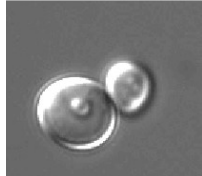


A high-throughput *Candida albicans* two hybrid (C2H) system

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Prof. Van Dijck

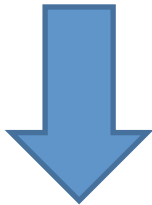
Candida albicans

- Fungus



- Commensal organism
- Potential pathogen => no problem (?)
- Immuno compromised (HIV eg.)

(Spruw/thrush)



**4th Hospital-acquired
bloodstream infection
40% mortality!!**



WHY?

- \$2,5 Billion/year
- 40-60% mortality
- 4th Hb-infection
- 10.000 deaths/year/US
- 16.000 deaths/EU/year
- #drugtargets limited

⇒ Find new drugs!
(very specific ones: PPIs)

Table 1. Implantable devices in which *Candida* biofilms develop most frequently

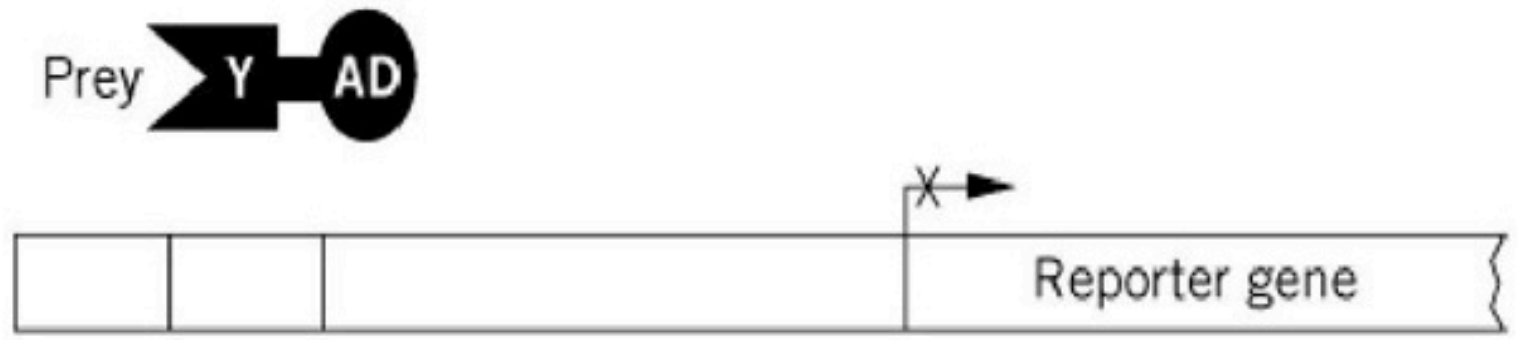
Device	Usage per year	Infection risk (%)	Main <i>Candida</i> species
Central and peripheral venous catheters	5 million	3–8	<i>albicans</i> <i>glabrata</i> <i>parapsilosis</i>
Hemodialysis and peritoneal dialysis catheters	240 000	1–20	<i>albicans</i> <i>parapsilosis</i>
Urinary catheters	Tens of millions	10–30	<i>albicans</i> <i>glabrata</i>
Endotracheal tubes	Millions	10–25	<i>albicans</i>
Intracardiac prosthetic devices	400 000	1–3	<i>albicans</i> <i>glabrata</i> <i>parapsilosis</i> <i>tropicalis</i>
Breast implants	130 000	1–2	<i>albicans</i>
Prosthetic joints	600 000	1–3	<i>parapsilosis</i> <i>albicans</i> <i>glabrata</i>
Neurosurgical shunts	40 000	6–15	<i>albicans</i>
Voice prostheses	Thousands	50–100	<i>albicans</i> <i>tropicalis</i>
Dentures	> 1 million	5–10	<i>albicans</i> <i>glabrata</i>

HOW

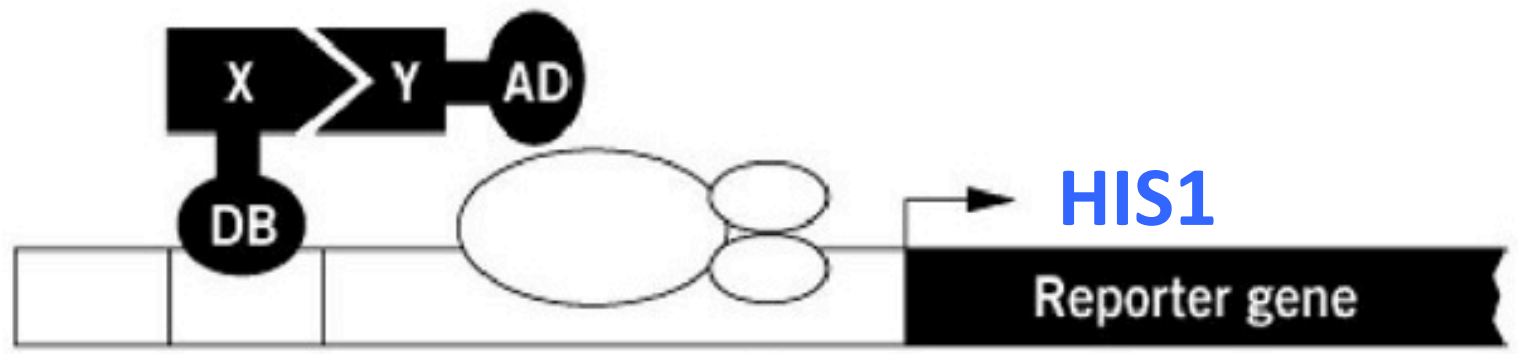
- Yeast two hybrid (*saccharomyces cerevisiae*)
(Remember: PPIs = drugtargets!)
- However: *C. albicans* translates CUG as serine rather than leucine
- Solution: Candida 2 hybrid (C2H) system



(b)



(c)

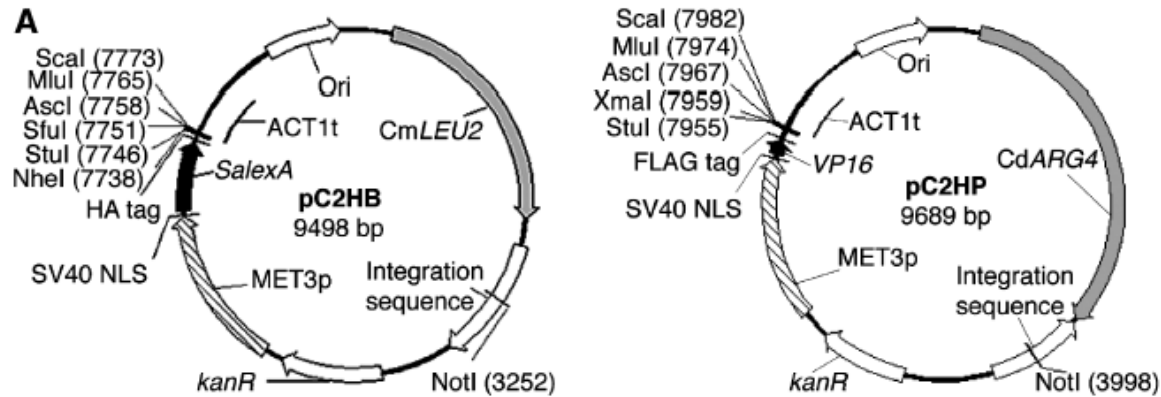


(d)

Construction ORFeome C2H library

Bait library: Leucine selection

Prey library: Arginine selection



Stylen *et al.*, 2010

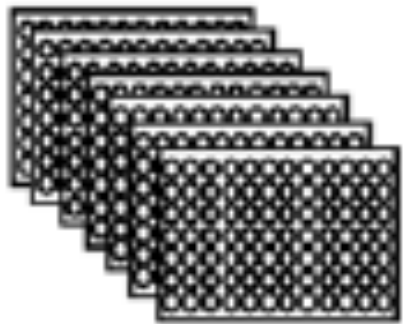
*Adapted with gatewaytechnology

*SN152 (his-leu-arg-)

Construction prey library

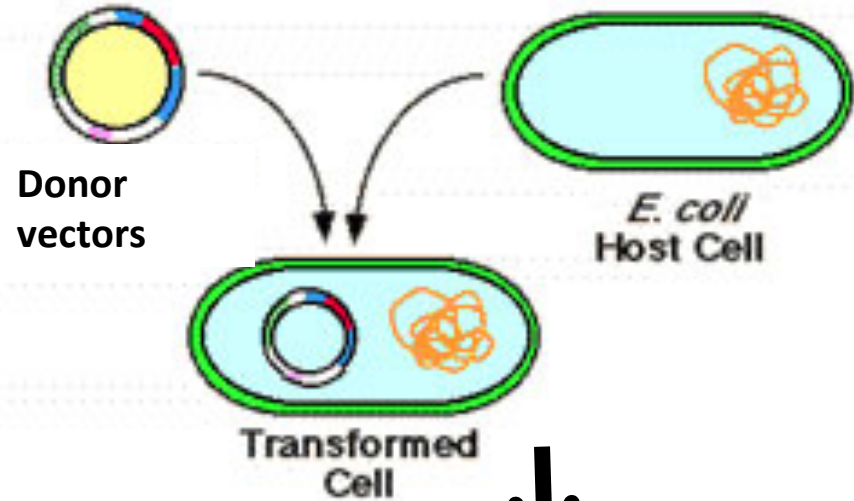
Plasmid DNA, 96 well plates

Donor vectors



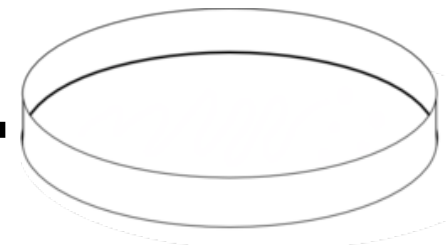
1

Transformation



2

Miniprep



LB + Gentamycin

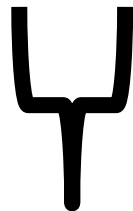
Donor vectors

+

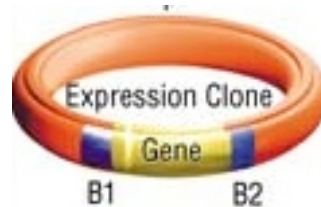


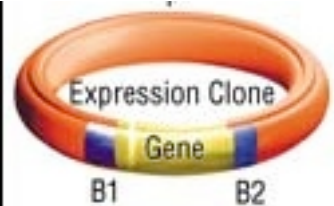
3

LR reaction

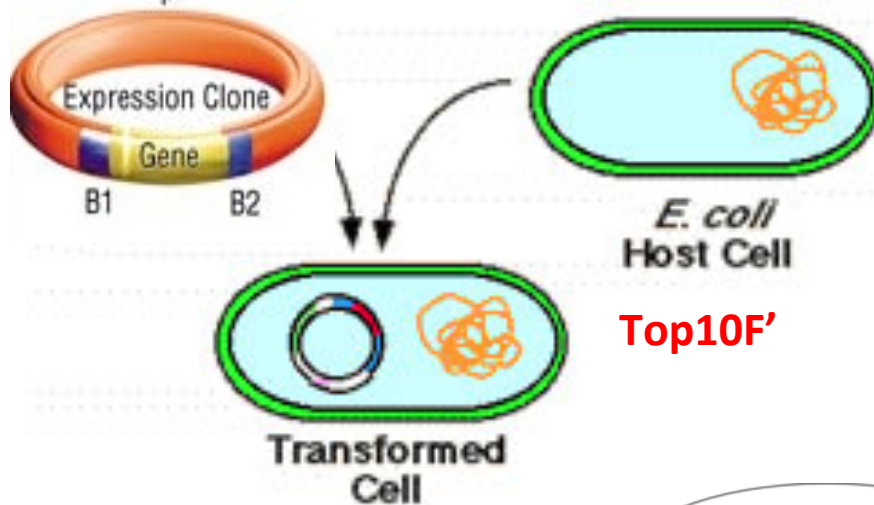


Expression vector



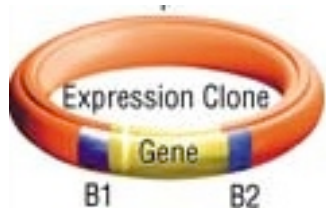


4 Transformation

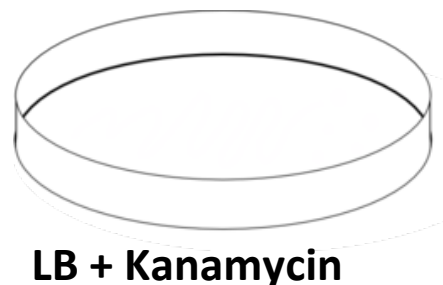


Expression vector

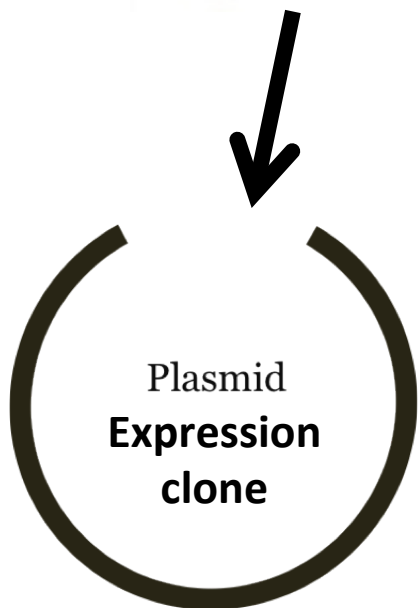
5 miniprep



With expression vector

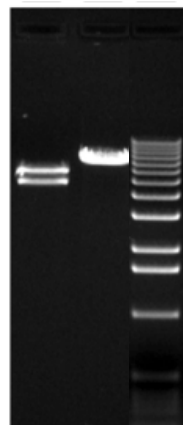


6 Digest with NOT I (gateway cassette + integration sequence)



Verification on gel

Bad-good-ladder

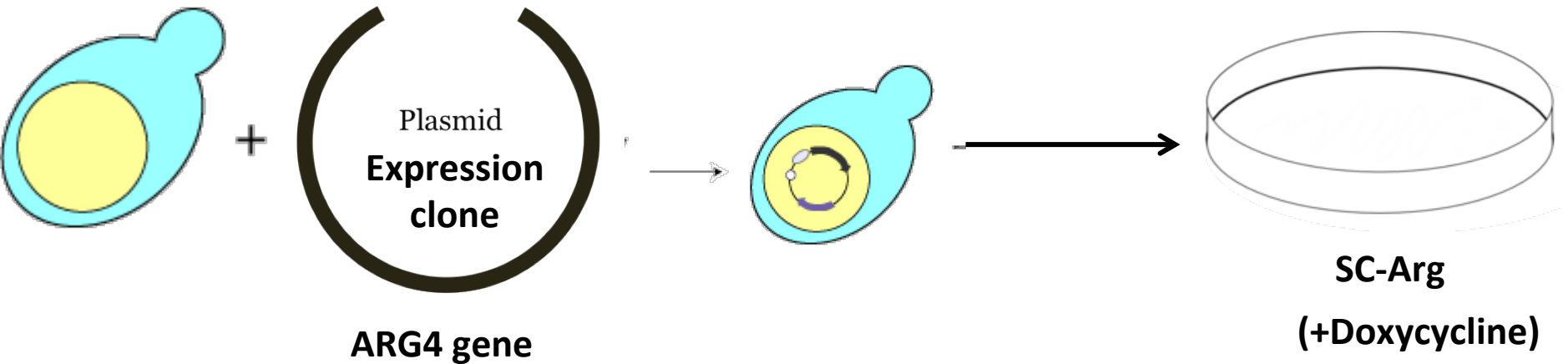


7 Transformation
Candida cells!

Genomic transformation

Continued...

7 Transformation candida cells

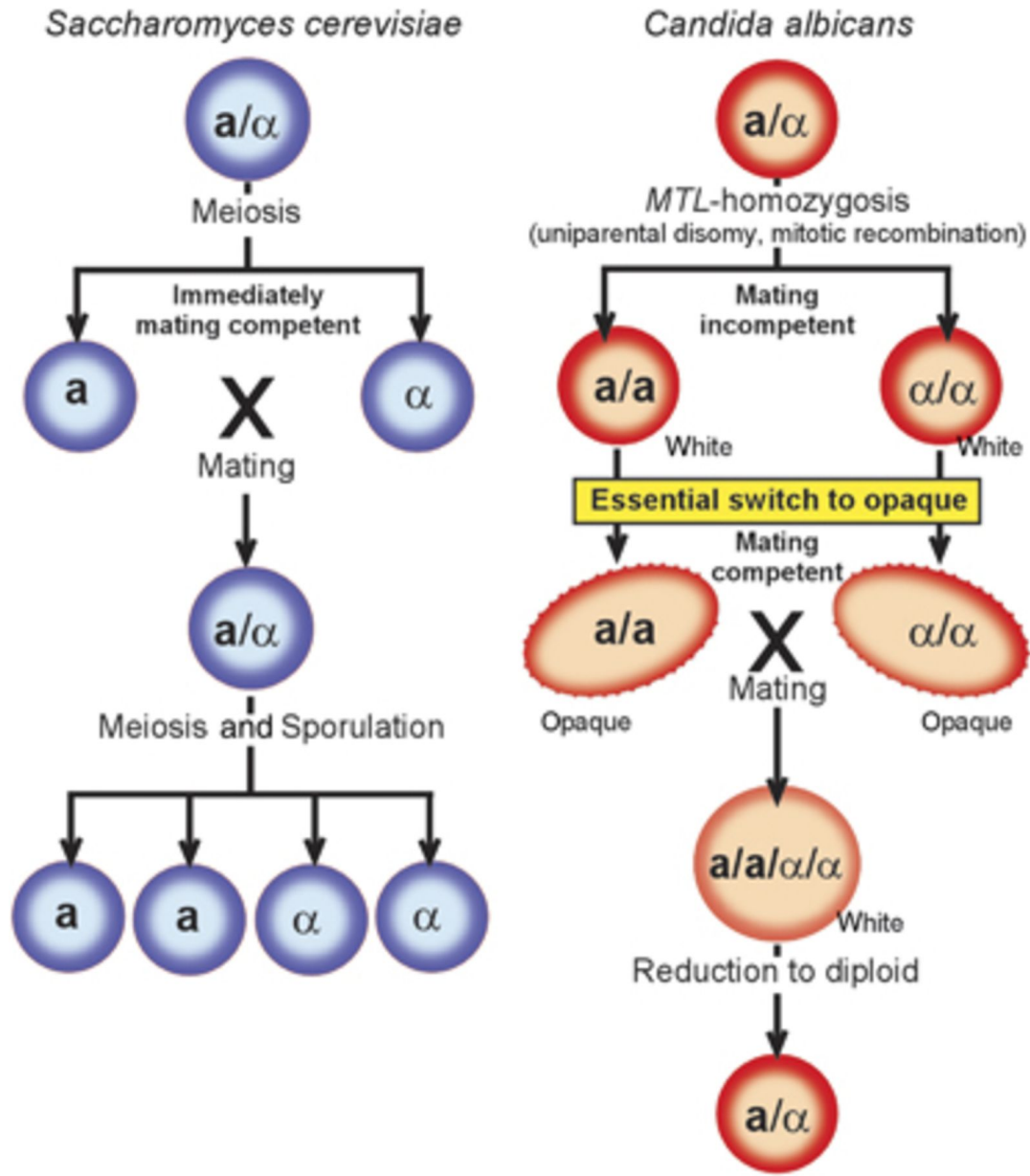


+ => storage -80°C

- => restart

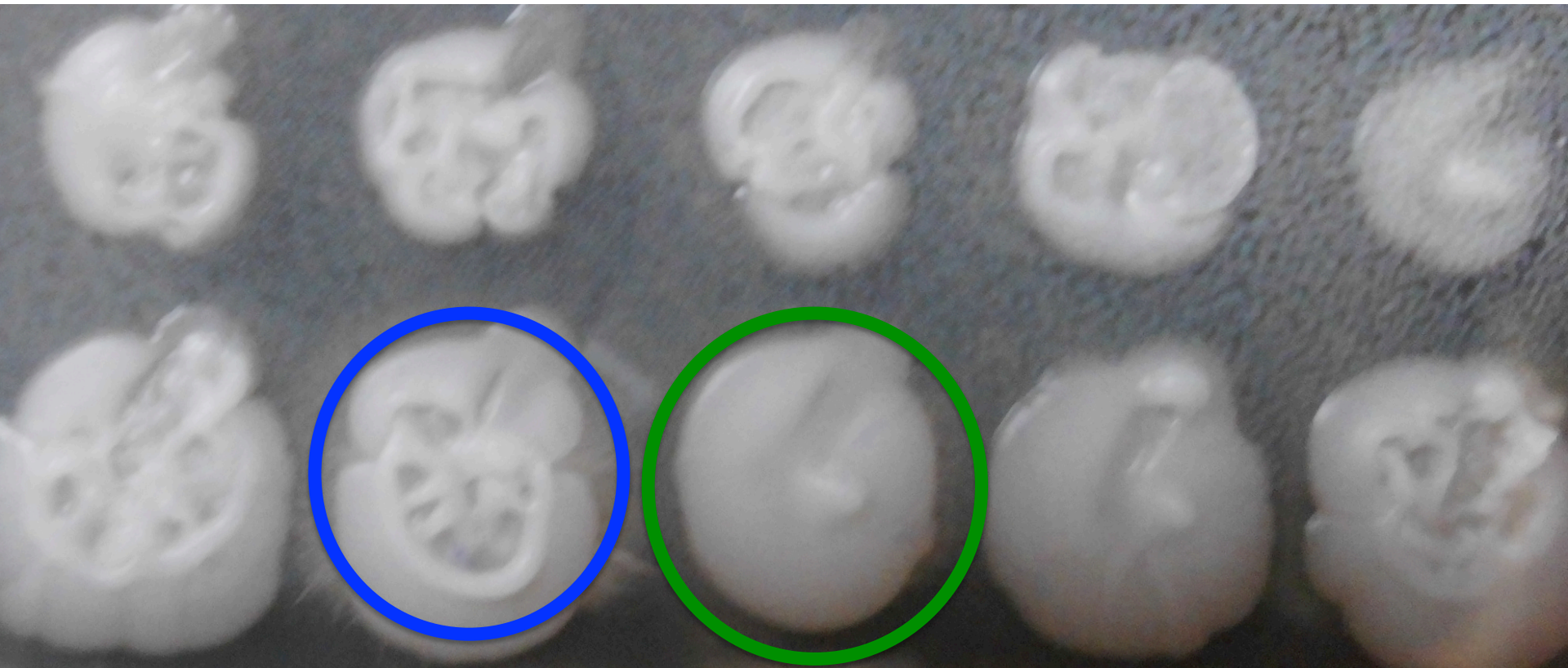
Control PCR: gDNA (12/96 per plate)

8 mating of cells



Mating

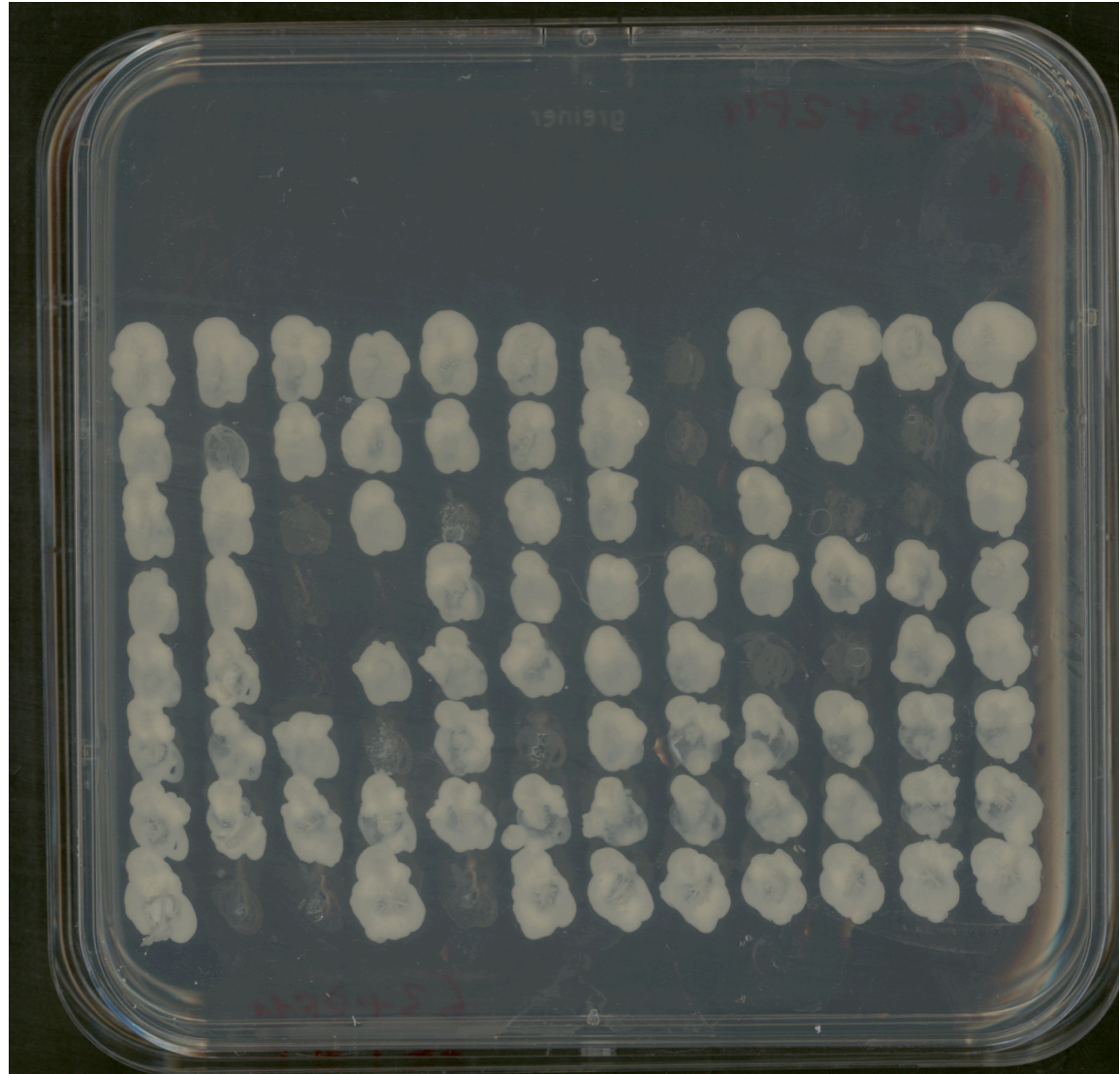
- Dry => Robot? No!
=> **Manual: frogger!**



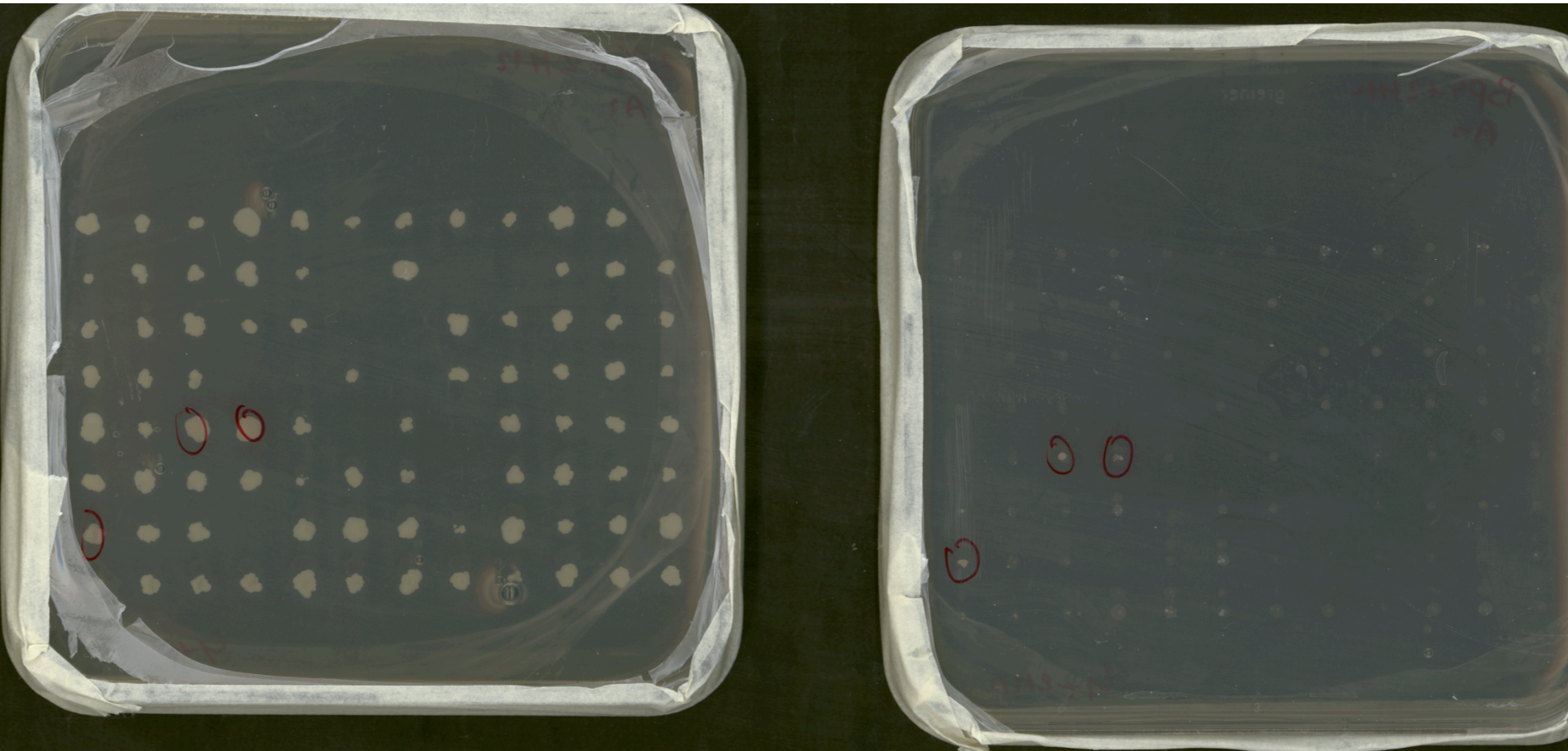
Selection after mating

Growth vs no growth

SC-Leu-Arg

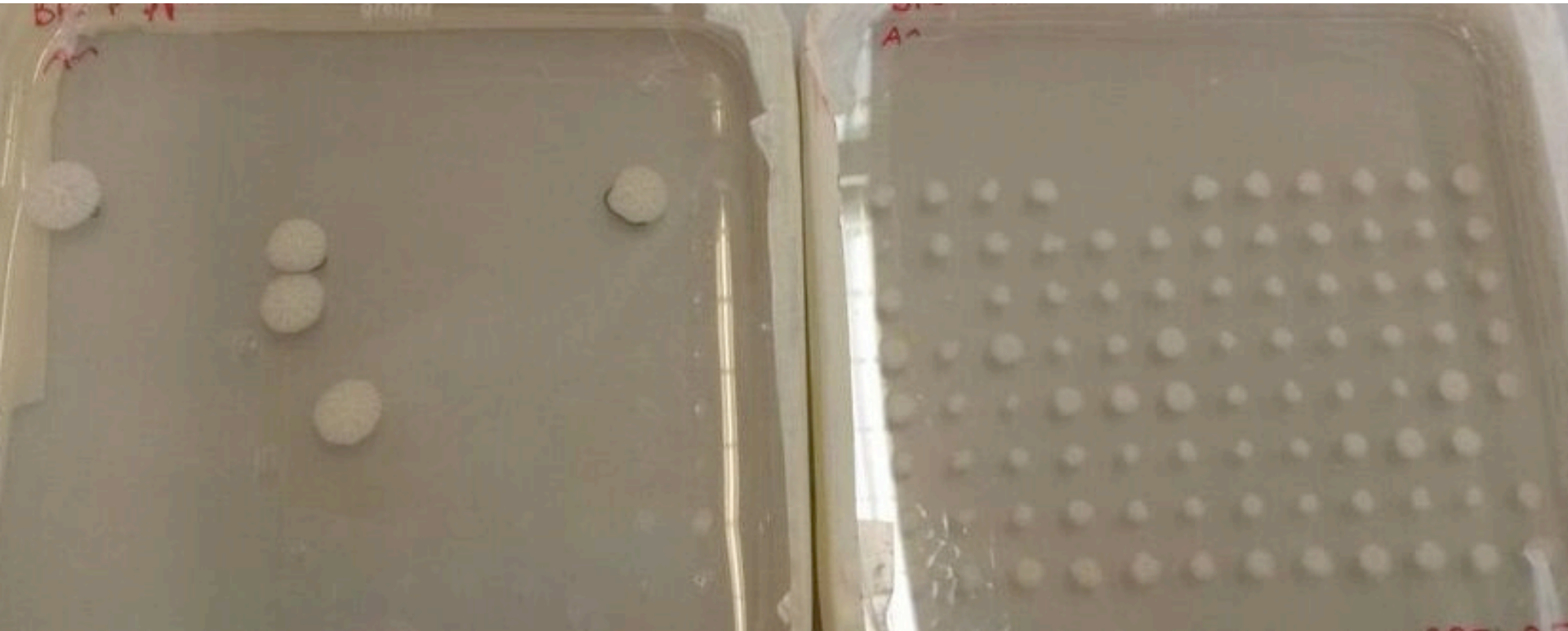


Selection Interaction (medium lacking his/meth)



No water vs water (diluting)
2 days

Selection Interaction (medium lacking his/meth)



Both pinned on sc-met-his medium but different suppliers,

Left: USBiologicals (7 days)

Right: Formedium (4 days)

So far...

- 1581 prey clones
 - 9 baits tested: totaling $1581 * 9 = 14\ 229$ interactions tested
- ⇒ 92% success rate for the mating
- ⇒ **32** (0,25%) potential interactions, of which 7 (0,055%, 22%) known to interact in yeast
- OR **4 (1)** !!
- ⇒ **Old** vs **NEW** medium

Thank you for your attention