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# A549V point mutation in Clcn4 (corresponding the human A555V PM) via a CRISPR/Cas9 approach

## Kur8012 / IM8012

Report by Marie-Christine Birling
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# Line Kur8012-14631-PM

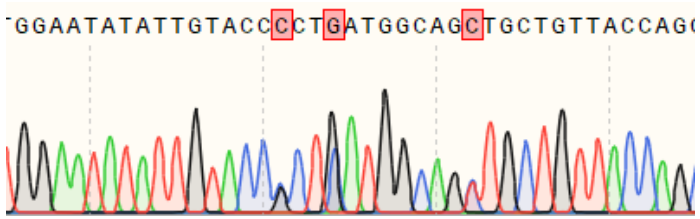
## F1 genotype

Sequence with PCR F2-R2
Primers are in orange
Expected PM in green
Silent mutation (for BsrBI diagnostic restriction site)

GGGATAAAATTATGATCTGTAAGCTTGTTTGCGTAAACCACACATTCATGGGGACTGAGGCTGGGCTTTTAAATCTGTCTTTTTTTATTACAGGTGGAGTGAC
TAGGATGACAGTGTCTCTGGTAGTCATCATGTTTGAACTGACTGGAGGTCTGGAATATATTGTACCGCTCATGGCAGTTGCTGTTACCAGCAAGTGGGTGGC
TGATGCCTTTGGGAAAGAAGGGATTTATGAAGCCCACATCCATCTGAATGGGTACCCATTTCTTGATGTGAAGGATGAGTTCACTCACCGTACCCTAGCCACT
GATGTGATGCGGCCCCGCAGGGGAGAACCACCTTTGTCAGTACTAACCCAGGACAGCATGACTGTGGAGGATGTGGAGACTC

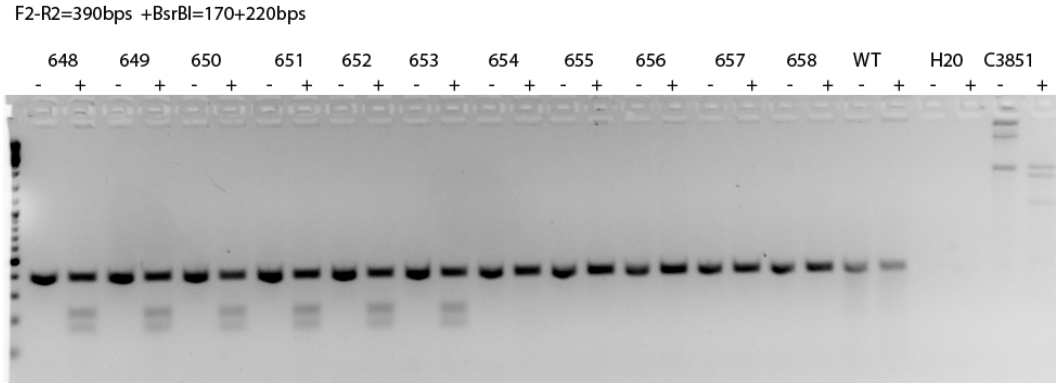
### Sanger sequencing

F1#217




GGAATATATTGTACCGCTCATGGCAGTTGCTGTTACCAG

### F1 genotyping

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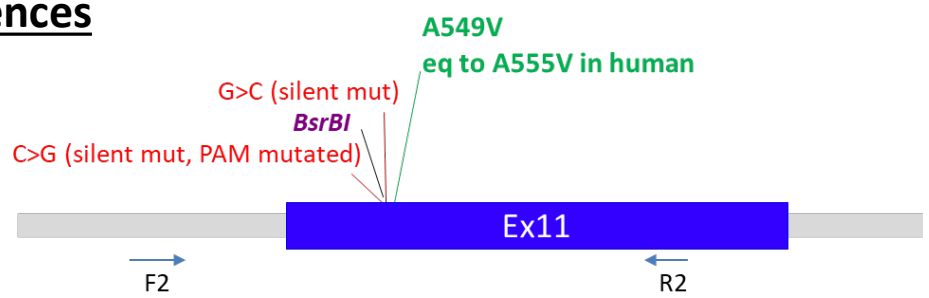
# Genotyping protocol

cnrs
Université de Strasbourg
Instituts thématiques Inserm Institut national de la santé et de la recherche médicale
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

# GENOTYPING INSTRUCTIONS

## Genotyping scheme and sequences




F2 and R2 are in orange
BsrBI restriction site in italic
Expected mutation in green
Silent mutations in red

GGGATAAAATTATGATCTGTAAGCTTGTTTGCGTAAACCACACATTCATGGGGACTGAGGCTGGGCTTTTAAATCTGTCTTTTTTTATTACAGGTGGAG
TGACTAGGATGACAGTGTCTCTGGTAGTCATCATGTTTGAACTGACTGGAGGTCTGGAATATATTGTA*CCGCTC*ATGGCAGTTGCTGTTACCAGCAAGT
GGGTGGCTGATGCCTTTGGGAAAGAAGGGATTTATGAAGCCCACATCCATCTGAATGGGTACCCATTTCTTGATGTGAAGGATGAGTTCACTCACCGTA
CCCTAGCCACTGATGTGATGCGGCCCCGCAGGGGAGAACCACCTTTGTCAGTACTAACCCAGGACAGCATGACTGTGGAGGATGTGGAGACTC

## PCR genotyping

| Primer ref. | Sequence | Amplification product size WT | Sizes observed if BsrBI restriction present (associated with asked PM) |
|---|---|---|---|
| F2 | GGGATAAAATTATGATCTGTAAGCT | 390 bps | 170 bps+ 220 bps |
| R2 | GAGTCTCCACATCCTCCACAGTCAT | | |

# GENOTYPING INSTRUCTIONS

## PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping F0 and F1 genotyping.

| **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 | 5 min | 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 | 0.2 µl |
| H20 | 7.8 µl |

This reaction is incubated 15 mins 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 2022/06/22

by Marie-Christine BIRLING, PhD

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