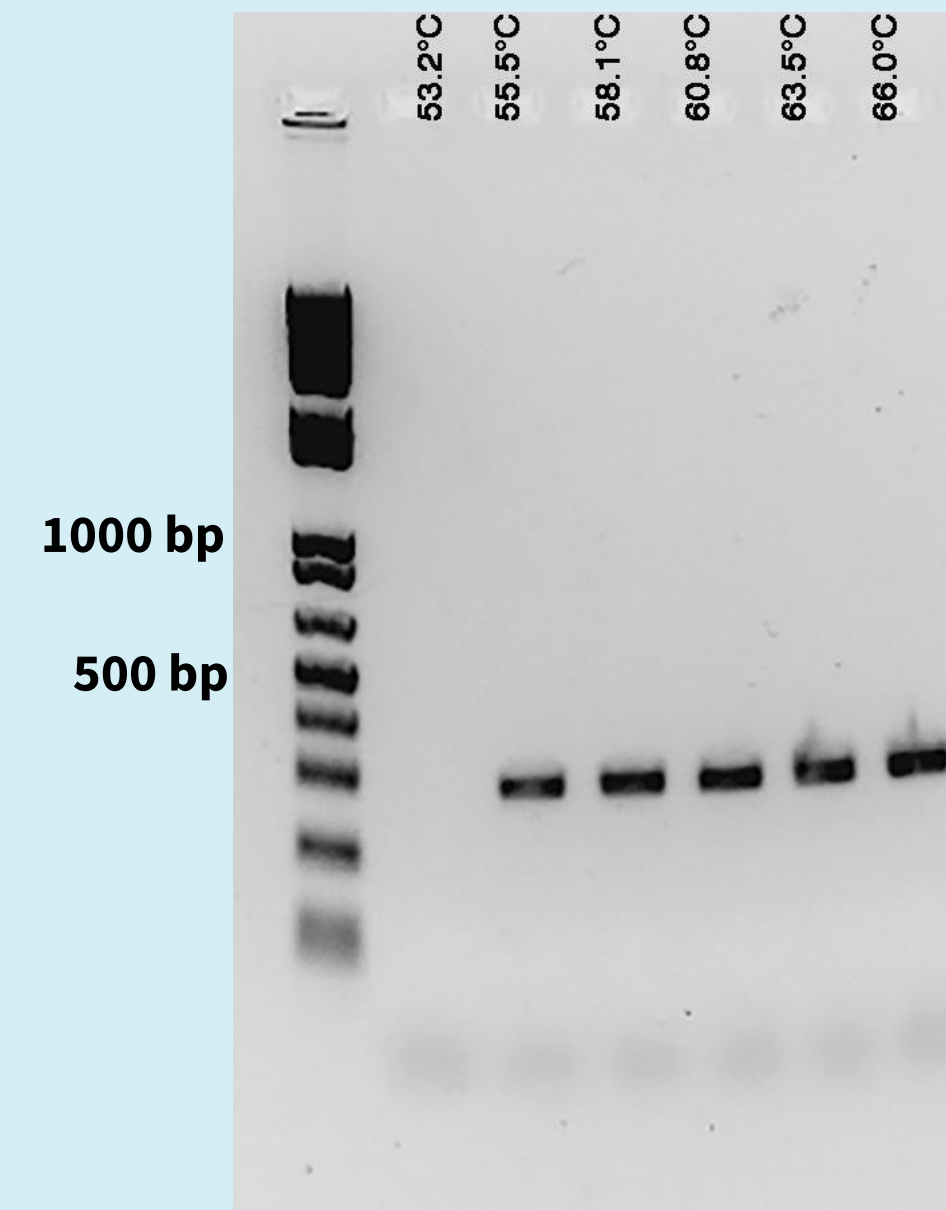


PROJECT DESCRIPTION

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
    
```

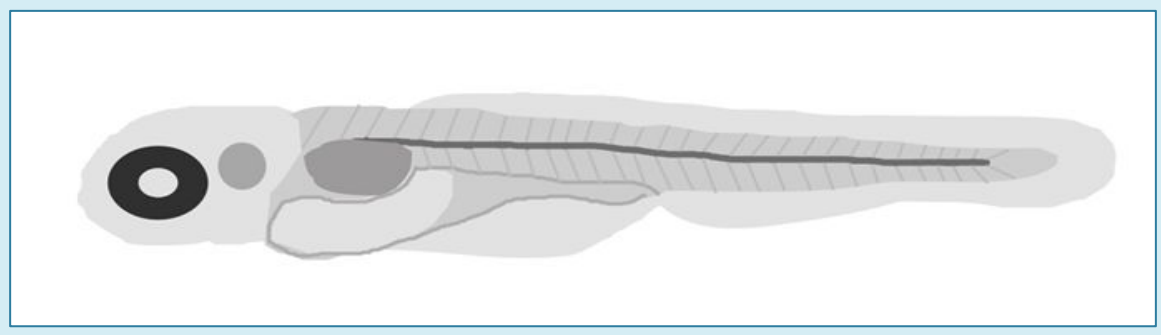
Fish #2

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 GTCAGAGCTCTCCTCCCGTGGTCTGCTGTCTGTGCTCCGCTATAGGT 214
Sbjct 129 GTCAGAGCTCTCCTCCCGTGGTCTGTTCTGCTGTCTGTGCTCCGCTATAGGT 182
    
```

Nicotine +



Behavior
Nicotine Seeker

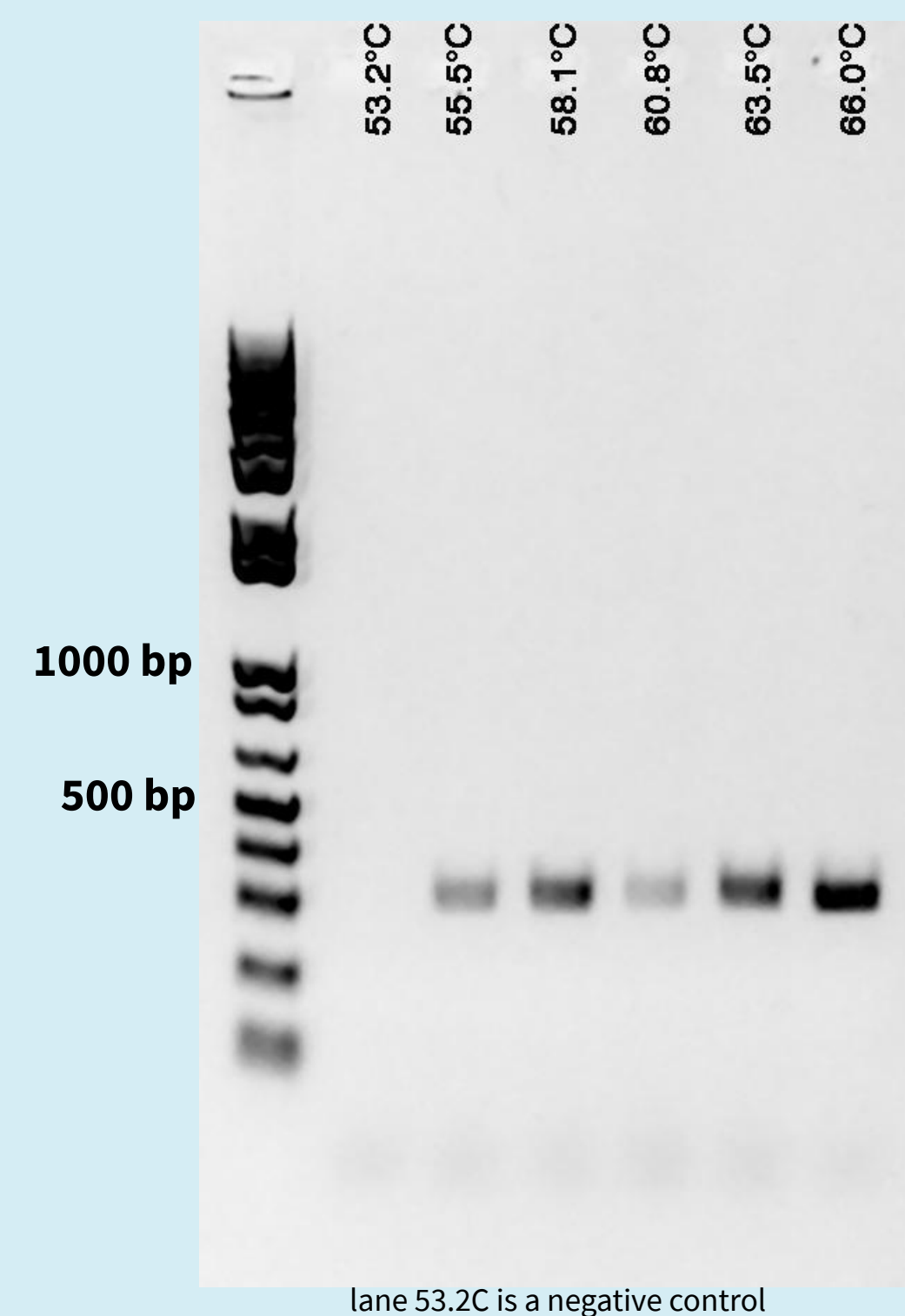
Behavior
Non-seeker

DNA Isolation,
PCR &
Sequencing

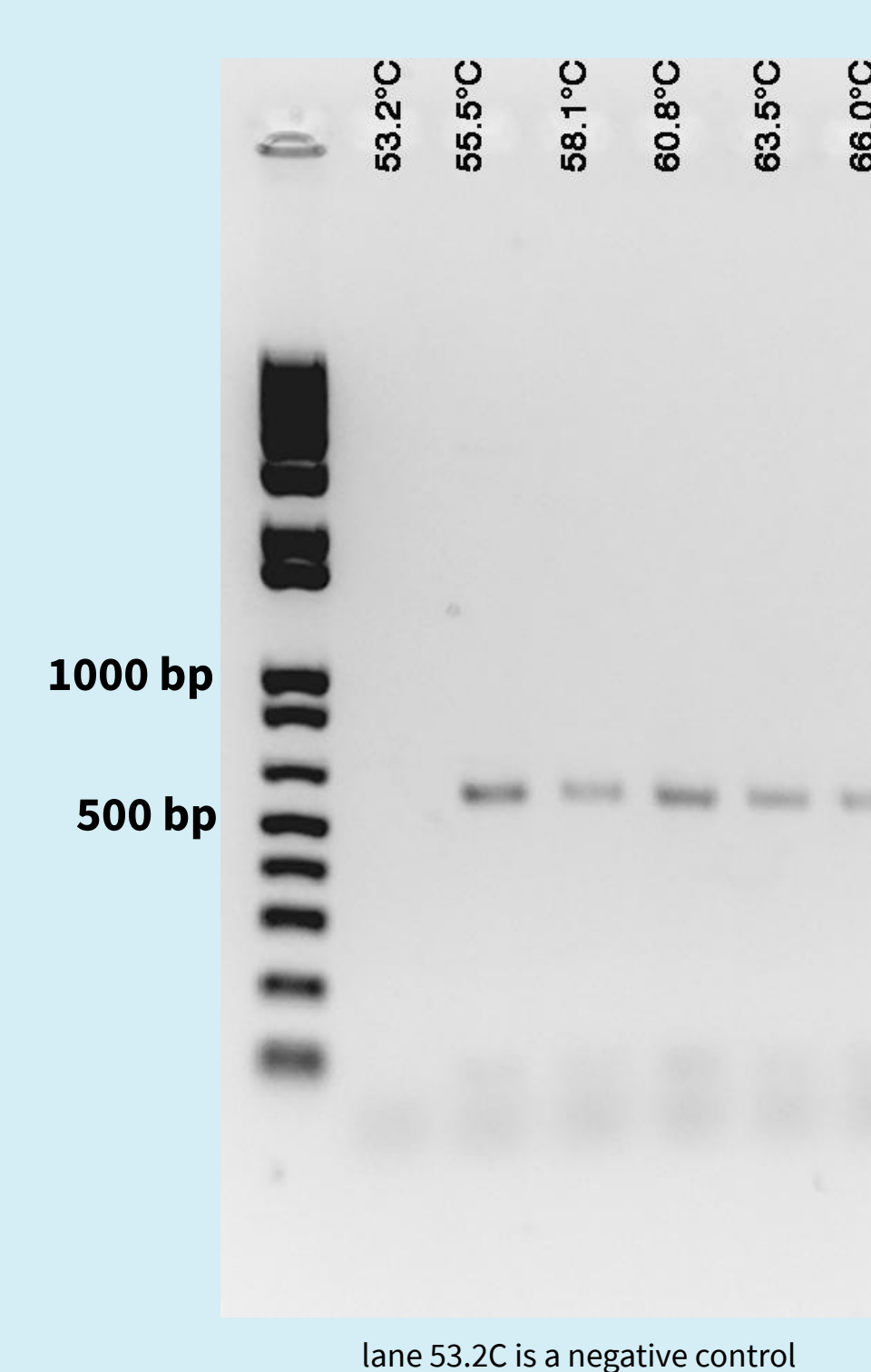


Sequence
Alignment
of SNP region

chrna3 PCR



cyp2y3 PCR



nol4lb PCR

