WCSB, Wageningen University	IBISBA-SOP-WU20
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: *MASC-PCR*

distribution list			
changes to prior version:			
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Instruction

MASC-PCR

1. Introduction / Purpose

Recombinant cells from MAGE experiments targeting selectable markers or a visible phenotype can be identified by plating them on appropriate selective or differential media. However, because most mutations do not present screenable or selectable phenotypes, multiplex allele-specific colony PCR (MASC-PCR) can be used to simultaneously interrogate the genotypes of many mutagenized loci. MASC-PCR affords single base-pair resolution and permits the detection of single-nucleotide polymorphisms. For each targeted locus, three primers are designed: (i) a forward primer specific to the WT (*f*WT) sequence, (ii) a forward primer specific to the mutant (*f*mut) and (iii) a reverse primer (*r*) common to both. The two forward primers only differ at their 3′-terminal bases, allowing for discrimination of SNPs with an allele- specific PCR. Two MASC-PCRs are required to screen each colony: one to assay the WT genotype using *f*WT and *r* primers, and the other to assay the mutant genotype using *f*mut and *r* primers. If the colony contains the mutant allele, an amplicon will only be produced by the *f*mut and *r* primers but not the *f*WT and *r* primers. For the WT allele, only *f*WT and *r* primers will produce an amplicon.

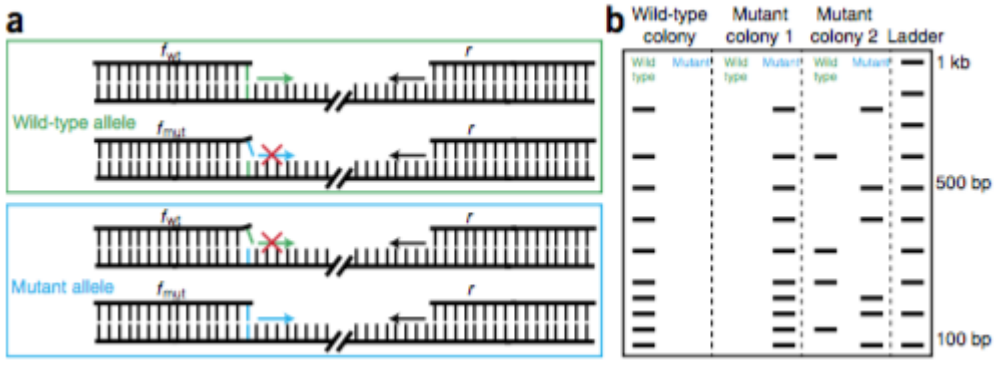

Figure 1. Genotype assays by MASC-PCR. (a) fmut and fWT primers share a common r primer and are identical, except for the 3'-terminal base, which can either anneal to the mutant or the wild-type allele, respectively. At the optimal Tm, extension occurs only if the forward primer is an exact match for its target site. (b) Examples of binary MASC-PCR results.

Keywords: MASC-PCR, MASC

2. Equipment and chemicals

2.1. Equipment

- PCR machine
- Agarose gel electrophoresis apparatus

2.2. Chemicals

- Phire 5x Green Buffer (Thermo Scientific)
- Phire polymerase (Thermo Scientific)

2.3. Other materials

Plate with test colonies

S*pecial consumables*

- dNTPs
- Primer *fWT*
- Primer *fmut*
- Primer *rev*
- Sterile 96-well culture plates

3. Media and Buffers

- **LB (1 L):** 10 g Tryptone + 5 g Yeast Extract + 10 g NaCl (Sterilization by autoclaving)

4. Procedures

Prepare serial tenfold dilutions of the liquid culture that you want to analyze.

Spread 50 µl of each dilution on plates selective for the mutagenized strain. Incubate the plates at 30°C O/N until colonies appear.

Use pipette tips or loops to pick colonies from each sample, and then transfer each colony to 10 µl of dH_2O. Use the colony in solution as DNA template for each PCR reaction mix (WT and mut), which will include the next components:

Table 1. MASC-PCR reaction mix. (1) The primer FW will vary depending on the reaction either fWT or fmut; (2) If multiplex MASC-PCR, different set of primers will be included in the reactions to get different amplicons; and, (3) If multiplex MASC-PCR, the volume of added dH_2O has to be adapted to the number of set of primers.

Reaction mixture (20 µl)	
Phire 5x Green Buffer	4 µl
dNTPs	0.4 µl
Primer FW [1, 2]	0.5 µl
Primer RV [2]	0.5 µl
Phire polymerase	0.4 µl
dH_2O [3]	13.2 µl
DNA template	1 µl

Set up the following PCR program described in Table 2.

Table 2. MASC-PCR program

Cycles	1	25			1	1
Temperature	98°C	98°C	Annealing temperature of the primers	72°C	72°C	12°C
Time	10 min	10 s	10 s	30 s per kb of the amplicon	2 min	∞

Prepare a 1% (wt/vol) agarose gel to run the samples.

Once the PCR reactions are finished, load them into the gel in an electrophoresis chamber using the DNA ladder as reference. Run the electrophoresis for 45 minutes at 100 V.

Visualize the resulting agarose gel to determine the genotype of each colony.

5. Remarks / troubleshooting

Optional: Freeze and store the pure cultures for the colonies that screened correctly, and then confirm genotype with Sanger sequencing.

6. Biosafety

No biosafety issues were associated with this protocol.

7. Acknowledgements

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.