Thoughts on Perturb-Seq data analysis approaches and pipelines

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Perturb-Seq data in the context of the Perturbation Catalogue

- For MAVE and CRISPR assays, we are lucky to have curated repositories (MaveDB, DepMap) with good quality, highly processed datasets.
- Perturb-Seq experiments are more complex: repositories like scPerturb aggregate dozens of studies but provide mostly raw expression counts.
- Interpretation and downstream analysis is left to the user, which can be quite complex.

Perturb-Seq data essence

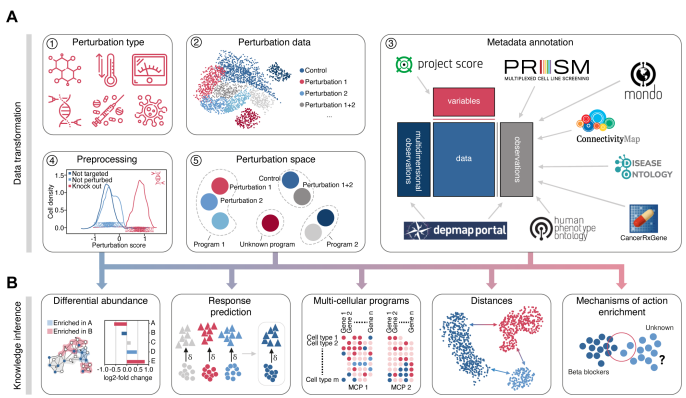
- Each observation is roughly: perturbing *gene X* in a specific cell type/tissue under set conditions yields a given gene expression profile per cell.
- Data characteristics:
  - High noise levels;
  - Pronounced batch effects;
  - Large scale: thousands of cells per perturbation, multiple conditions;
  - Raw counts require normalization, filtering, summarization, enrichment/differential expression/etc.
- Raw Perturb-Seq matrices need some systematic processing to become useful for Perturbation Catalogue users.

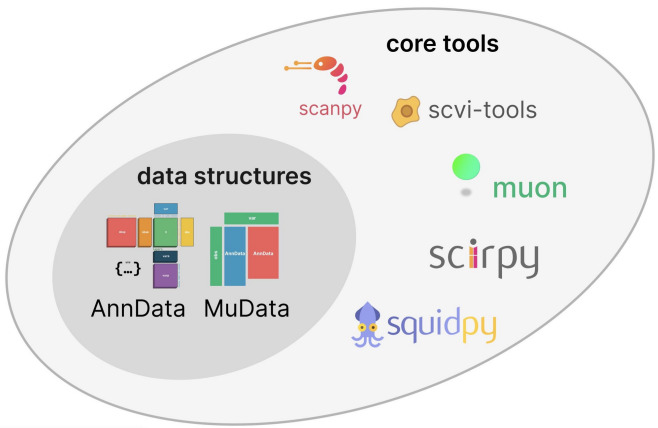
Possible processing approaches for Perturb-Seq data

- Specialized pipelines exist for rigorous statistical analysis:
  - **Python:** MIMOSCA, MAESTRO (with partial AnnData compatibility).
  - **R:** SCEPTRE, Mixscape.
- Challenges:
  - Tools are highly specialized, may require steep learning curves.
  - For many, limited maintenance past the initial publication.
  - Poor compatibility with the broader Python ecosystem for single cell analysis.

scverse ecosystem

- We are a small team and aim to deliver an MVP fast; diving into deep technical pipelines may slow progress.
- **scverse** offers a unified, well maintained, rapidly evolving ecosystem for single-cell analysis.
- Specifically, the **pertpy** tool is designed to handle single-cell perturbation workflows start to end.
- **Current status:** We are not currently using scverse approach because they are making rapid changes and several of the tools are not compatible, but we will use them in the future once they stabilise.





Implementation Update: Curated Studies

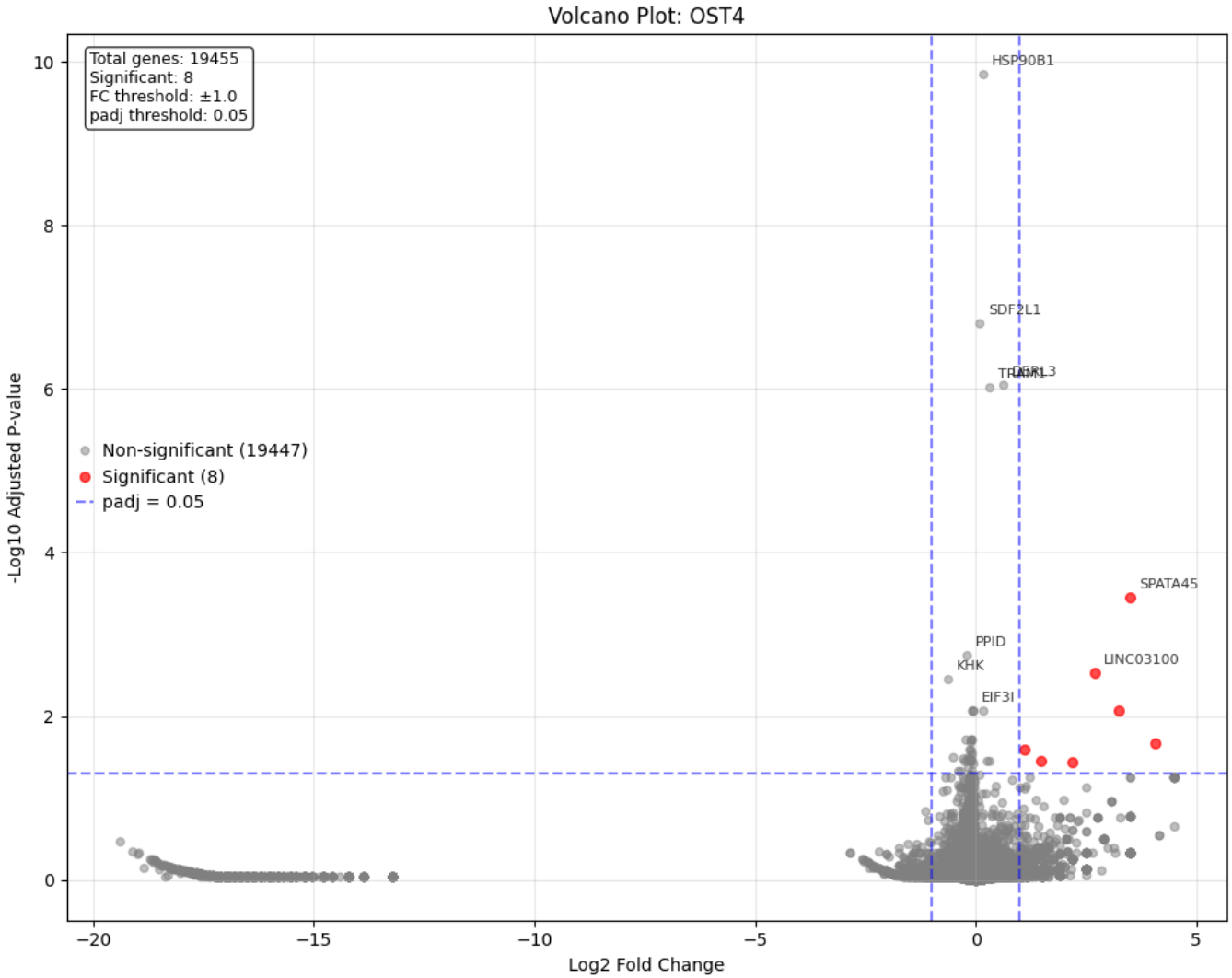
- **Progress:** 4 studies from scPerturb curated by Aleks - huge thanks!
- Curation process is now well established, unified, and will proceed even quicker in the future.
- Currently all studies have 1 cell type per study; in the future we'll curate more and larger studies.

Study	Size	Genes	Cells/Gene	Cell Type
adamson_2016_pilot	117M	7	500	lymphoblast
adamson_2016_upr_epistasis	479M	15	8-1500	lymphoblast
adamson_2016_upr_perturb_seq	1.8G	90	250-750	lymphoblast
datlinger_2017	132M	32	50-250	T cell

Processing approach: Pseudobulk Differential Expression

- **Input source:** scPerturb harmonised + curated to the common data schema (already implemented).
- **Strategy:** compute pseudobulk differential expression (simple and robust).
- **Workflow:**
  1. Group cells by control vs. perturbation within each cell type.
  2. Aggregate counts to pseudobulk profiles (mean expression per perturbation).
  3. Perform differential expression using t-tests with multiple testing correction.
  4. Apply filtering: adjusted p-value < 0.05, $|\log_2 \text{FC}| > 1$.
- **Implementation:** Parallel processing with expression thresholding and robust statistical testing.

Example Volcano Plot



Example volcano plot showing differential expression results for one perturbation.

Analysis Results and Filtering

- **User-facing results:**
  - "Perturbing gene X induces significant changes in genes Y, Z..."
  - "Expression of gene A is most strongly altered by perturbations in genes B, C..."
- **Filtering statistics:**
  - Records written: 29,476
  - Records skipped: 2,803,366
  - Filter criteria: padj ≤ 0.05, —log2FC— ≥ 1.0

Next Steps and Future Directions

- **Immediate:**
  - Continue curating additional studies from scPerturb.
  - Optimize processing pipeline for larger datasets.
  - Implement user interface for browsing results.
- **Future enhancements:**
  - Support for multi-cell-type studies.
  - Integration with pathway enrichment analysis.
  - Advanced visualization tools.
  - Cell-type-specific perturbation effects.
- **Questions/Discussion:**
  - Alternative analysis approaches?
  - Priority datasets for curation?