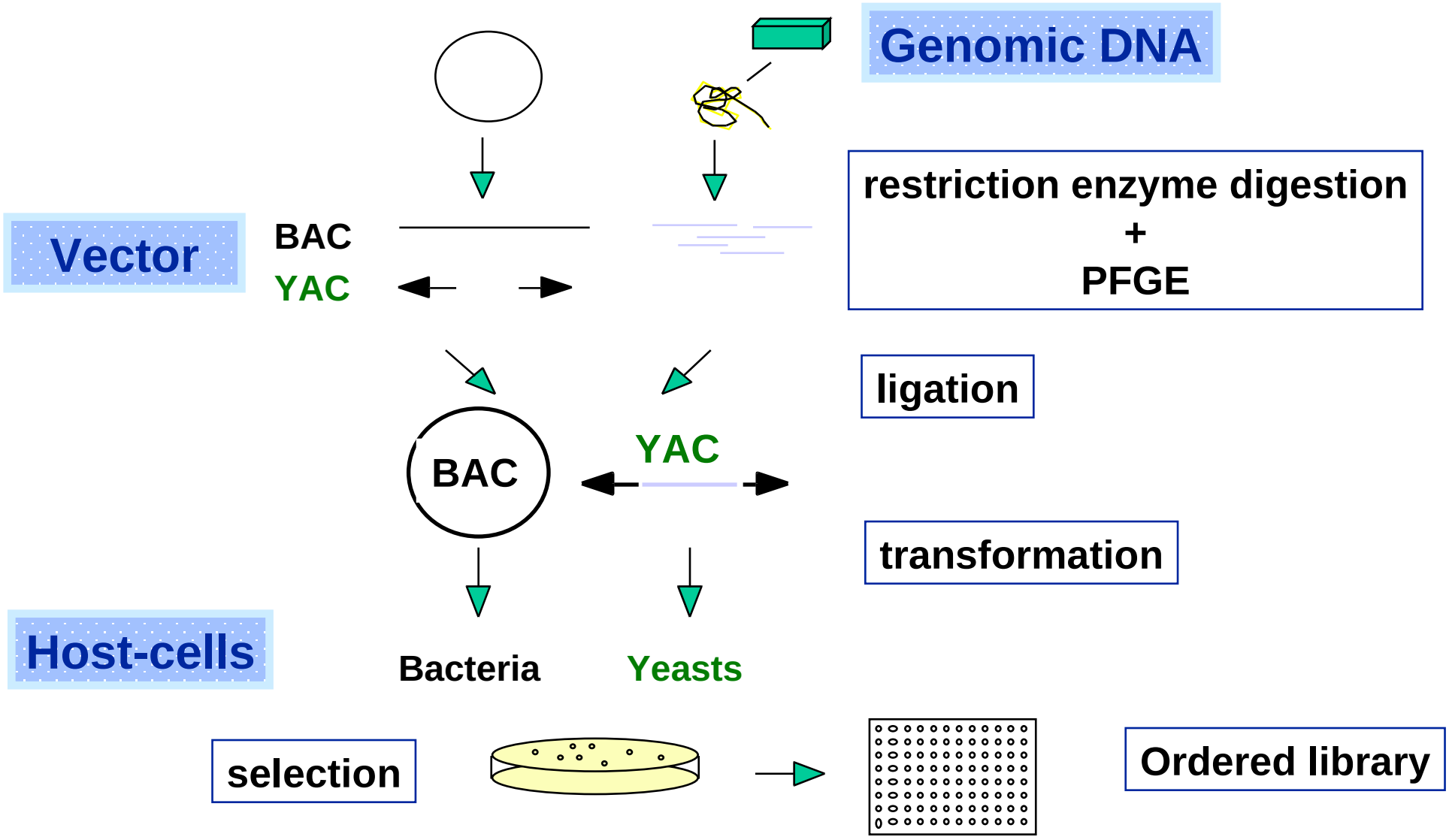
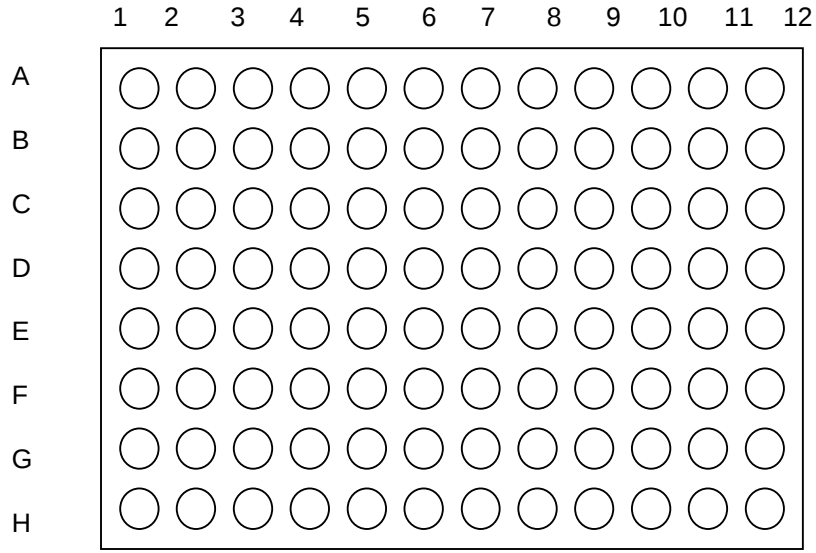
Organization of the libraries

For PCR screening

Recall : Construction of a library (YAC and BAC)



Library = Ordered collection of large insert genomic DNA fragments cloned in BACs or YACs



Set of 96-well or 384-well plates

All stored at -80°C

Each clone is defined by an address :
plate number, column number and row number

Library screening
How to identify a clone ?

- Hybridization on HD membranes (clones are spotted)
  - Recent production
  - Membranes currently reserved to LREG and LGBC (intern use)

- PCR Screening : Clone pooling

PCR Screening : Clone pooling

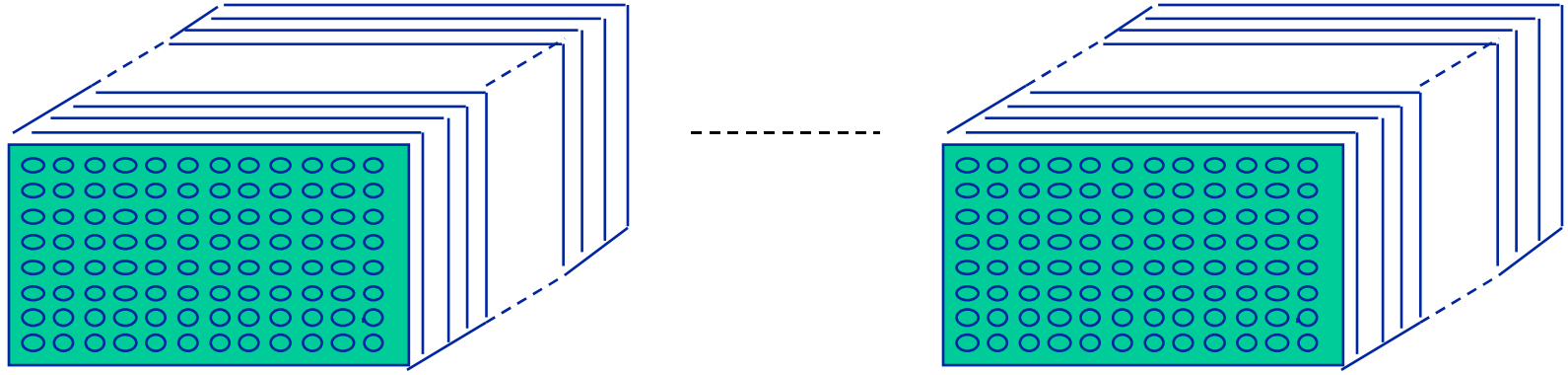
- Aim : To reduce the number of PCRs
- Several ways of pooling
- We chose :
  - 3D pooling
  - 20 or 24 96-well plates per pool
  - possible to pool by hand (pooling must be done with clones in 96-well plates)

Clone pooling

First organization level: Superpools

Groups of 20 plates : all the clones are mixed

96 clones X 20 plates = 1920 clones / superpool



For 100,000 clones

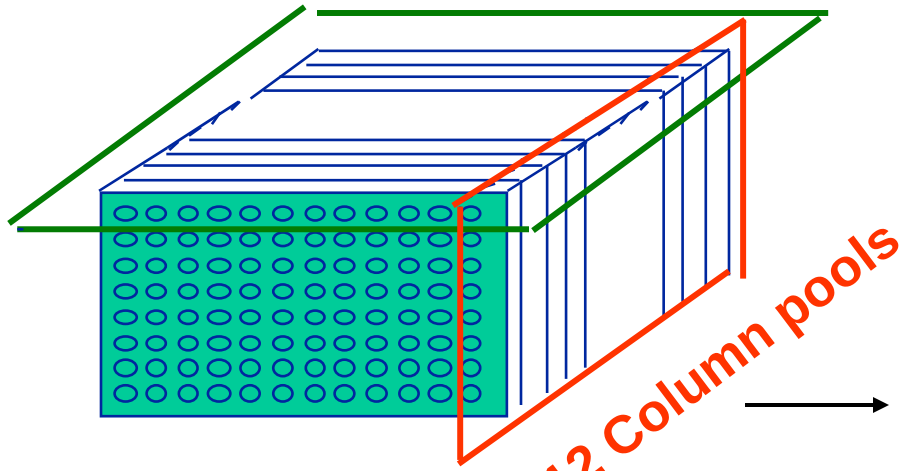
superpool 1

superpool 52

Clone pooling
Second organization level: pools

In one group of 20 plates

8 Row pools → **12X20=240 clones / pool**



12 Column pools → **8X20 = 160 clones / pool**

20 Plate pools → **96 clones / pool**

Preparation of DNA pools and superpools

All pools are centrifuged (bacteria)

Row pools and Column pools + 3m of TE

boiled to prepare DNA → **3 ml**

Plate pools + 5 ml of TE

3 ml are boiled to prepare DNA → **3 ml**

2 ml to make the **Superpool**

The 2 ml of all the pool plates are mixed

Centrifugation, pellet + 10 ml of TE

boiled to prepare DNA → **10 ml**

Dilutions for PCR

1:10 in 96 well plates

→ pools plates, rows and columns : 30 ml each

→ superpools : 100 ml each

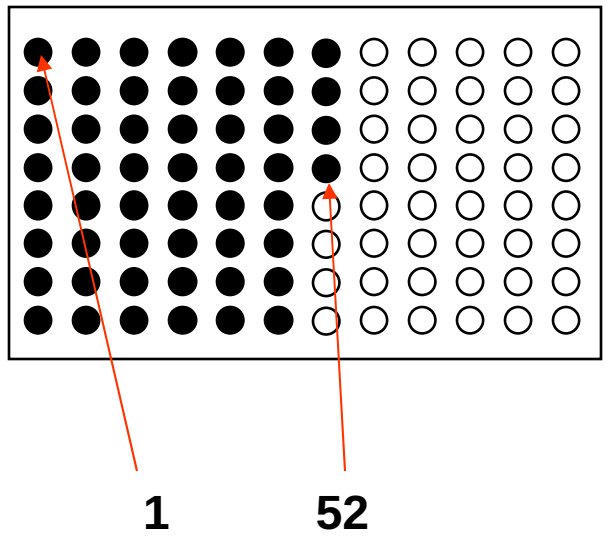
volume of DNA in one PCR : 1 µl

→ 30000 PCRs for each pool

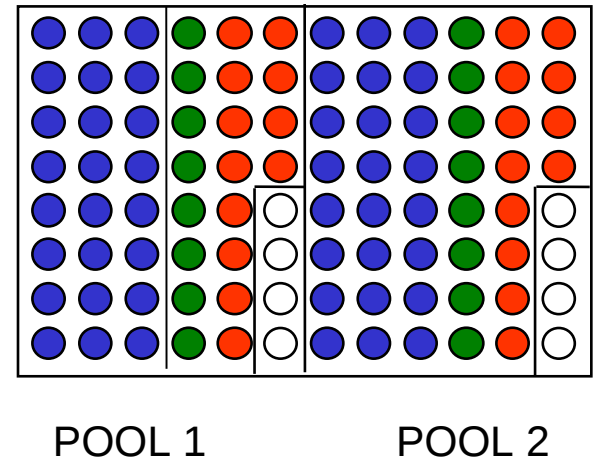
→100000 PCRs for each superpool

Enough DNA for many years of screening

Storage of DNA pools and superpools



superpools



pools

Plates 1 to 24

Rows A to H

Columns 1 to 12

Stored at -20°C

Possible to work with multichannel pipettes

Library screening by PCR

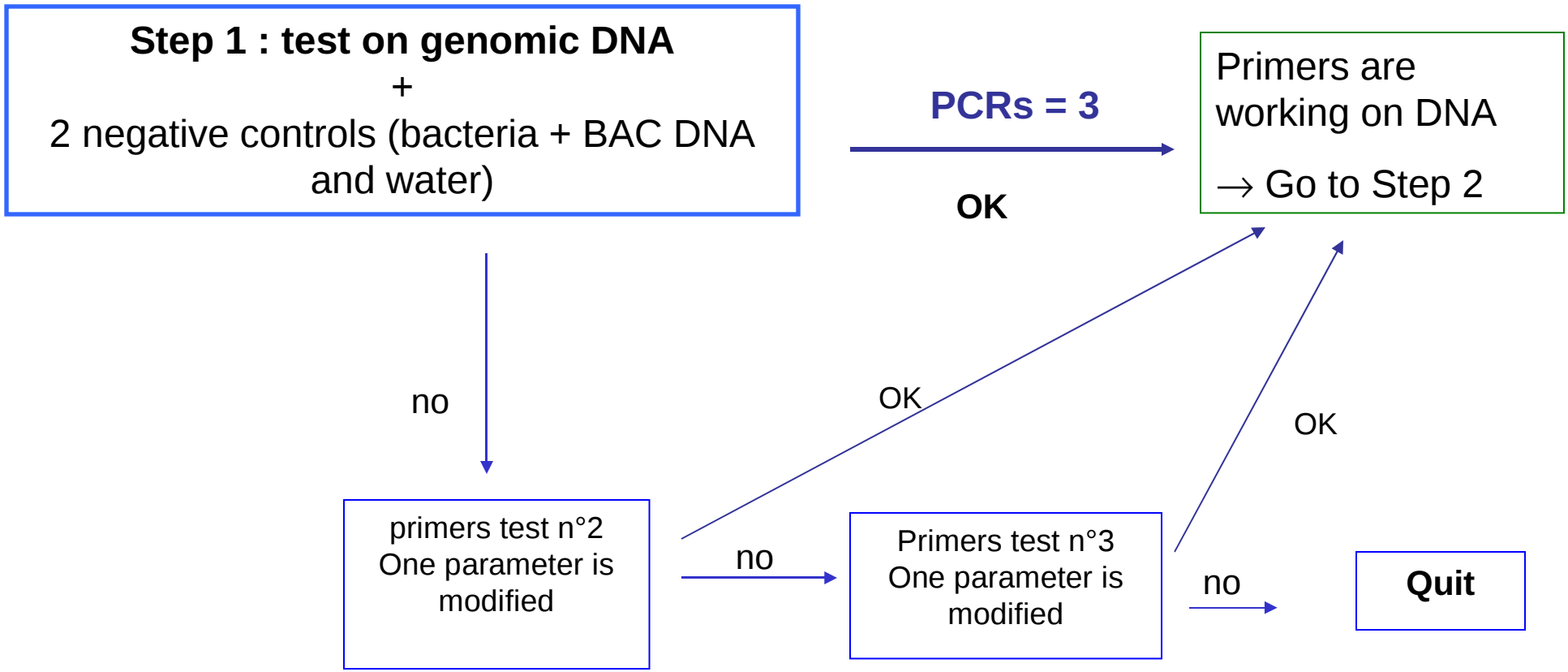
Example :

100,000 clones

52 Superpools (20 plates / Superpool)

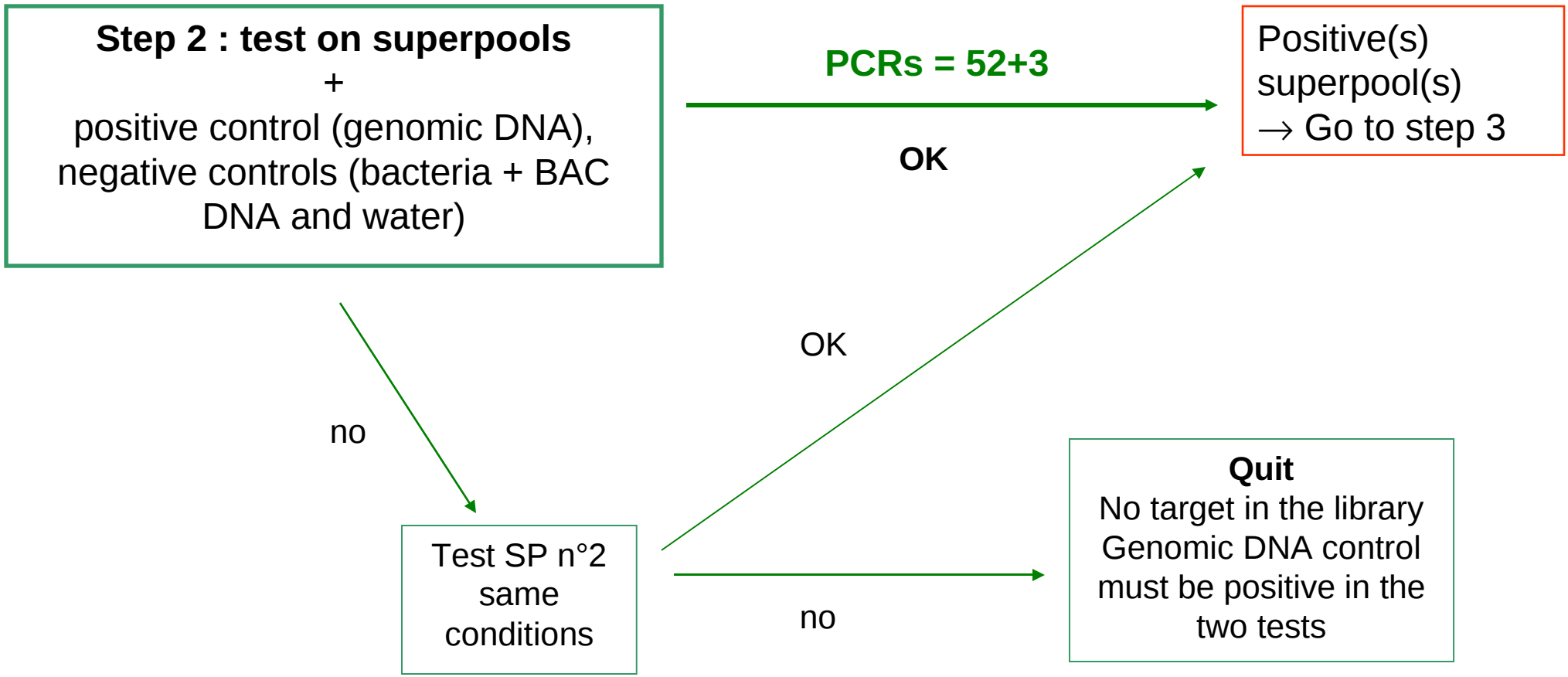
PCR screening of a library : 4 steps (minimum)

Step 1: Check PCR conditions on genomic DNA



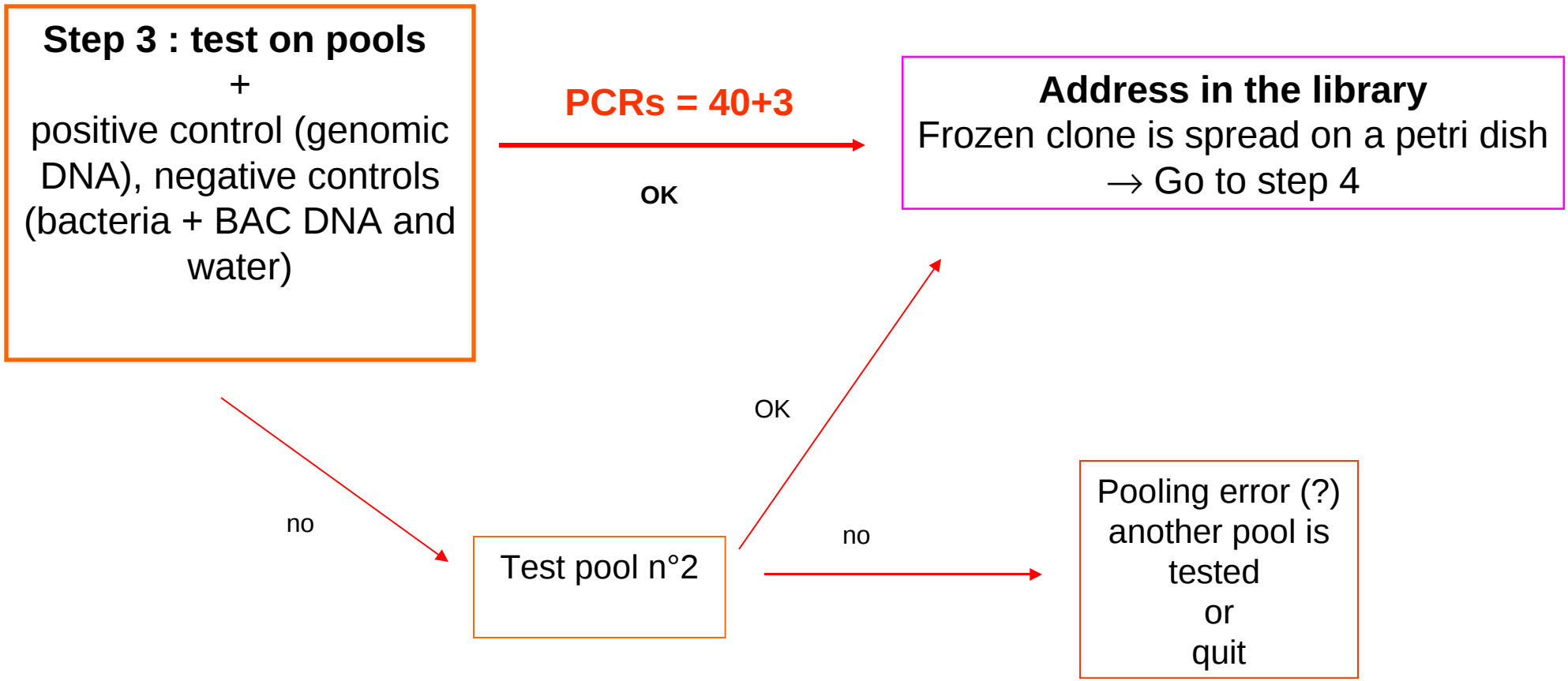
PCR screening of a library : 4 steps (minimum)

Step 2 : PCR on the DNA superpools



PCR screening of a library : 4 steps (minimum)

Step 3 : PCR on the DNA row, column and plate pools



PCR screening of a library : 4 steps (minimum)

Step 4 : PCR on isolated clones

Step 4 : PCR on isolated colonies

Test on 2 colonies + 3 controls

PCRs = 2+3

OK

Isolated colony ready to be sent

no

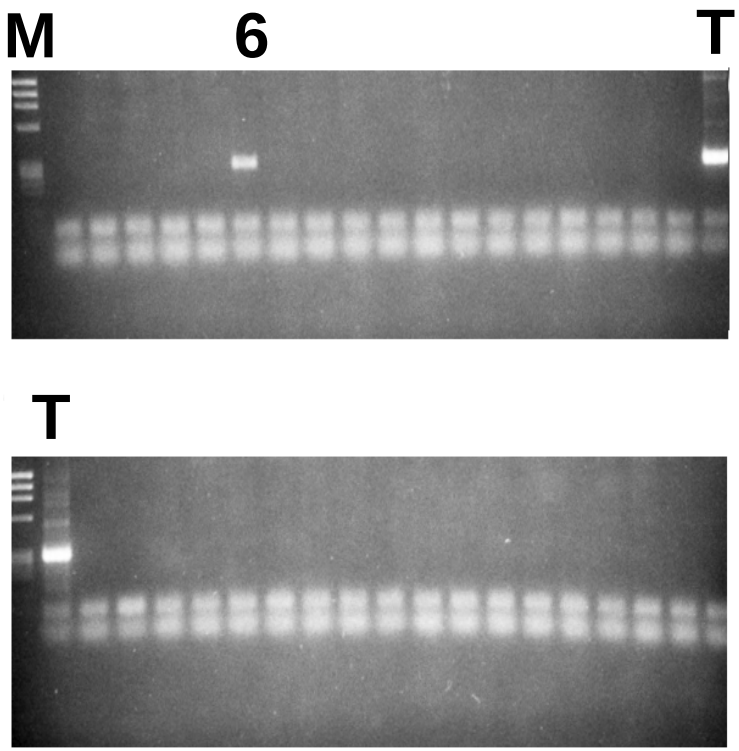
Pooling error (?)
another pool is tested or quit

⇒ 106 PCRs to find a clone

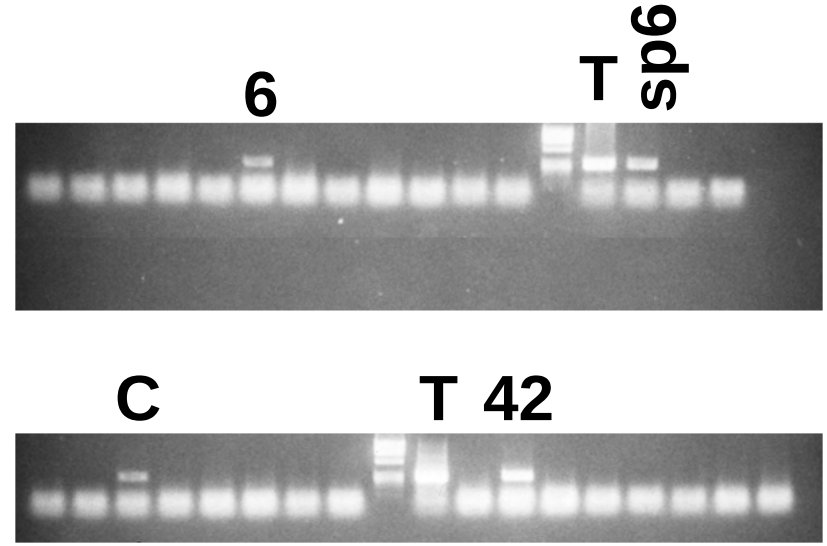
⇒ 48 PCRs only for an additional clone

Result on an agarose gel

Superpools



Pools



⇒ **clone address = 42C6**

Request for using the libraries

- Request usually arrive by e-mail to :karine.hugot@jouy.inra.fr
- Briefly :
  - Library to be screened
  - Description of the project (localization studies : one clone or contig studies : all the clones that can be found).
  - PCR conditions (annealing temp.)
  - 100 µl of each primer at 100 µM (100 pmoles/µl)
- Library screening and clone isolation are performed in 4 steps only by PCR

Request for using the libraries

- **Shipment :** Only Colonies are sent on an LB-agar-chloramphenicol stab

- **Payment (for laboratories outside Europe only)**

Labs are only billed for clone shipping charges (express mail)

- **Restrictions for using the clones**

Since the library screening is free of charge and the clones are available for the scientific community the following has to be mentioned in the publications :

- Reference of the library
- BAC-YAC Resource Center of the Animal Genetics Department of the INRA (acknowledgments section)
- The person who performed the screening can also be mentioned in the acknowledgment section or be part of the authors