Cre genotyping

The Cre genotyping is done by 1 PCR which amplifies the Cre gene. But since matings only take place between heterozygotes and wild types, progeny can only be heterozygotes or wild types.

Name	Oligo Sequence
PT178-Cre forward	TCCAATTTACTGACCGTACACCAA
PT179-Cre reverse	CCTGATCCTGGCAATTTCGGCTA

Prepare the mix on ice.

PCR mix per sample.

	(µl)
H_2O (Fresh Milli-Q)	6.5
Buffer	1
$MgCl_2$	0.5
dNTP	1
primers 178 x 179	1
Enzyme (Taq-Pol.)	0.1
Template DNA	1

Mix by pipetting

PCR program: 3-step PCR.

95 °C - 1 minute		
95 °C - 10 seconds 60 °C - 10 sec 72 °C - 30 sec	}	30 cycles
72 °C - 3 min		
4 °C - ∞		

Expected size:
550 basepairs

Results:
No fragment → mouse is a wildtype
Fragment of 550 basepairs → mouse is a heterozygote

Remarks:
Use buffer, $MgCl_2$ and enzyme from the same kit (Lot#).
Buffer, $MgCl_2$, dNTP and primers are stored in fridge 3 in Eric's PCR Equipment Box, the Taq-Polymerase is stored in freezer 5, top drawer on the left side in Eric's Box.
Don't forget the control samples. There is a special plate with control samples for al PCRs in fridge 3 in a box (which normally contains 96-Well Optical Reaction Plates for purifying DNA). This box is called **DNA Eric.**