GHENT UNIVERSITY

CRIG

OncoRNALab

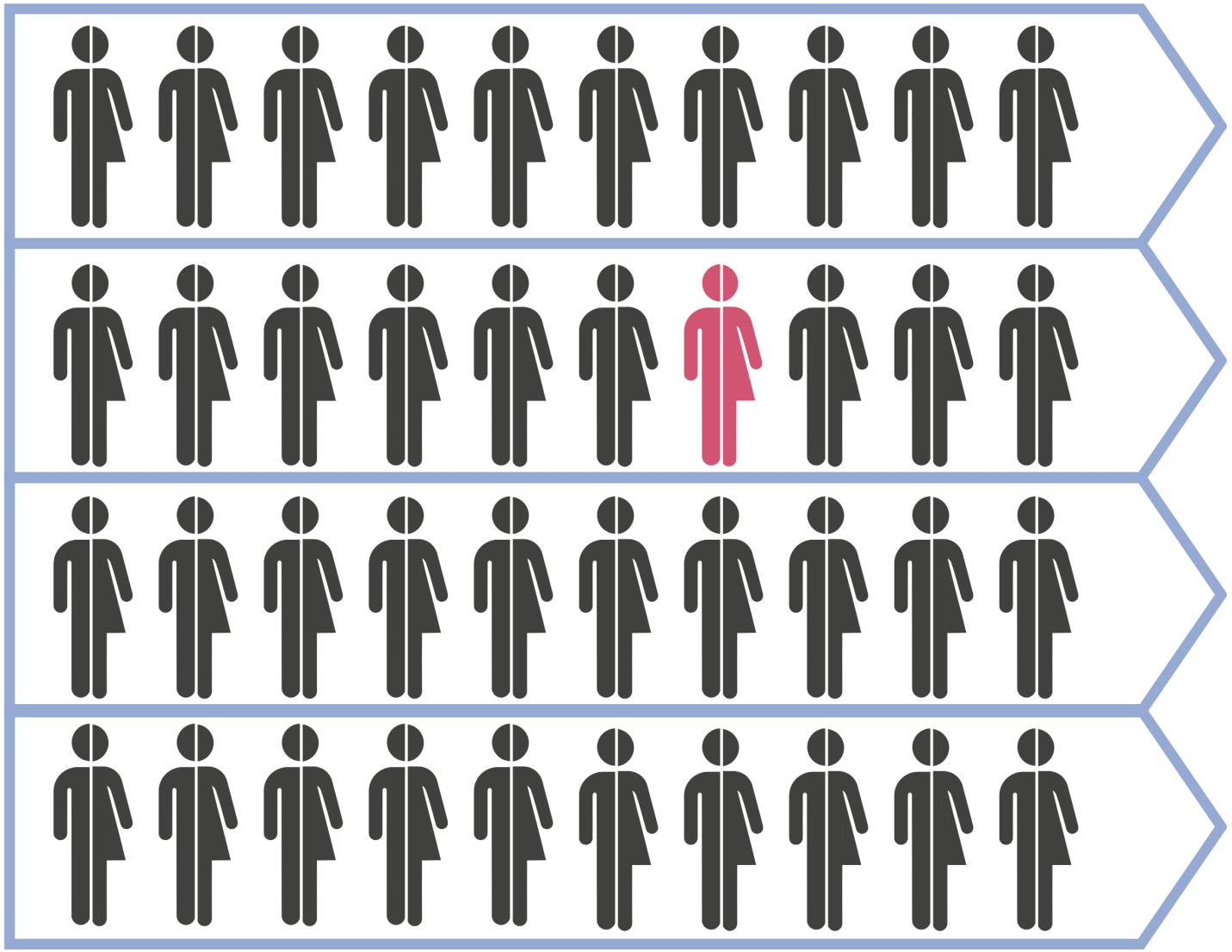
COVID-19 sample pooling: to pool or not to pool?

Jasper Verwilt

September 23, 2020

Test everybody!
40 people, 40 tests

Many tests… to find one patient…



Let's make pools!

1. Test each pool as a whole
2. Test samples from positive pool individually

40 people,

4 + 10 = 14 tests

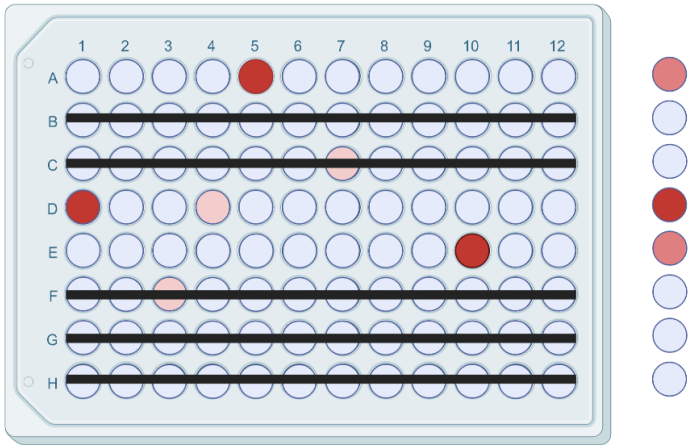
Why pooling?

1. Massive population screening
2. Lower cost
3. Quicker results for more people

Pooling strategies

1. One-time pooling (1D)
2. 2D-pooling
3. Sequential pooling
4. *P-BEST* or *Tapestry*

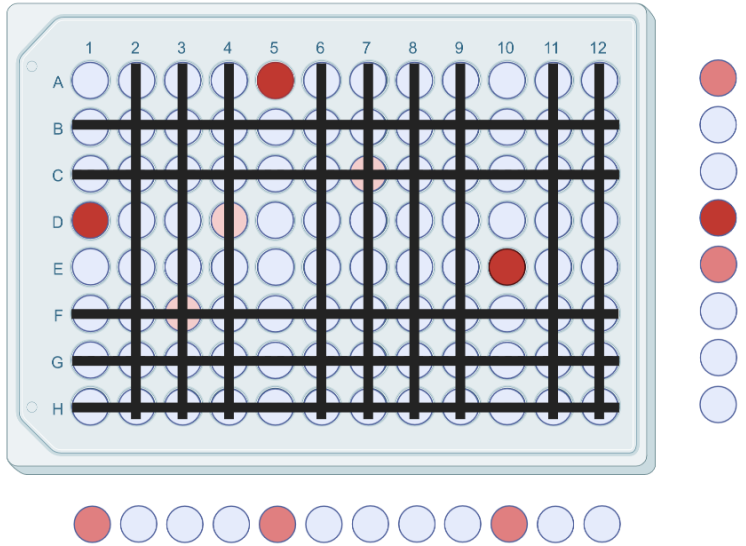
Pooling strategies



1. **One-time pooling (1D)**
    1. Make pools and test
    2. Positive pools tested individually
2. **2D-pooling**
3. **Sequential pooling**
4. ***P-BEST* or *Tapestry***

Pooling strategies

1. One-time pooling (1D)
2. **2D-pooling**
    1. Arrange in grid
    2. Make pools over columns and rows
    3. Test pools
    4. Remove negative rows and columns
    5. Test individual samples that remain
3. Sequential pooling
4. *P-BEST* or *Tapestry*



Sinnott-Armstrong, Nasa, Daniel Klein, and Brendan Hickey. "Evaluation of group testing for SARS-CoV-2 RNA." medRxiv (2020).

Pooling strategies

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3. Sequential pooling
4. *P-BEST and Tapestry*

Pooling strategies

1. One-time pooling (1D)
2. 2D-pooling
3. **Sequential pooling**
   - Go back to the same samples over and over
4. ***P-BEST and Tapestry***
   - Complex pipetting regimes

Not practical

Pooling strategies

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2. 2D-pooling
3. ~~Sequential pooling~~
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   - Complex pipetting regimes

Not practical

Pooling strategies

1. One-time pooling (1D)
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3. ~~Sequential pooling~~
4. ~~*P-BEST* or *Tapestry*~~

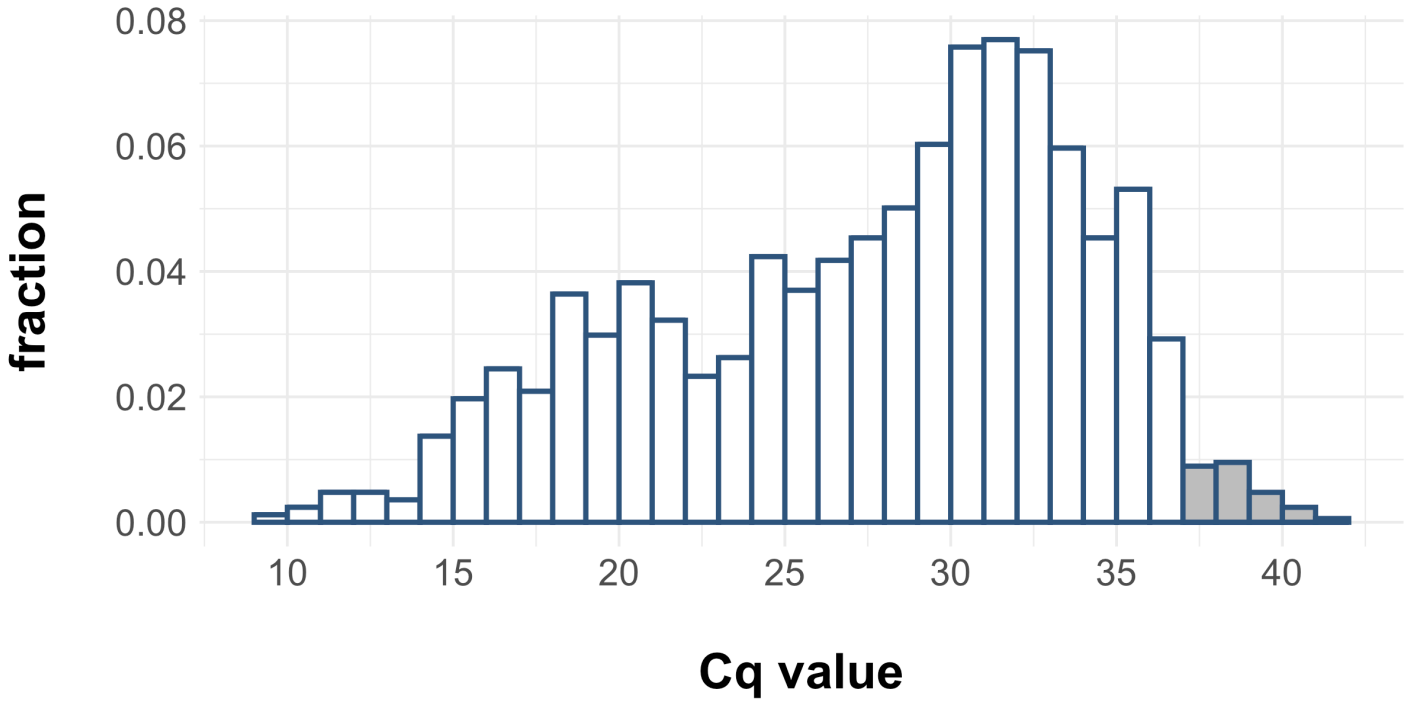
Simulation approach

1. **Use viral load proxy (Cq value) instead of binary absent/present (negative/positive)**
   - Dilution during pooling: *false-negatives*
2. **Screening**
   1. High number of samples
   2. Low number of infected/positive people
3. **Practical pools**
   - Think about microtiter plates (4, 8, 12, 16, 24)
4. **Use real life data**

Real-life data

- **Real Cq values from national testing platform**
- **Full** 96-well RNA plates with **less than 10 positives** and **good controls**

Mimic low prevalence screening



Experimental set-up

1. **No. of samples:** 100 000 samples
2. **Prevalence:** 0.01% to 10%
3. **Strategy:**
   - 1D-pooling: 4, 8, 12, 16, 24 samples
   - 2D-pooling: 8x12, 12x16, 16x24 matrix
4. **Replicate simulations:** 5

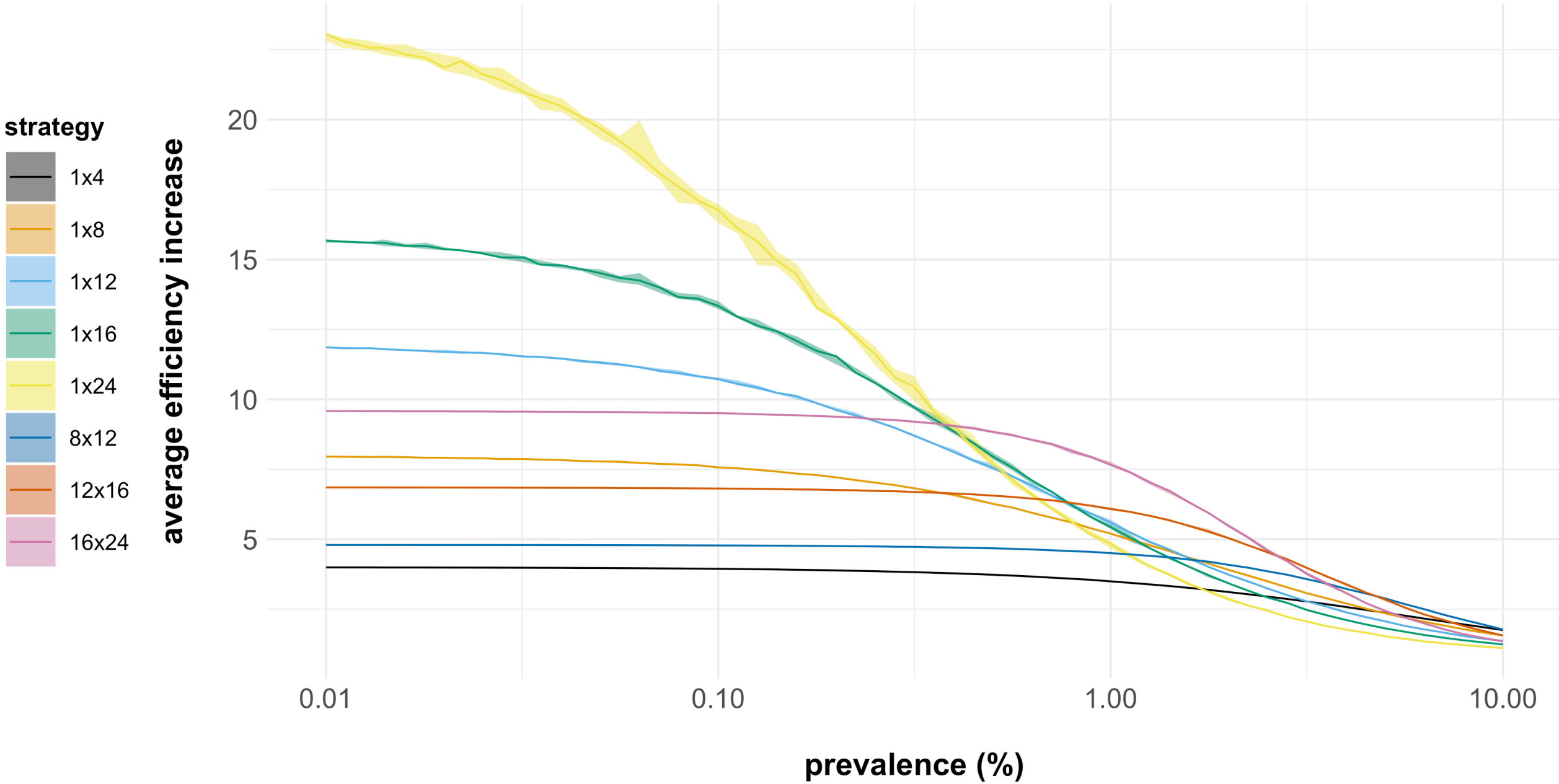
Output

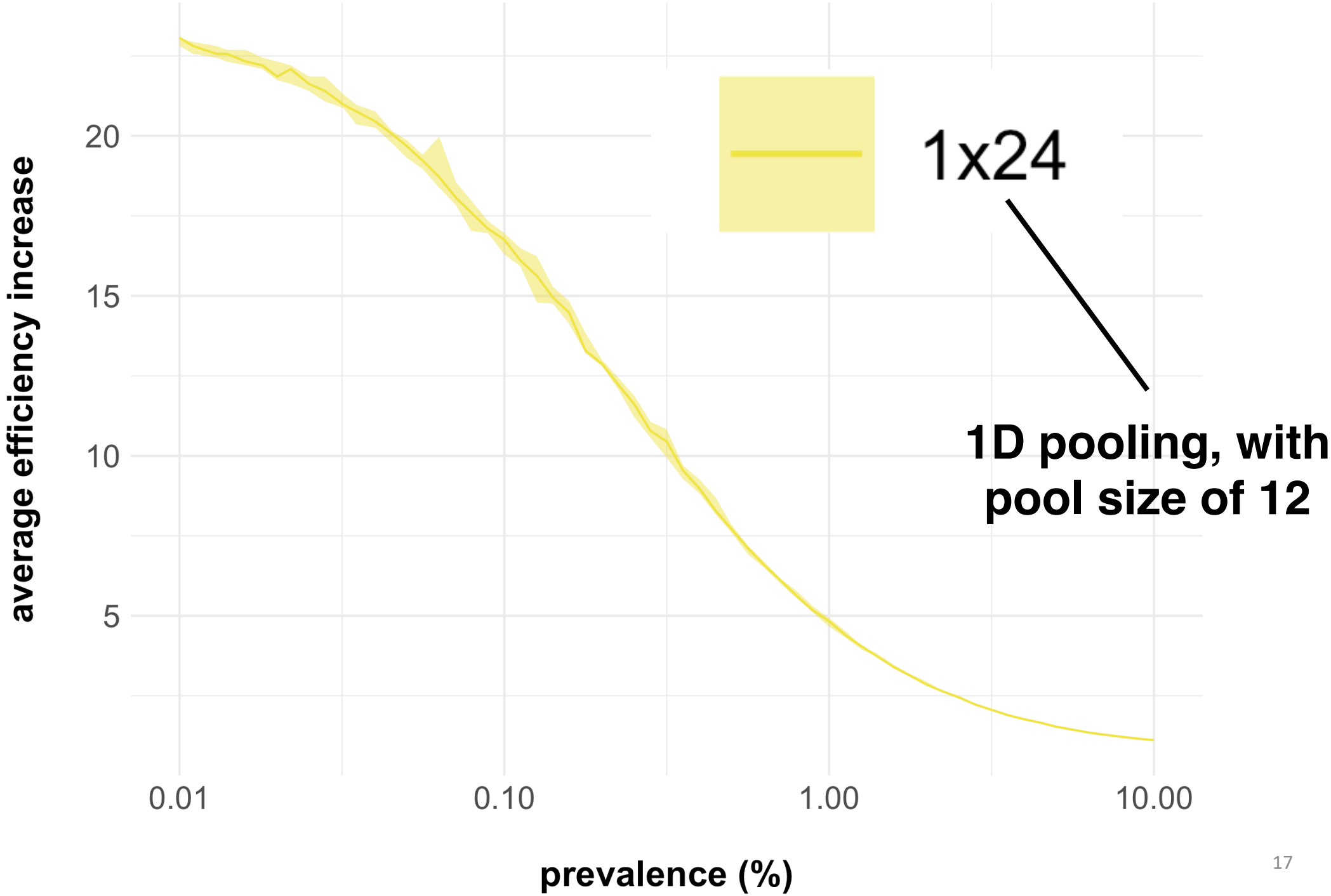
- **Efficiency increase**

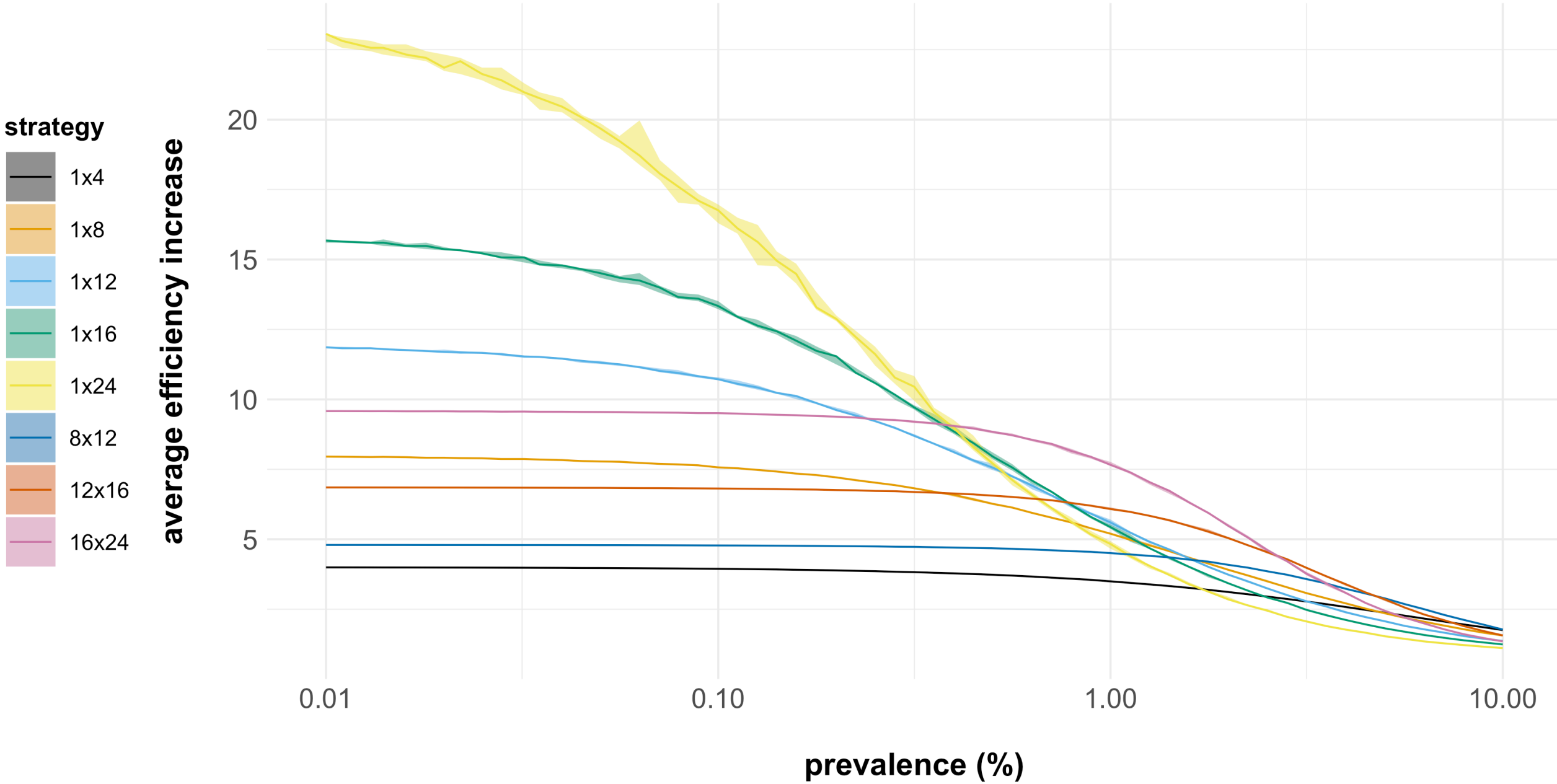
$$\frac{\#\ of\ tests\ without\ pooling}{\#\ of\ tests\ with\ pooling}$$

- **Sensitivity**

$$\frac{\#\ of\ true\ positives}{\#\ of\ true\ positives + \#\ of\ false\ negatives}$$


strategy
1x4
1x8
1x12
1x16
1x24
8x12
12x16
16x24
average efficiency increase
20
15
10
5
0.01
0.10
1.00
10.00
prevalence (%)


average efficiency increase
20
15
10
5
0.01
0.10
1.00
10.00
prevalence (%)
1x24
1D pooling, with
pool size of 12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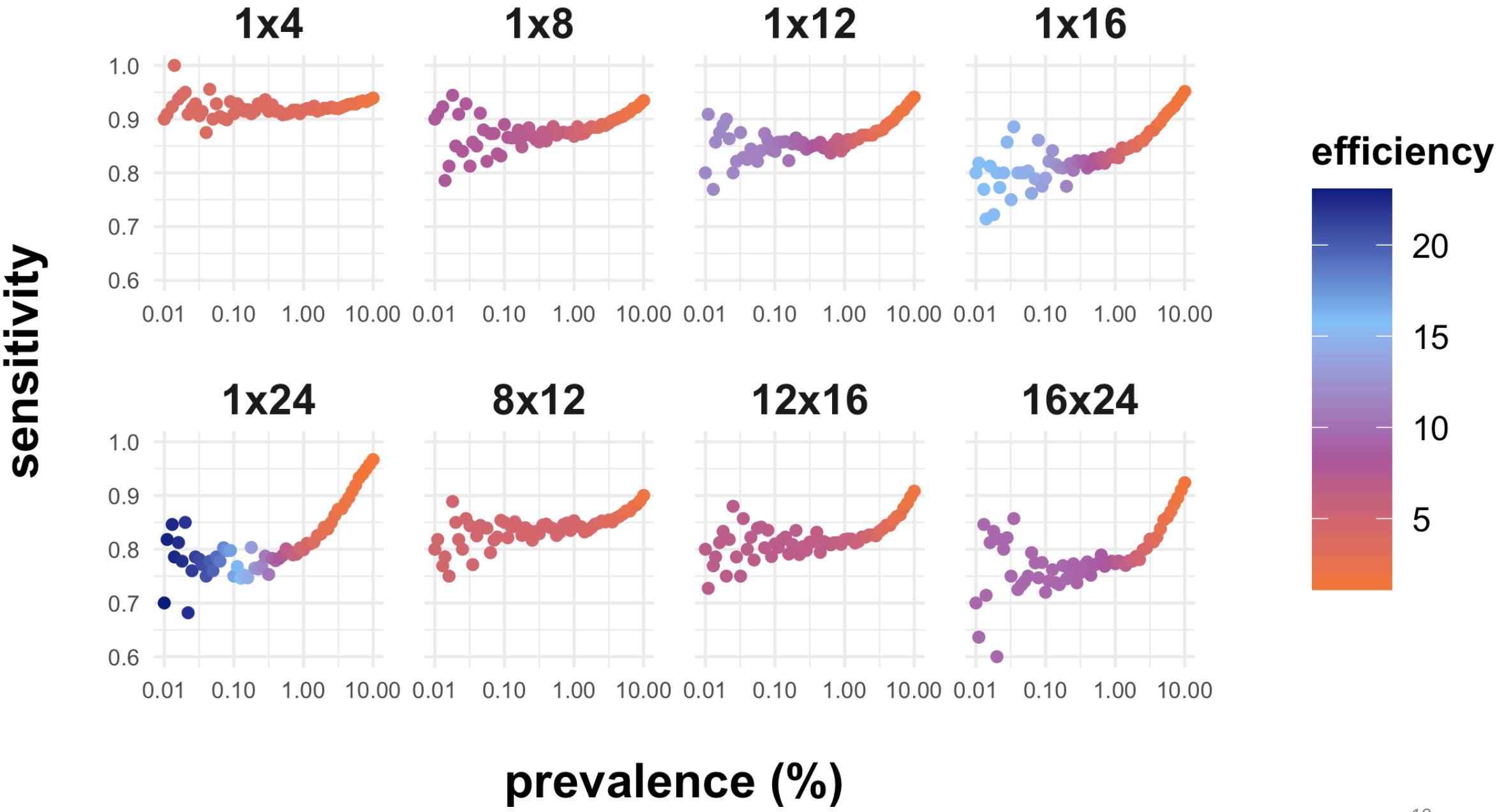
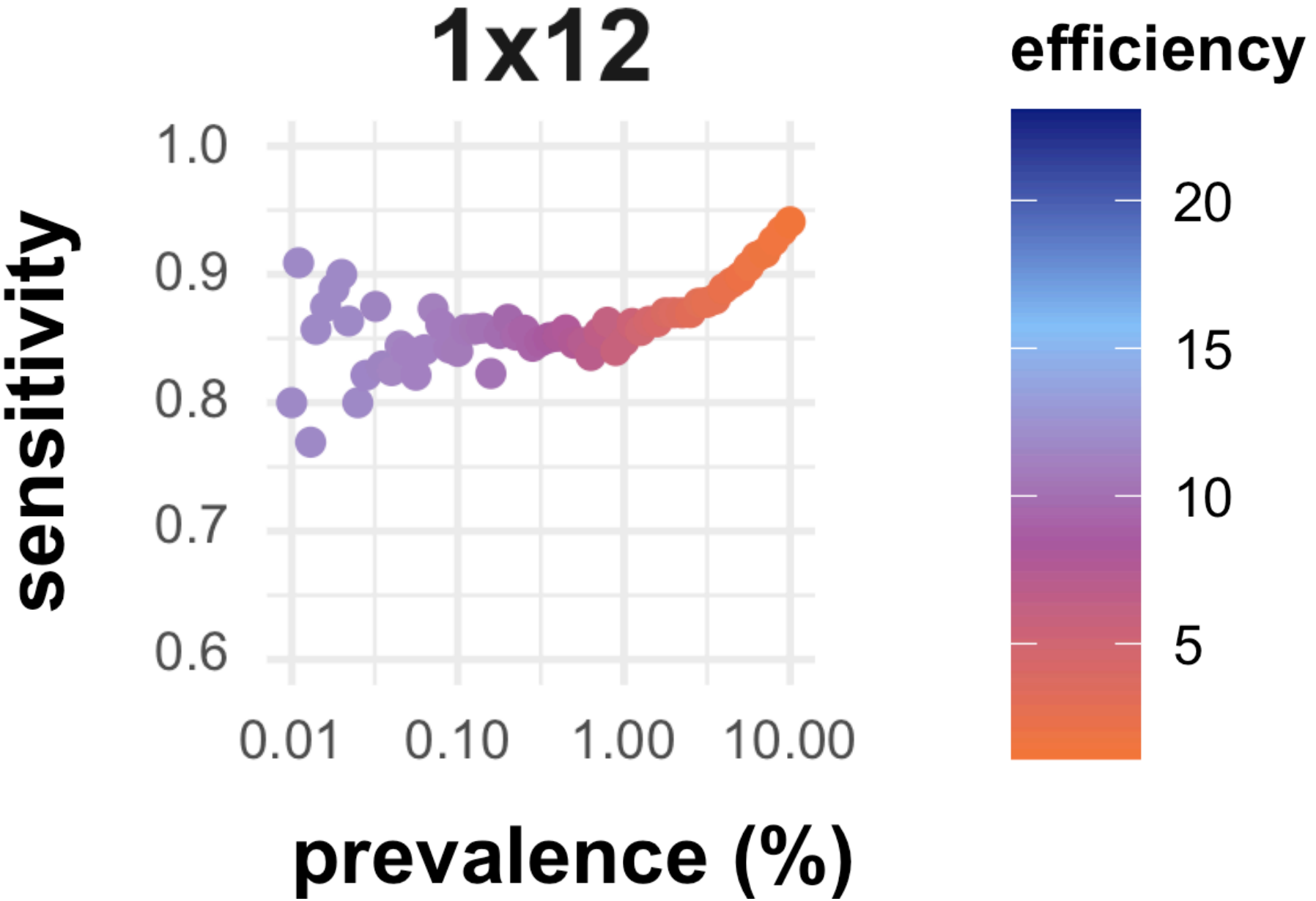
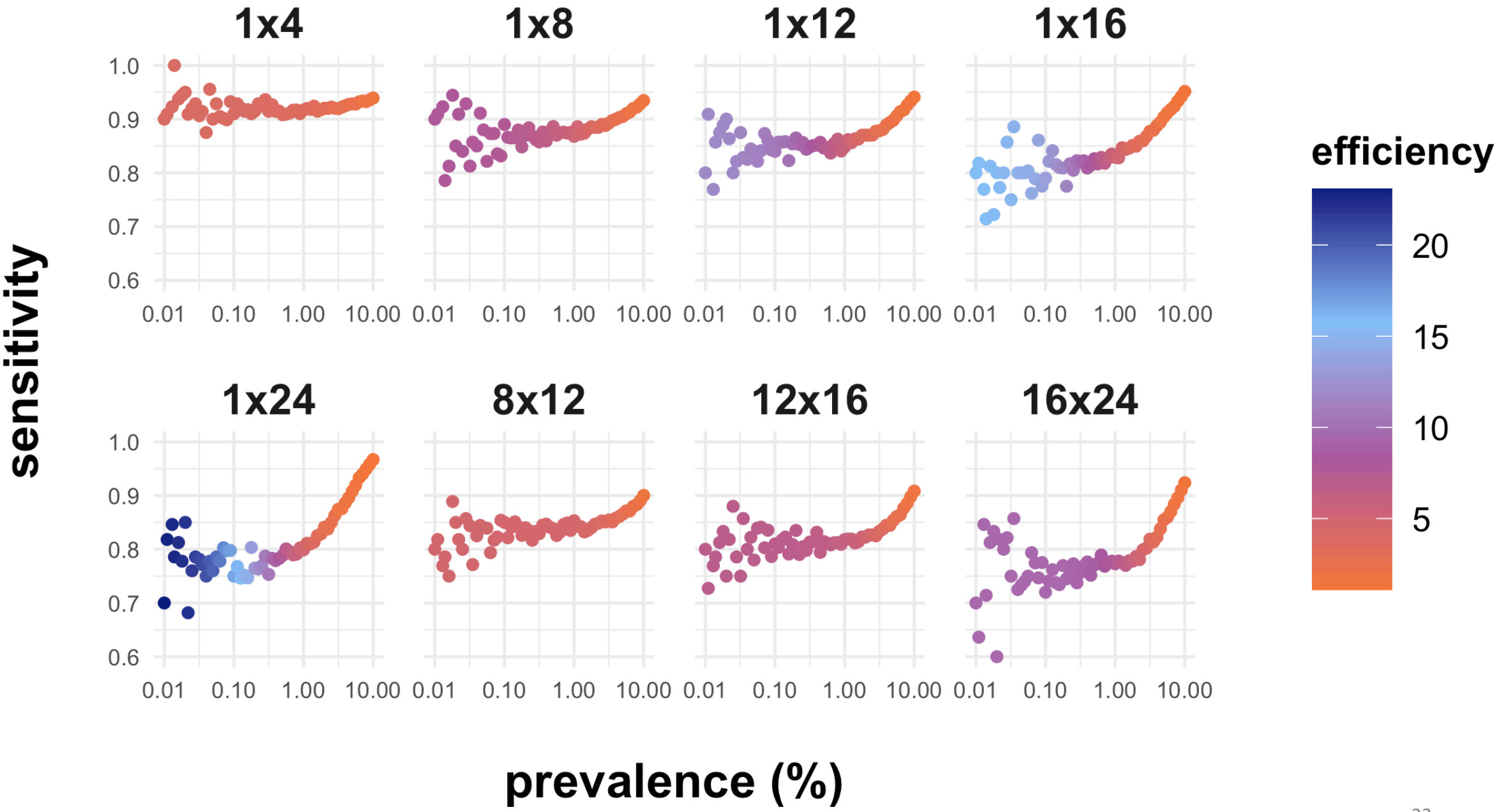

1x4
1x8
1x12
1x16
1x24
8x12
12x16
16x24
1.0
0.9
0.8
0.7
0.6
0.01
0.10
1.00
10.00
sensitivity
prevalence (%)
efficiency
20
15
10
5


1x12
efficiency
sensitivity
1.0
0.9
0.8
0.7
0.6
0.01
0.10
1.00
10.00
prevalence (%)
20
15
10
5

‘Rescue’

Low viral load samples, that in a pool would test negative due to dilution, can test positive when a high viral load sample is present in the same pool


1x4
1x8
1x12
1x16
1x24
8x12
12x16
16x24
sensitivity
prevalence (%)
efficiency
1.0
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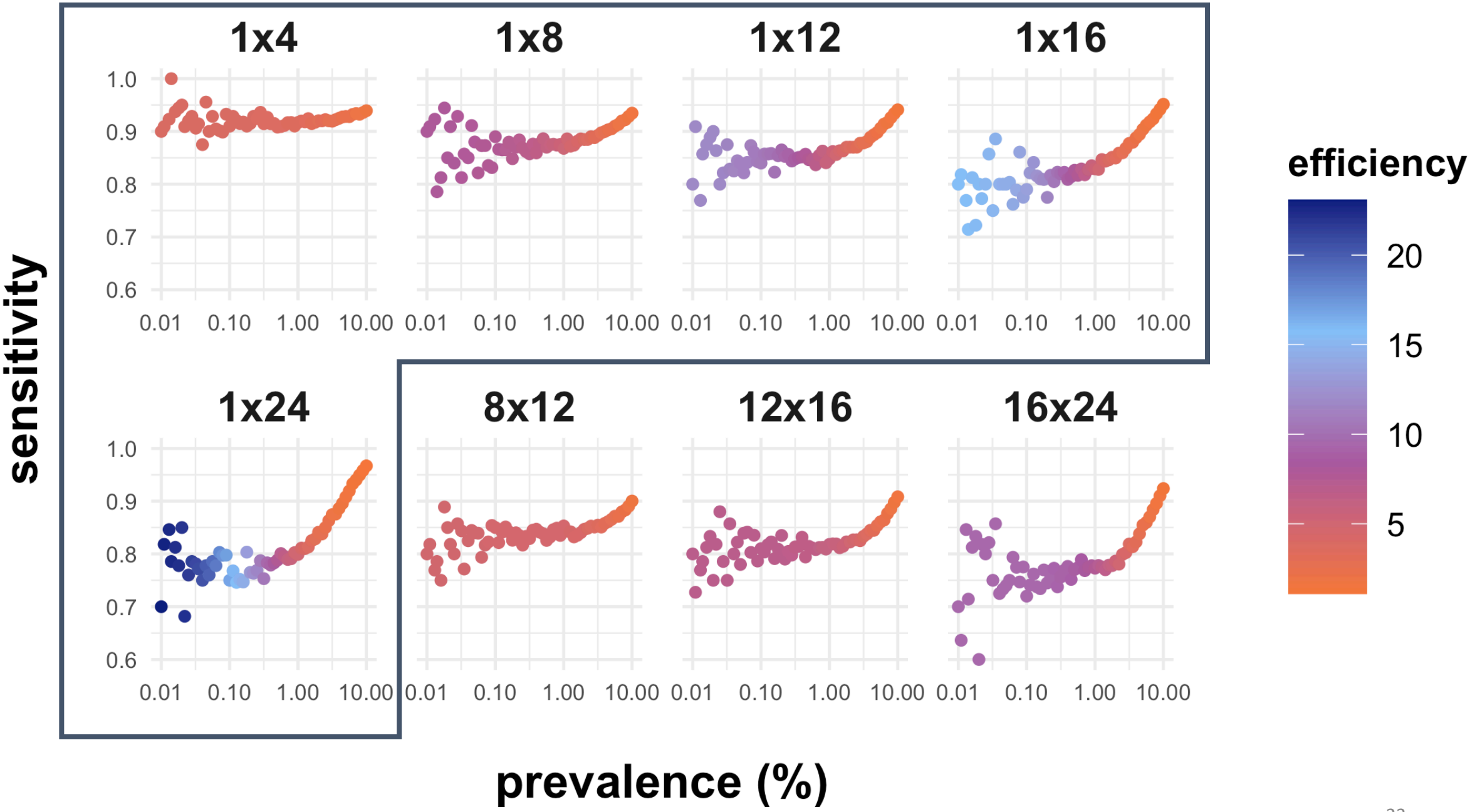
0.7
0.6
0.01
0.10
1.00
10.00
20
15
10
5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0.9
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0.01
0.10
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10.00
sensitivity
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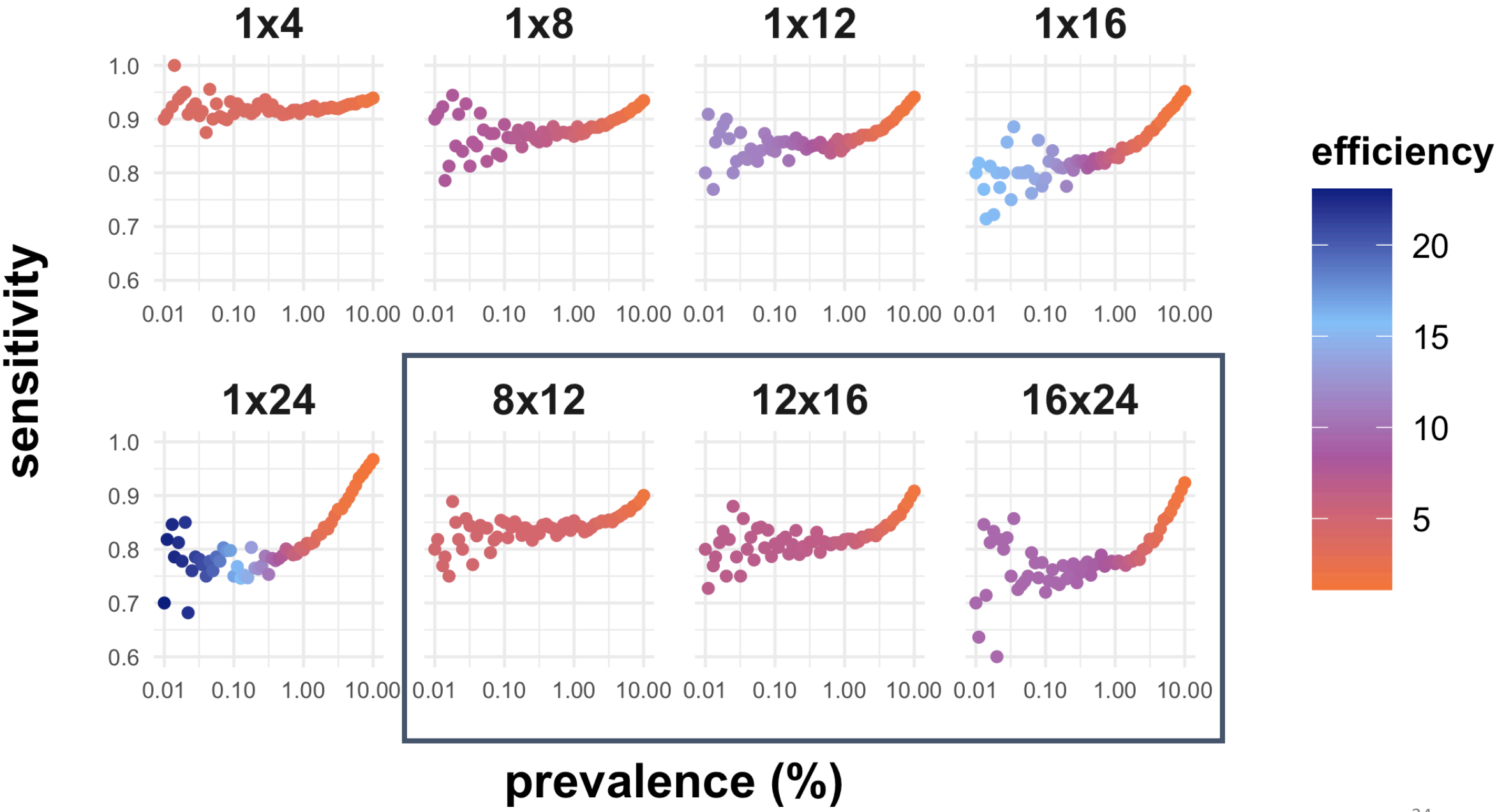
15
10
5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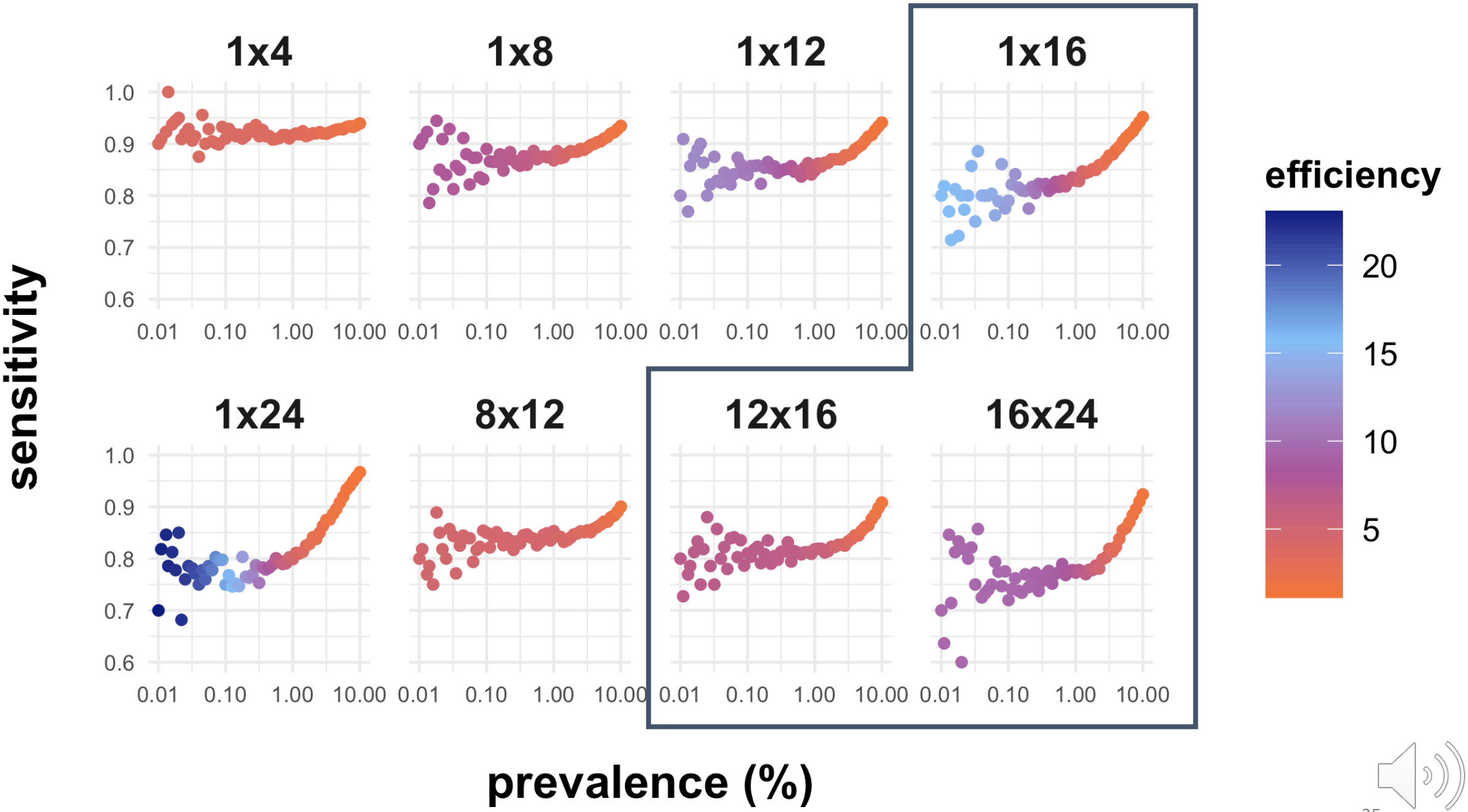
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10.00
sensitivity
prevalence (%)
efficiency
20
15
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5

Take-aways

1. At *high prevalence*, **2D is more efficient**
2. At *low prevalence*, **1D is more efficient**
3. Large pools have **better efficiency**
4. Large pools have **more false-negatives**

Evaluation of efficiency and sensitivity of 1D and 2D sample pooling strategies for diagnostic screening purposes

Jasper Verwilt, Pieter Mestdagh, Jo Vandesompele



medRχiv https://www.medrxiv.org/content/10.1101/2020.07.17.20152702v2

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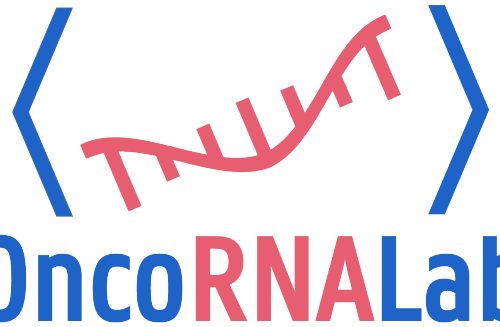
GENETICA GENT



- Jo Vandesompele
- Pieter Mestdagh
- Alexander Reinartz
- OncoRNALab

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