# Smart-pooling for interactome mapping

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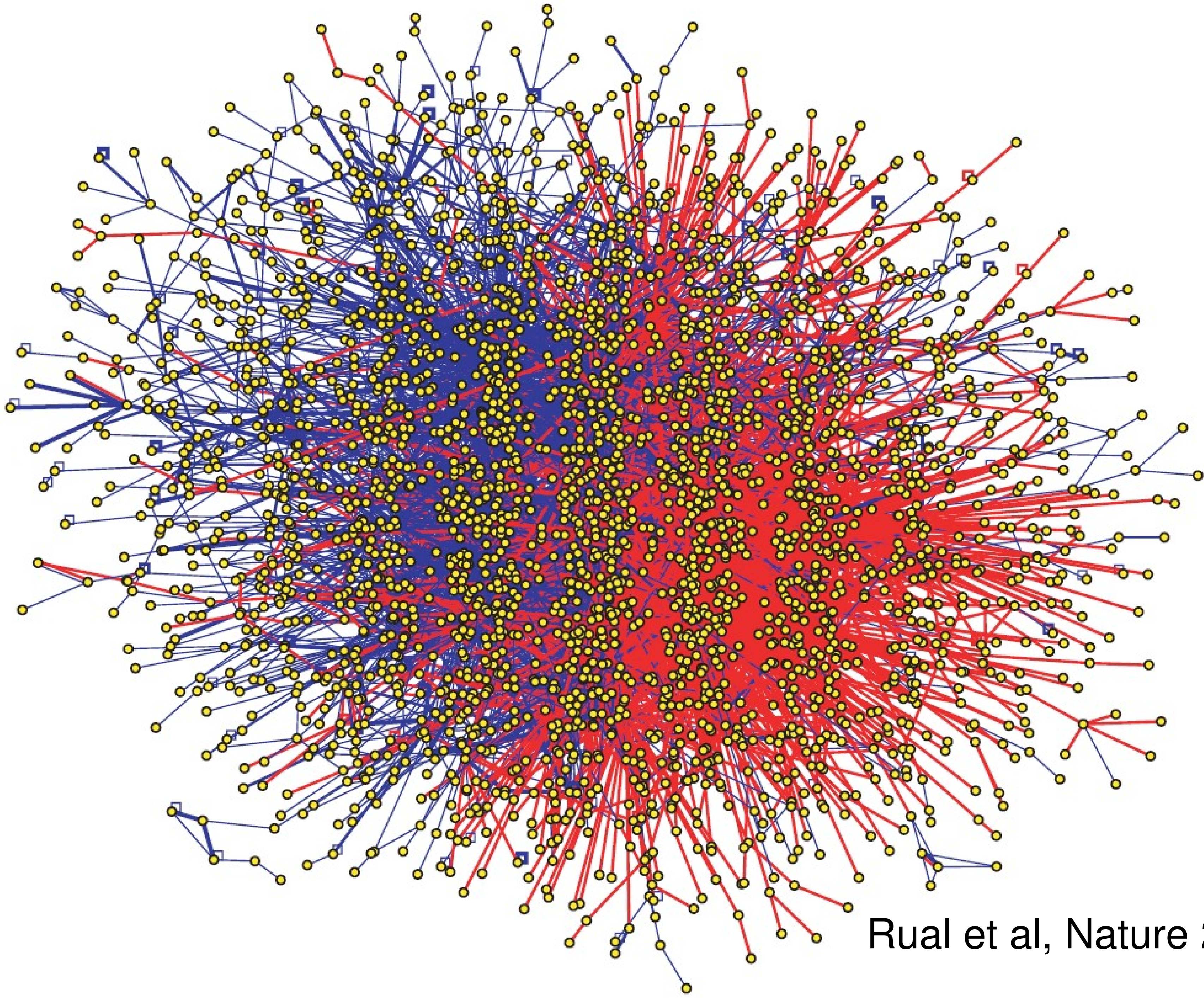


Rual et al, Nature 2005

## CCSB-HI1

Assay: yest two-hybrid (Y2H)

Space: 8100x8100

2800 interactions

125 retested by co-AP: ~80% success

-> few (technical) false positives, but many false negatives

Protocol:

- one bait against mini-pools of 188 preys, 96-well format
- identification by sequencing
- pairwise retests

# Smart-pooling

Y2H and many other HT experiments:

- basic **yes-or-no test** to a large collection of "objects"
- **low-frequency** positives
- **experimental noise**

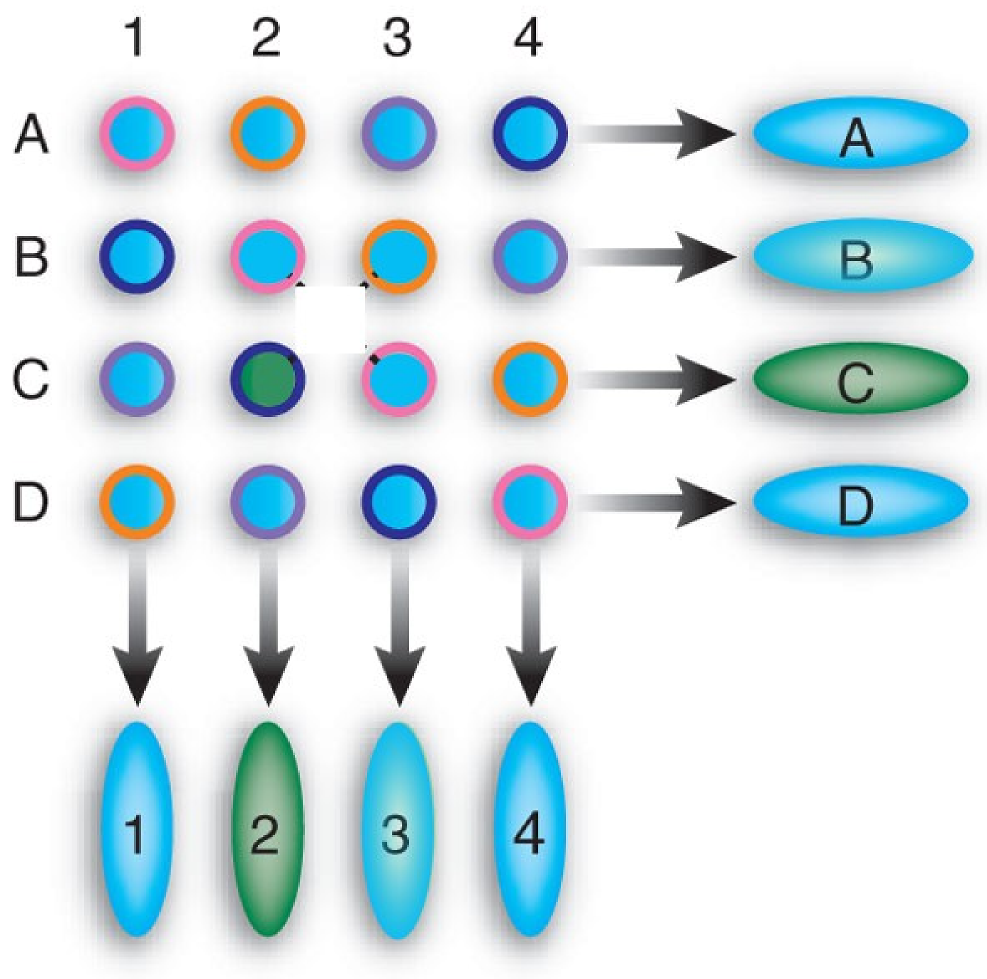
**Smart-pooling:** increase **efficiency**, **accuracy** and **coverage**, provided that

- objects individually available (eg ORFeome)
- basic assay works on pools (logical OR)
- Cherry-picking robot...

**Method:**

- **small number** of **redundant** pools
- **direct identification** (eg no sequencing in Y2H)
- deal with **false positives & negatives**

# Example: rows-and-columns design



16 probes (A1-D4)

one pool per row (A-D) & column (1-4)

If **C and 2 positive**, then C2 is the only positive probe.

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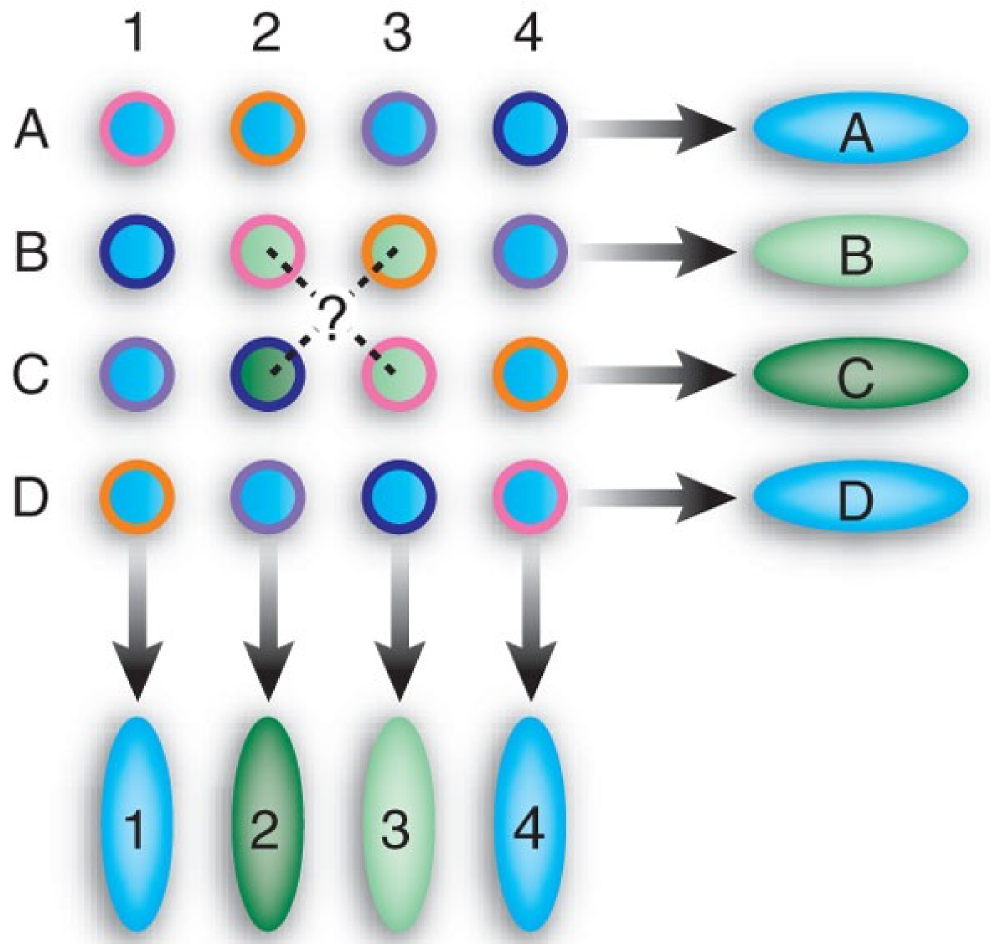

16 probes (A1-D4)

one pool per row (A-D) & column (1-4)

If **C and 2 positive**, then C2 is the only positive probe.

But if **B and 3 also positive**, the two solutions (B2 and C3) or (B3 and C2) cannot be distinguished.

Resolved by adding 4 'diagonal' pools.

Still, not a great design!

(from: Thierry-Mieg N. Pooling in systems biology becomes smart. Nat Methods. 2006 Mar;3(3):161-2.)

# The pooling problem

- Pooling problem (Combinatorial Group Testing problem) (n,t,E):
  - $\mathcal{A}_n$ a set of Boolean variables (n≈100-$10^4$)
  - t = max number of positives (≈1-10)
  - E = max number of errors (≈1-40% of tests)

Pool: subset of $\mathcal{A}_n$ , value=OR

Goal: build a set of **v** pools

- v as small as possible
- guarantee correction of errors & identification of positives

# Matrix representation

v×n Boolean matrix: M(i,j) true ⇔ pool i contains variable j

Example: n=9, $\mathcal{A}_9 = \{0, 1, \ldots, 8\}$ :

$$\begin{array}{c|c} & \text{pools} \\ \left[\begin{array}{ccccccccc} 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \end{array}\right] & \begin{array}{c} \{0, 3, 6\} \\ \{1, 4, 7\} \\ \{2, 5, 8\} \end{array} \end{array}$$

"layer" = partition of $\mathcal{A}_n$

## Shifted Transversal Design: idea

"Transversal" construction: layers

"Shift" variables from layer to layer

- Limit co-occurrence of variables
- Constant-sized intersections between pools

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STD(n;q;k) : **n** variables, **q** prime, $q < n$, **k** number of layers ($k \leq q+1$)

- First q layers: symmetric construction, q pools of size n/q or 1+n/q
- If $k=q+1$ : additional singular layer, up to q pools of heterogeneous sizes

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Let:

- $\sigma_q$ circular permutation on $\{0,1\}^q$ : $\sigma_q \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_q \end{bmatrix} = \begin{bmatrix} x_q \\ x_1 \\ \vdots \\ x_{q-1} \end{bmatrix}$
- $\Gamma(q,n) = \min\{\gamma \mid q^{\gamma+1} \geq n\}$

# STD construction

$\forall$ j $\in$ {0,...,q}: $M_j$ qxn Boolean matrix, representing layer L(j)

columns $C_{j,0}, \ldots, C_{j,n-1}$ :

$C_{0,0} = \begin{bmatrix} 1 \\ 0 \\ \vdots \\ 0 \end{bmatrix}$, and $\forall i \in \{0, \ldots, n\}$ $C_{j,i} = \sigma_q^{s(i,j)}(C_{0,0})$ where:

- if j < q : $s(i,j) = \sum_{c=0}^{\Gamma} j^c \left\lfloor \frac{i}{q^c} \right\rfloor$
- If j = q (singular layer) : $s(i,q) = \left\lfloor \frac{i}{q^\Gamma} \right\rfloor$

For k $\in$ {1,...,q+1} , STD(n;q;k) = L(0) $\cup$ ... $\cup$ L(k-1)

# STD example: n=9, q=3

$$M_0 = \begin{bmatrix} 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \end{bmatrix}$$

L(0) = {{0,3,6},{1,4,7},{2,5,8}}

$$M_1 = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 0 \end{bmatrix}$$

L(1) = {{0,5,7},{1,3,8},{2,4,6}}

$$M_2 = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \end{bmatrix}$$

L(2) = {{0,4,8},{1,5,6},{2,3,7}}

$$M_3 = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \end{bmatrix}$$

L(3) = {{0,1,2},{3,4,5},{6,7,8}}

STD(n=9;q=3;k=2) = L(0) $\cup$ L(1)

# STD example: n=9 to 27, q=3

n=9, q=3, third layer (j=2): $M_2 = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \end{bmatrix}$

n=27, q=3, j=2: $s(i,j) = \sum_{c=0}^{\Gamma} j^c \left[\!\left[ \frac{i}{q^c} \right]\!\right] = i + 2\left[\!\left[ \frac{i}{3} \right]\!\right] + 4\left[\!\left[ \frac{i}{9} \right]\!\right]$

+1

+(1+j)

$+(1+j+j^2)$

$$M_2 = \left[\begin{array}{ccccccccc|ccccccccc|ccccccccc} 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \end{array}\right]$$

# STD Properties

- Theorem: number of pools that contain any 2 variables is at most $\Gamma(q,n)$
- Proof: layers j = roots of non-zero polynomial on GF(q) of degree at most $\Gamma$
- Example: n=9, q=3

L(0) = {{0,3,6}, {1,4,7}, {2,5,8}}
L(1) = {{0,5,7}, {1,3,8}, {2,4,6}}
L(2) = {{0,4,8}, {1,5,6}, {2,3,7}}
L(3) = {{0,1,2}, {3,4,5}, {6,7,8}}

0 appears exactly once ($\Gamma$=1) with each other variable.

# A solution to the pooling problem

- **Corollary:** If there are **at most t positive variables** in $\mathcal{A}_n$ and **at most E false positive and E false negative observations**: STD(n;q;k) is a solution, when choosing q prime such that $t \cdot \Gamma(q,n)+2 \cdot E \leq q$, and $k = t \cdot \Gamma + 2 \cdot E + 1$

- Constructive proof: exhibit a simple algorithm that works
  Algorithm relies on knowledge of E

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- Constructive proof: exhibit a simple algorithm that works
  Algorithm relies on knowledge of E

▶ STD is sound

▶ Allows to compare with other published designs: favorable (on numerical examples)

# Even redistribution of variables

**Theorem:** Let m ≤ k ≤ q and consider $\{P_1,\ldots,P_m\} \subset STD(n;q;k)$, each belonging to a different layer. Then:

$$\lambda_m \le \left|\bigcap_{h=1}^{m} P_h\right| \le \lambda_m + 1 \text{ , where } \qquad \lambda_m = \sum_{c=m}^{\Gamma} \left( \left[\!\left[ \frac{n-1}{q^c} \right]\!\right] \% \, q \right) q^{c-m}$$

**Notes:**

- $\lambda_m$ depends only on m, not on the choice of the pools $P_1,\ldots,P_m$

→ every pool, and every intersection between 2 or more pools, is redistributed evenly in each remaining layer

- L(q) does not work (k ≤ q)

# Using STD

- In practice: tolerate a few ambiguous variables → many fewer pools
  Example: n=10000, t=5, error-rate 1%
  - guarantee requires 483 pools
  - when tolerating up to 10 ambiguous variables, 143 pools prove sufficient

- Given (n,t,E-rates) and “ambiguity tolerance”, find optimal parameter values by simulation

- Difficulty: “decode” observed pool values

## Interpreting smart-pooling results

Decoding an observation: a combinatorial optimization problem

Difficult for general solvers (eg integer linear programming)

▶ Interpool: an algorithm to solve it

- Branch-and-bound
- Exact
- Fast (usually)
- GNU GPL

Manuscript under review

## Validation

- Pilot project: 100 baits x 940 preys

Varied subspace of CCSB-HI1: many interactions, hubs, auto-activators...

Choosing the design: simulations with interpool

## STD(940;13;13), 10% FPR

| Positives | FNR | TPs missed | Retests | Simulations | Time |
|---|---|---|---|---|---|
| 2 | 10% | 0 | 2.26 | 10000 | 1m |
|  | 20% | 0 | 2.26 |  | 1m |
|  | 30% | 1.2% | 2.27 |  | 4m |
| 3 | 10% | 0 | 3.57 | 10000 | 4m |
|  | 20% | 0.4% | 3.58 |  | 33m |
|  | 30% | 3.4% | 3.60 |  | 2h |
| 4 | 10% | 0 | 5.06 | 10000 | 32m |
|  | 20% | 1.0% | 5.11 | 10000 | 10h39m |
|  | 30% | 6.2% | 5.26 | 7500 | 2d11h |
| 5 | 10% | 0.1% | 6.71 | 10000 | 4h |
|  | 20% | 1.7% | 6.94 | 1000 | 12h47m |
|  | 30% | 12.9% | 7.88 | 300 | 3d10h |

TPs missed and Retests: upper bounds of the 95% confidence intervals

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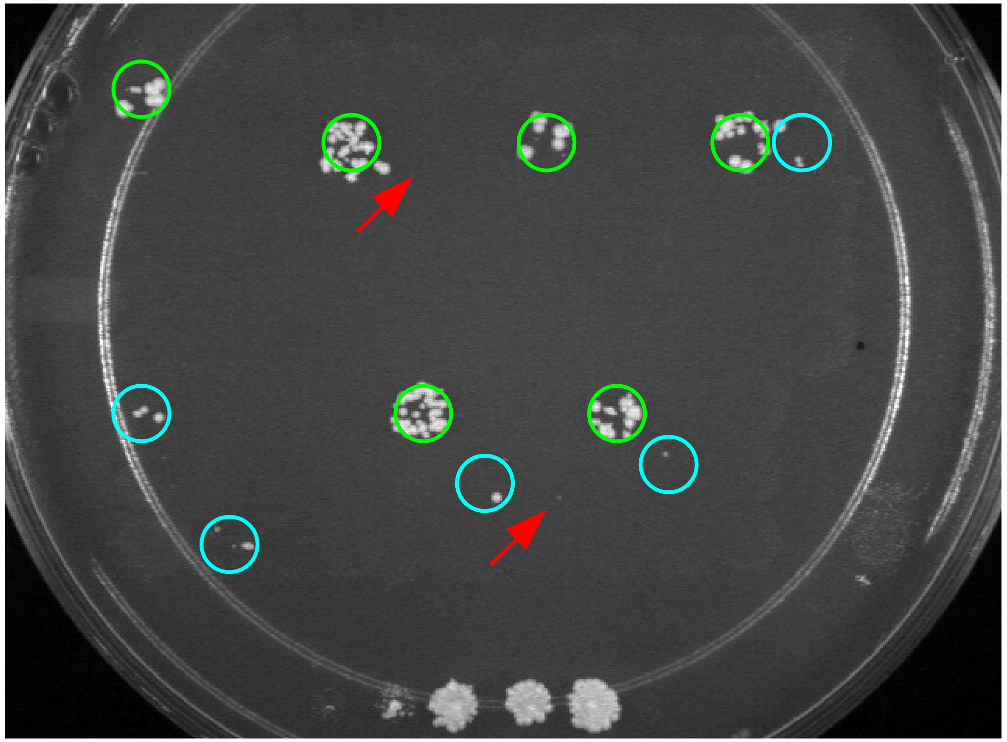
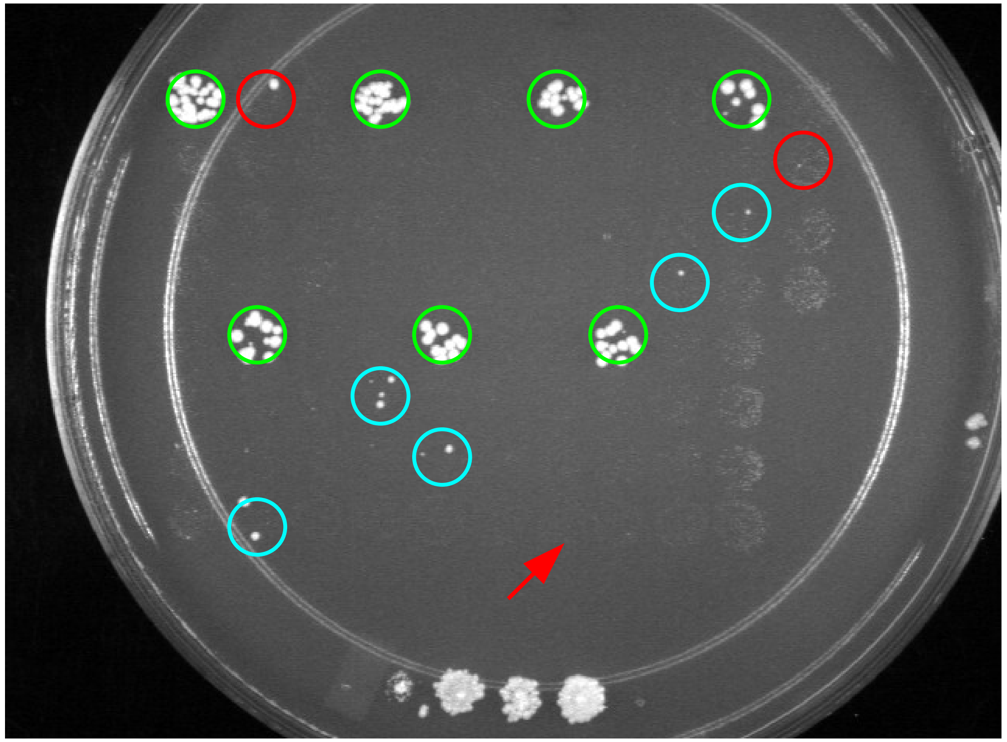
Varied subspace of CCSB-HI1: many interactions, hubs, auto-activators...

- Smart-pooled the 940 preys according to STD(940;13;13)
  - 169 pools, 73 preys in each pool
  - each prey is in 13 pools
  - at most 2 pools contain any pair

  $\rightarrow$ 3 pools for identification, 10 pools for errors and multiple positives
- Screened each bait against the 169 pools, scored positive pools
- Decoded the patterns of positive pools (interpool) -> putative positives
- Pairwise retests

# Example with one bait





Circles: spots scored positive.

Decoding finds:

- 2 interactors: green (no FNs), and blue (3 FNs = red arrows)
- 2 FPs (red circles)

## Results

- Identified 65 putative interactions

- Retest: 60 passed, 3 failed, 2 unconfirmed (auto-activation in the retest)
  $\rightarrow$ **Specificity between 92% and 95%**

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- 60 confirmed = 36 CCSB-HI1 + 24 novel

▶ **Recall of CCSB-HI1 data**: the 36 represent 73% of CCSB-HI1, or 84% when excluding the two hardest baits (strong hub, auto-activator)

▶ **Sensitivity vs CCSB-HI1**: Difficult because subspace strongly biased

Low estimate: **172% higher sensitivity**

High estimate: **325% higher sensitivity**

## Summary

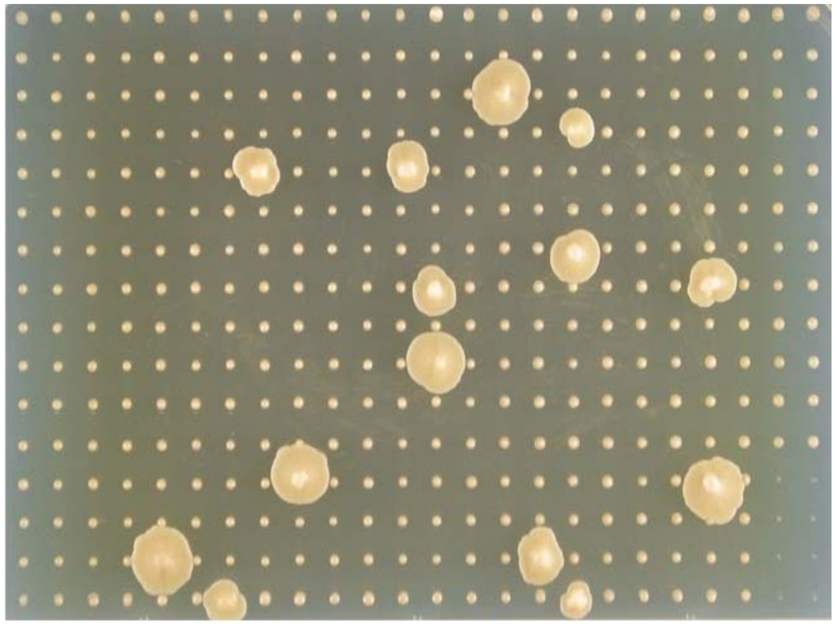
- **STD** (the Shifted Transversal Design) is a **flexible and efficient family of pooling designs**. On paper and in silico, STD performs very well.

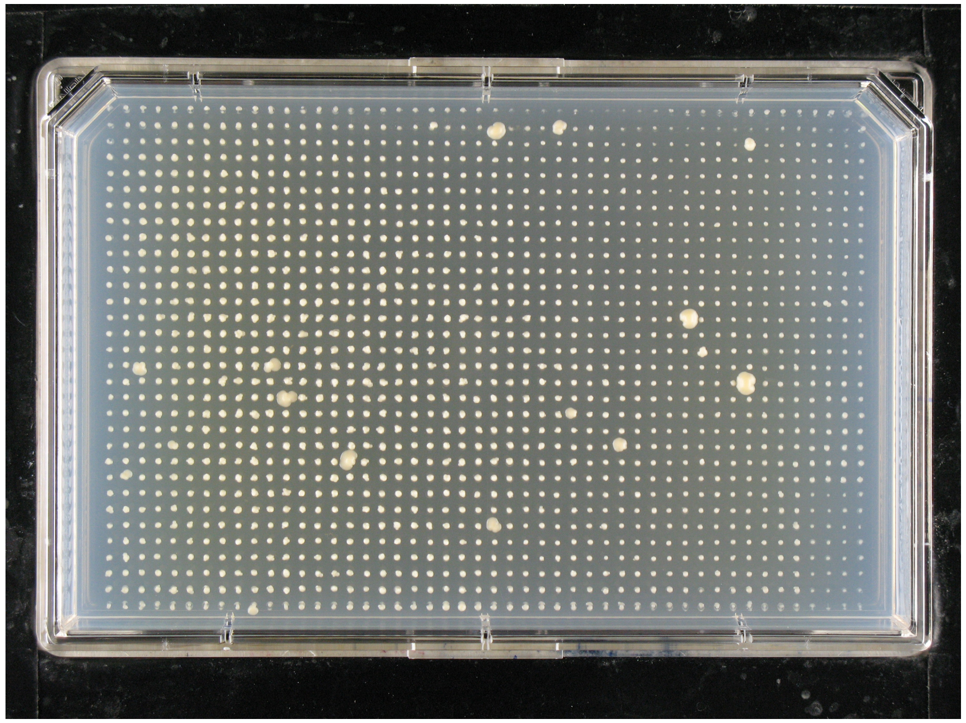
- **Interpool** is a **fast exact decoding algorithm**. Useful both for choosing a design (simulations) and for interpreting experimental results. Open source.

- **Smart-pooling really works** for HT-Y2H: it is efficient, sensitive and specific.

**Current work:** scaling up to the complete *C. elegans* ORFeome, using denser formats (384 and 1536)

Takes advantage of STD symmetries: build micro-pools, then combine at will





# Acknowledgments

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Thierry-Mieg N. A new pooling strategy for high-throughput screening: the Shifted Transversal Design. BMC Bioinformatics 2006, 7:28.

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