Cre recombinase mouse line genotyping protocol

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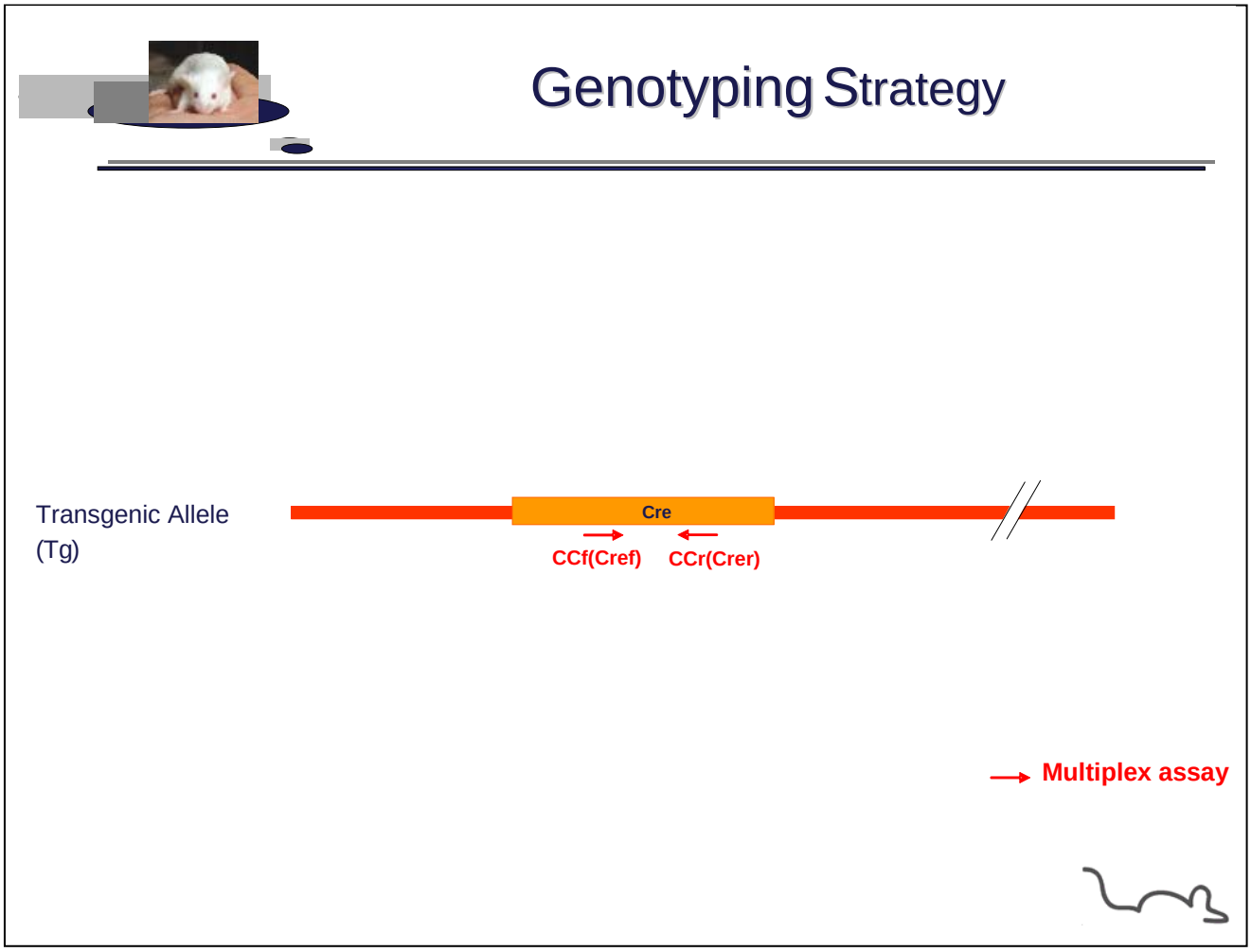
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Cre recombinase** Transgenic model, cre construct (Tg) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping

Multiplex CCf/CCr *	Cf	4029	ACTGGGATCTTCGAACTCTTTGGAC
	Cr	4030	GATGTTGGGGCACTGCTCATTCACC
	Cref	4045	CCATCTGCCACCAGCCAG
	Crer	4046	TCGCCATCTTCCAGCAGG

PCR fragments expected size (bp):

	Region analyzed	**Primers used**	Position on the primer (see the map above)	Transgenic gene detected (Tg)	No transgenic gene detected (WT)
Multiplex* CCf/CCr	Control assay	4029-4030	Cf / Cr	420	420
	Cre specific PCR	4045-4046	Cref / Crer	281	---

--- No Amplicon should be obtained

* Detection of cre transgene is done using a multiplex assay using a primer pair specific to the cre sequence and a primer pair specific to Cpxm1 gene (used as a positive control).

1.2. PCR protocol

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

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

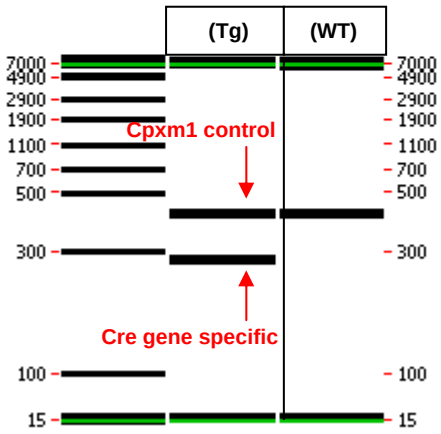
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.