Thoughts on Perturb-Seq data analysis approaches and pipelines

Kirill Tsukanov
Senior Full Stack Developer & Data Engineer
ktsukanov@ebi.ac.uk

Perturbation Catalogue WP1/2/3 Technical Meeting

2025-04-29

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

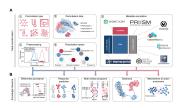
- ► Each observation is roughly: perturbing gene X in a specific cell type/tissue under set conditions yields a given gene expression profile per cell.
- Data characteristics:
 - High noise levels;
 - Pronounced batch effects;
 - Large scale: thousands of cells per perturbation, multiple conditions;
 - ► 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.





Suggested processing approach for Perturb-Seq

- ▶ Input source: scPerturb harmonised + curated to the common data schema (already in progress by Aleks).
- Proposed strategy: compute pseudobulk differential expression (because simple and robust).
- Workflow:
 - 1. Group cells by control vs. perturbation within each cell type.
 - 2. Aggregate counts to pseudobulk profiles.
 - 3. Perform differential expression using pertpy facilities.
- 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..."
- Any other ideas?