Building confidence in regulatory acceptance of in vitro high throughput transcriptomics - evaluating false positive rates across models

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Background and aims

- **High-throughput transcriptomics** (HTTr) can be used to profile the dose-dependent bioactivity of a substance and often provides a conservative estimate, e.g., [1].
- Acceptance of these methods is contingent on understanding the uncertainties and variance in both the experimental methods and the **analysis workflow**.
- Here we present a method to investigate the assessment of **false positive rate (FPR)** of several methodologies for dose-response analysis at both the probe/gene and pathway/signature level: tcplfit2 (v0.1.6), httrpathway (v0.2)+ tcplfit2, BMDExpress2 and BIFROST[2].

Methods

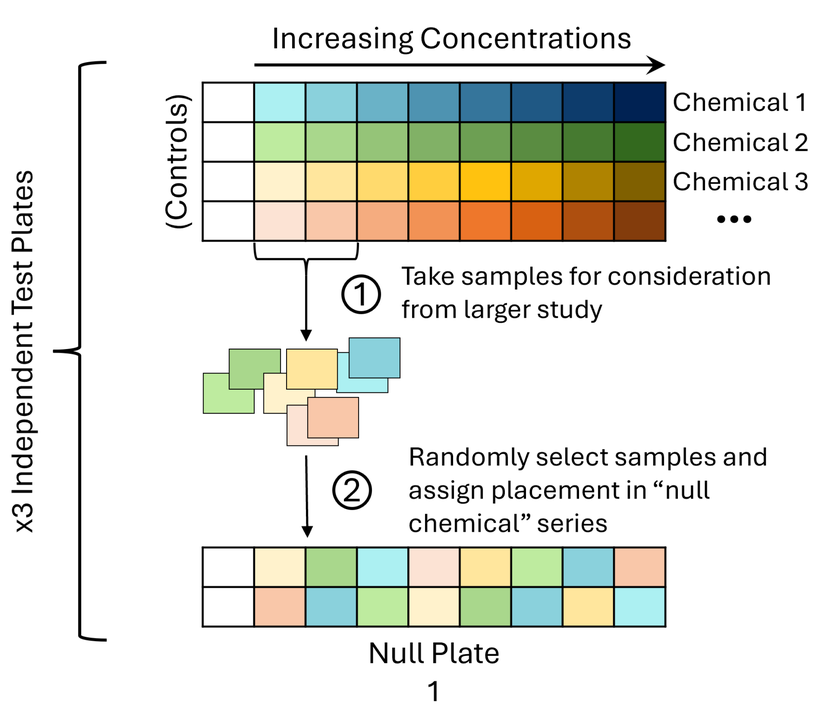


Figure 1. "Null chemical" generation

We generated "**null chemicals**" i.e., mock dose-response series where no dose-responsive behaviour is expected using TempO-seq in MCF7 cells. These profiles were generated in two ways:

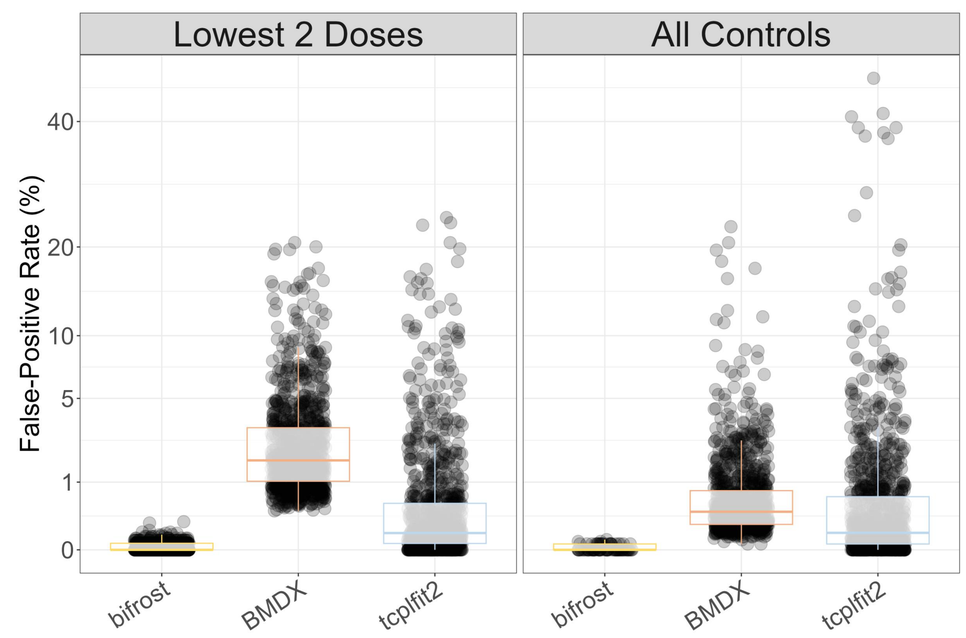
1) **Random sampling** of **lowest two doses** of 44 test chemicals [3]; and,

2) Random sampling from 63 **vehicle control** (DMSO) samples [4].

For each methodology 1000 null chemicals were generated (Figure 1), and "hit calls" based on previously published thresholds for each workflow were defined as false positives.

Results - False Positive Rate (FPR) estimation

Probe level



		Min	Mean	Median	Max
Lowest 2 Doses	BIFROST	0	0.004	0	0.173
	BMDX	0.331	2.778	1.744	20.584
	tcplfit2	0	1.008	0.061	24.074
All Controls	BIFROST	0	0.002	0	0.023
	BMDX	0.007	0.899	0.316	22.770
	tcplfit2	0	1.235	0.063	48.486

Figure 2. False-Positive rate distributions for each probe-level fitting method based on 1000 null chemicals (lowest two doses) and all controls BMDExpress2 (BMDX) and tcplfit2 and 100 nulls for BIFROST with control dataset.

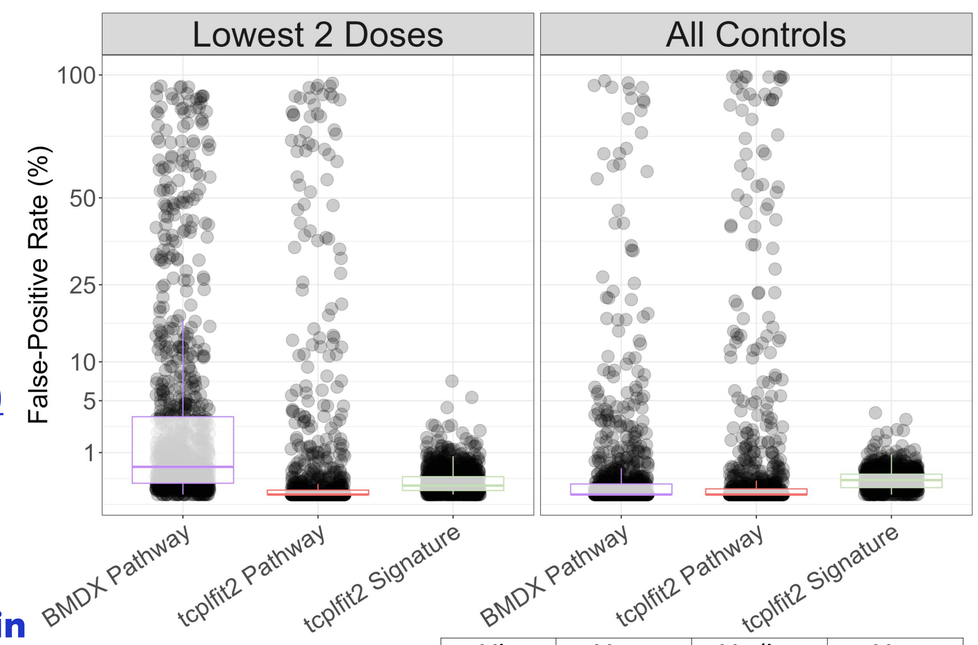
Probe level (Figure2)

Our results show average **FPR rates <3%** for all methods with **BIFROST having the lowest FPR** in each null chemical set whereas the **other methods showed a skewed distribution** with maximum FPR ~20-50% depending on dataset

Pathway/Signature level

Pathway/Signature level (Figure 3)

The **median for both BMDX Pathway and tcplfit2 Pathway is low** (Lowest 2 Doses – 0.435% BMDX, 0% tcplfit2- Pathway, All Controls – 0%), **the range is large in both null chemical sets (0-90+%)**. The **FPR for tcplfit2 Signature is low** on average with the means of the two null chemical sets being close (Lowest 2 Doses – 0.045%, All Controls – 0.118%). **The range of FPR for tcplfit2 Signature is much smaller for both null chemical sets.**



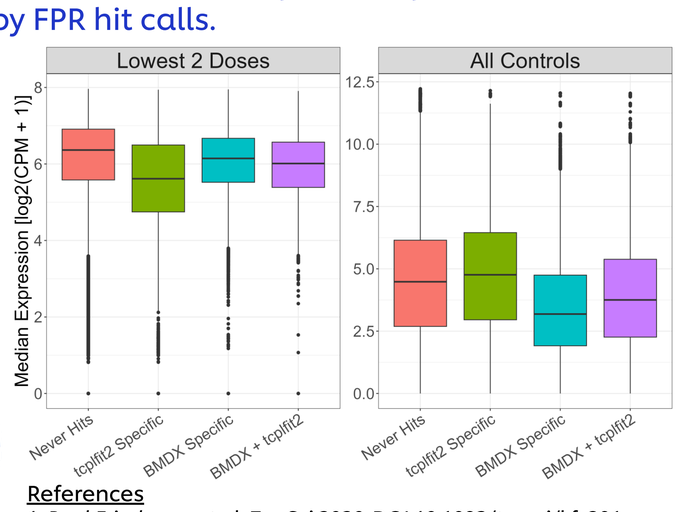
		Min	Mean	Median	Max
Lowest 2 Doses	BMDX	0	8.513	0.435	94.745
	tcplfit2-Path	0	3.701	0	96.059
	tcplfit2- Sig	0	0.194	0.045	7.307
All Controls	BMDX	0	2.363	0	97.228
	tcplfit2-Path	0	3.805	0	99.565
	Tcplfit2-Sig	0	0.183	0.118	3.789

Figure 3. False-Positive rate distributions for each pathway/signature fitting method, BMDExpress2 using pathway method (BMDX Pathway), tcplfit2 using BMDExpress2-style aggregation into pathways (tcplfit2 Pathway) and Httrpathway + tcplfit2 (tcplfit2 Signature). BIFROST generated too few hits on all null chemicals to perform BMDExpress2-style aggregation.

Characterisation of FPR

Figure 4 (below right). Distributions of median probe expression within null chemicals group by FPR hit calls.

In each null chemical set, probes which were called a hit tended to have a similar average expression compared to those that were never called a hit.



References

1: *Paul Friedman*, et al. *Tox Sci 2020*. DOI:10.1093/toxsci/kfz201
2: Reynolds, et al. *Com Tox 2020* DOI:10.1016/j.comtox.2020.100138
3: *Harrill, et al. Tox Sci 2021. DOI: 10.1093/toxsci/kfab009*
4: *Middleton, et al. Tox Sci 2022. DOI: 10.1093/toxsci/kfac068*

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Conclusions

- At probe level: low average FPR with a subset of null chemicals showing elevated FPR in all methods except BIFROST. This was the case in both datasets and is therefore unlikely to be a result of some wells having residual activity.
- At pathway/signature level: aggregating the data after dose-response analysis leads to higher FPR than performing dose-response on signatures (httrpathway + tcplfit2)
- Our results indicate that using the lowest two doses in this design is suitable for FPR estimation and suggest that "null chemicals" can be used to establish chemical-level bioactivity thresholds.
- FPR rates are not driven by lowly expressed probes
- FPR alone should not be used to select or optimise a method

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