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EXCELLENCE IN MOUSE PHENOGENOMICS

Sprague Dawley Clcn4 rat cKO genotyping protocol Clcn4^{em3Ics}

Ics internal code Kur8011

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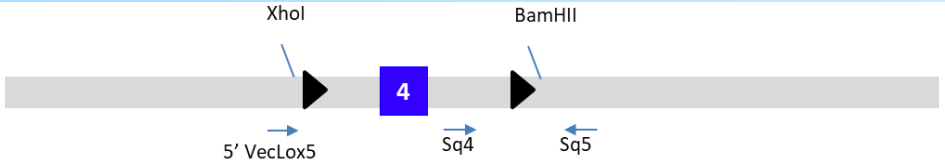
GENOTYPING INSTRUCTIONS

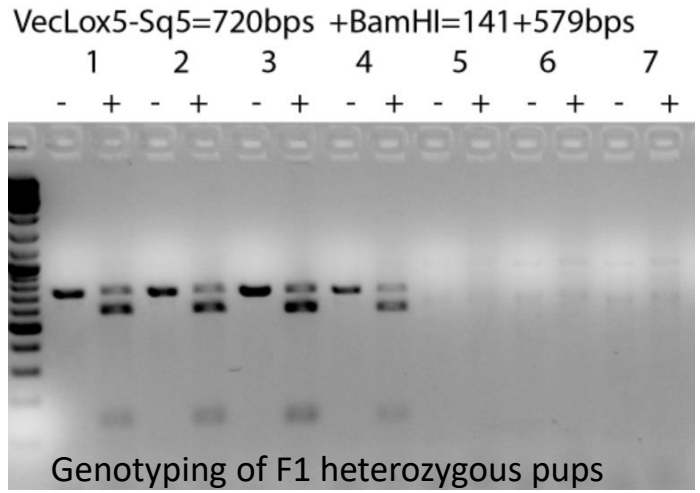
Sequence with PCR VecLox5-sq5
Oligos are in orange
Exon 4 in blue
LoxP are in black, bold and underline



CTTCTCGAGCTTAAGGTCGACCTGCAG**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**TTAATTAAGACTGGGAATTTAACGGCTTCATTATATTCATCTTCTCAGTCTCTTTGTTTCCTGAGGAATCTTCTACTTCA
GAAGCATGAACTAGAAAGTGTGTTCTGATCAGTTGCTGTCTGTCCTTCATTGCCAGATAACCAGCAAAAGTAAAGAGTCTATTTGGGAGTTCATCAAGAGTCTGCTGGACGCATGGTCAGGATGGGTGGTGATGCT
ACTTATTGGTCTGCTAGCAGGTATGTGTGTTGTGCTCCTAGCACCCCCTCCCCAAACTCCATATGTTTGGGAGACAATGAAGCTGTGGAATATTCATCTTCCATTTGAATGCCAAGTATTTACTGCTTGAACCACTGAG
ACTAAGGCAAGAGCCTGATCTTAACTGTGGCACCAAAGGCCAAAGCAAAGATGGCTCACAAAACAACTACGGGATTTTGATGTTGTAATTAACAGTGCTGCTTGACCCTGTCATCCACACCACCGGTG**ATAACTTC**
GTATAATGTATGCTATACGAAGTTATGGATCCGCTGCATAGGTTTATGACCACCCTGAGCTTTTACTCAAAGCACACATTTTATATTCTGTTATACCTGGCTATGTGTGGGGTTACAAATACAGAAGTGTTATAATCTGCA
AGCATCAAACACCACATGTGATGGGCT

PCR genotyping strategy

Primer ref.	Sequence	Amplification product size for the WT allele	Amplification product for cKO allele	Amplification product for cKO allele + BamHI
VecLox5	CTTCTCGAGCTTAAGGTCGACCTGC	672 bps	720 bps	141 + 579 bps
sq5	AGCCCATCACATGTGGTG			
sq4	TTTACTGCTTGAACCACT	277 bps	325 bps	/
sq5	AGCCCATCACATGTGGTG			



Genotyping of F1 heterozygous pups

Sequence of the WT PCR (control WT allele) sq4-sq5

TTTACTGCTTGAACCACTGAGACTAAGGCAAGAGCCTGATCTTAACTGTGGCACCAAAGGCCAAAGCAAAGATGGCTCACAAAACAACTACGGGATTTTGATGTTGTAATTAACAGTGCTGCTT
GACCCTGTCATCCACACGCTGCATAGGTTTATGACCACCCTGAGCTTTTACTCAAAGCACACATTTTATATTCTGTTATACCTGGCTATGTGTGGGGTTACAAATACAGAAGTGTTATAATCTGCAAG
CATCAAACACCACATGTGATGGGCT

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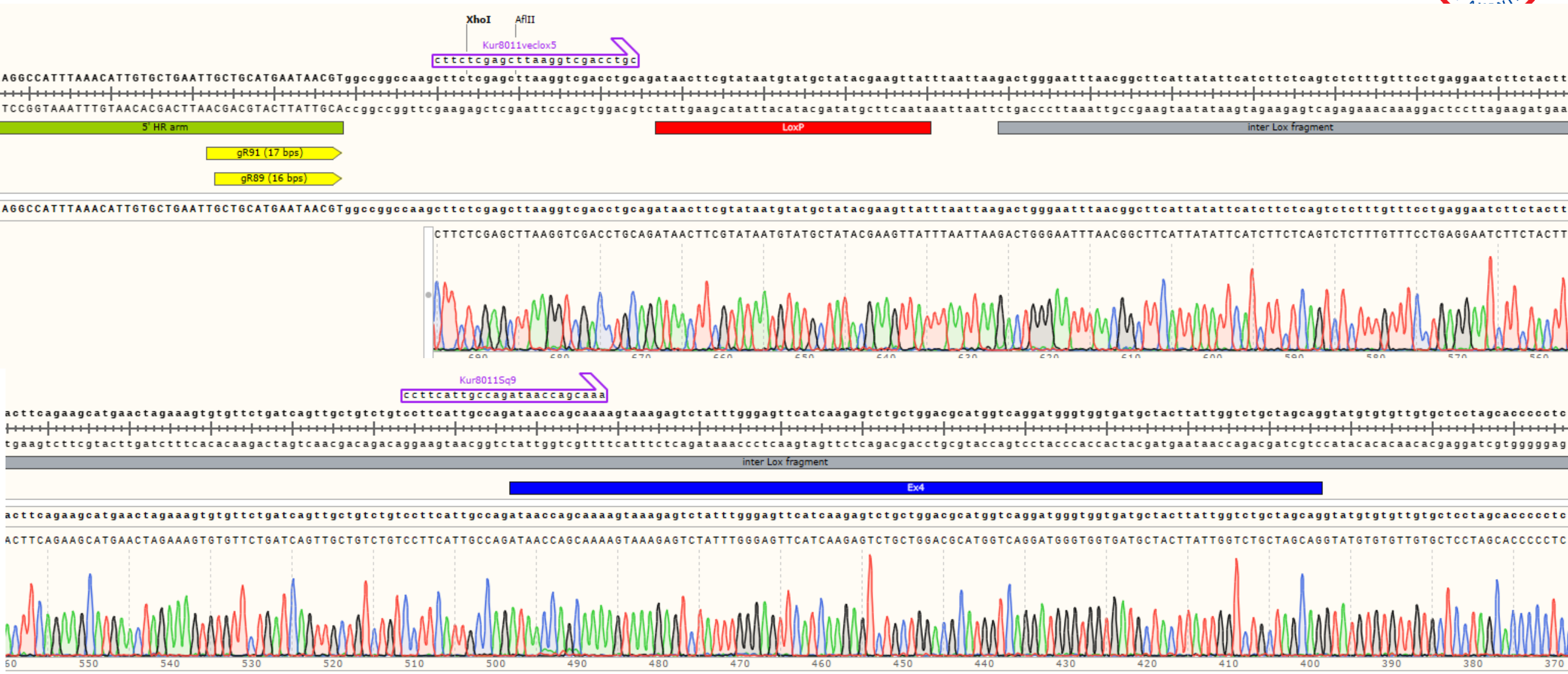
Sanger sequencing of F1 pups



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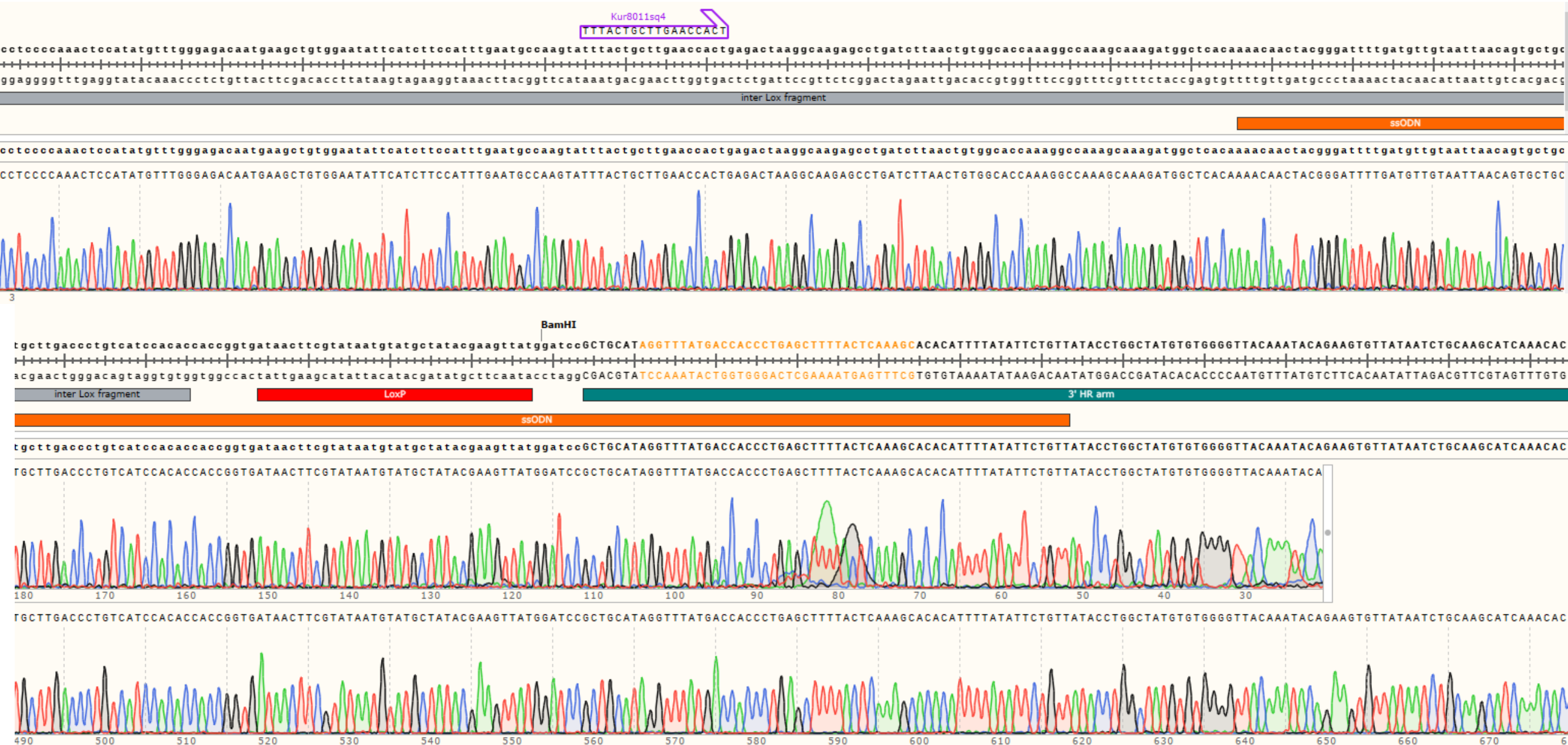
Sanger sequencing of F1 pups



GENOTYPING INSTRUCTIONS

PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping.

Lysis buffer: 50 µl DNA Extract All Reagents – appliedbiosystems, Ref 4402616 (25 µl Lysis buffer +25 µl stabilising buffer)

Reagents:	**Volume (per sample):**
- Phusion HS (Thermo Scientific) 5X Buffer	4 µl
- 10mM dNTP	0.4 µl
- 5’ primer (100 µM)	0.1 µl
- 3’ primer (100 µM)	0.1 µl
- DNA (lysate 1/10)	2 µl
- Phusion Hot Start II	0.2 µl
- Sterile H2O	up to 20 µl

Cycling conditions

Temp	Time	#Cycles
96°C	5min	1
96°C	8s	30
62°C	10s	
68°C	45s	
68°C	5min	1
12°C	5min	1

Digestion protocol	**Volume / sample**
PCR product	10 µl
Buffer 10X	2 µl
Restriction enzyme BamHI	0.2 µl
H_20	7.8 µl

This reaction is incubated **1hr at 37°C** then leaded on a 3% agarose. The 10 µl left over PCR reaction serves as negative control

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REPORT REDACTION & VALIDATION

Report performed on 2023/04/18

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