01_open_and_split

October 1, 2024

[1]:
```
# Autoreaload
%load_ext autoreload
%autoreload 2
```

[2]:
```
# Imports
import pandas as pd
import re
import scanpy as sc
from scipy.sparse import csr_matrix
from IPython.display import Markdown as md, display
from helper import add_top_column
```

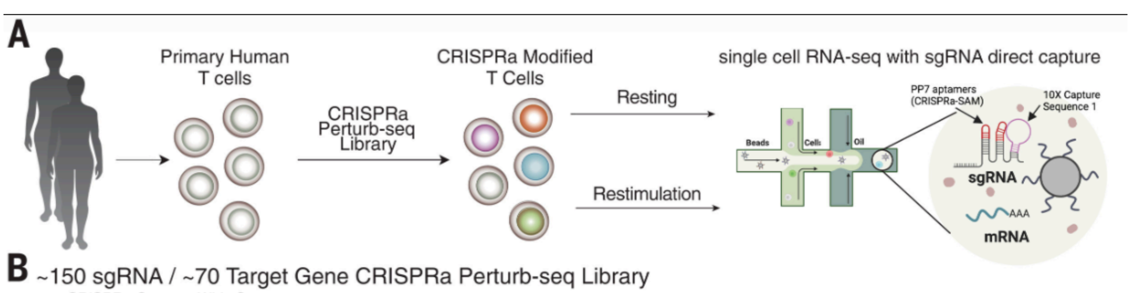
0.0.1 Paper

1 CRISPR activation and interference screens decode stimulation responses in primary human T cells

https://www.science.org/doi/10.1126/science.abj4008#body-ref-R56-1

2 Data

- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190604
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE174292
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190846
- https://zenodo.org/records/5784651 (R Repository with results)



We performed CRISPRa Perturb-seq characterization of regulators of stimulation responses in ~56,000 primary human T cells, targeting 70 hits and controls from our genome-wide CRISPRa cytokine screens (Fig. 4, A and B, and fig. S17, A to C). First, we confirmed that sgRNAs led to significant increases in the expression of their target genes (fig. S17D). Next, uniform manifold approximation and projection (UMAP) dimensionality reduction revealed discrete separation of the resting and restimulated cells (fig. S17E) and showed relatively even distribution of cells from two donors (Fig. 4C and fig. S17F). Gene signatures allowed us to resolve most T cells as either CD4+ or CD8+ (Fig. 4D and fig. S17, G and H). Thus, we generated a high-quality CRISPRa Perturb-seq dataset.

2.0.1 Data experimento:

- ~150 sgRNA
- ~ 70 Target Gene
- ~56,000 primary human T cells

3 Data Loading and preprocess

3.0.1 Raw Data (scRNA-seq)

[3]:
```
# Open data file
adata = sc.read_10x_mtx(
    '../data/GSE190604/',
    prefix='GSE190604_',
    cache=True,
    gex_only=False, # Only gene expression
    # make_unique=True
)
```

[4]:
```
# Cell Barcodes
adata.obs
```

[4]:
```
Empty DataFrame
Columns: []
Index: [AAACCCACAACAAGAT-1, AAACCCACAACGGCTC-1, AAACCCACACAGAAGC-1,
AAACCCACACCCTGTT-1, AAACCCACACTATGTG-1, AAACCCAGTACAGGTG-1, AAACCCAGTATGAGAT-1,
AAACCCAGTTACGATC-1, AAACCCATCGGCATTA-1, AAACCCATCTACCTTA-1, AAACCCATCTGTGCAA-1,
AAACGAAAGAACTCCT-1, AAACGAAAGCATGCAG-1, AAACGAAAGCTATCCA-1, AAACGAAAGGAGTATT-1,
AAACGAAAGTCGCCCA-1, AAACGAAAGTGGACTG-1, AAACGAACAACTGCCG-1, AAACGAACAAGTTTGC-1,
AAACGAACAGAAATCA-1, AAACGAACAGCTTCGG-1, AAACGAACAGTCGGTC-1, AAACGAACAGTTAAAG-1,
AAACGAAGTCACTTAG-1, AAACGAAGTCTCGCGA-1, AAACGAAGTGTCCTAA-1, AAACGAAGTTCATCTT-1,
AAACGAATCAAGCCAT-1, AAACGCTAGAAGTCTA-1, AAACGCTAGAGCATTA-1, AAACGCTAGCCACAAG-1,
AAACGCTAGTACAGCG-1, AAACGCTAGTACGAGC-1, AAACGCTAGTGGCGAT-1, AAACGCTAGTTGCATC-1,
AAACGCTCAGCGTACC-1, AAACGCTCATCAGCTA-1, AAACGCTGTCACGACC-1, AAACGCTGTGTAACGG-1,
AAACGCTGTTGATGTC-1, AAACGCTTCCAAGCAT-1, AAACGCTTCGGTAGGA-1, AAAGAACAGCCTCTTC-1,
AAAGAACAGCCTGTGC-1, AAAGAACAGCTCCGAC-1, AAAGAACAGGACTATA-1, AAAGAACAGTGCAACG-1,
AAAGAACAGTTGTACC-1, AAAGAACCAGTCTCTC-1, AAAGAACCATATCTCT-1, AAAGAACGTCGTGGTC-1,
AAAGAACGTTACGATC-1, AAAGAACTCAACACCA-1, AAAGAACTCAACGTGT-1, AAAGAACTCAGTAGGG-1,
AAAGAACTCCACAGCG-1, AAAGAACTCTATCGTT-1, AAAGGATAGAGAACCC-1, AAAGGATAGATGAAGG-1,
AAAGGATAGCATCAAA-1, AAAGGATAGGTTGCCC-1, AAAGGATCACGCACCA-1, AAAGGATCAGTTGTCA-1,
```

```
AAAGGATCAGTTGTTG-1, AAAGGATGTAGGACCA-1, AAAGGATGTATGACAA-1, AAAGGATGTGGACCAA-1,
AAAGGATGTTACGTAC-1, AAAGGATTCAGCGCAC-1, AAAGGATTCTAACGGT-1, AAAGGATTCTAGTGTG-1,
AAAGGGCAGATTGGGC-1, AAAGGGCCAACCACAT-1, AAAGGGCCAAGACTGG-1, AAAGGGCCAGAGTGTG-1,
AAAGGGCCAGGACTTT-1, AAAGGGCCAGGGTCTC-1, AAAGGGCCAGTGACCC-1, AAAGGGCCATCAACCA-1,
AAAGGGCCATGCAGCC-1, AAAGGGCGTCCGAAGA-1, AAAGGGCGTCTCGCGA-1, AAAGGGCGTGAACGGT-1,
AAAGGGCTCGCTTGCT-1, AAAGGGCTCGGTAGAG-1, AAAGGGCTCTGCATGA-1, AAAGGTAAGGGCCCTT-1,
AAAGGTACATACAGAA-1, AAAGGTACATTGTCGA-1, AAAGGTAGTAACATAG-1, AAAGGTAGTACGACTT-1,
AAAGGTAGTCACCTTC-1, AAAGGTAGTCACTGAT-1, AAAGGTAGTGACTGAG-1, AAAGTCCAGAACTGAT-1,
AAAGTCCAGGGCAAGG-1, AAAGTCCAGGGCTGAT-1, AAAGTCCCACGACCTG-1, AAAGTCCCACGATTCA-1,
AAAGTCCCAGACCTAT-1, …]

[103805 rows x 0 columns]
```

[5]:
```
display(md(f"""#### Raw data has {adata.shape[1]} genes and {adata.shape[0]}␣
↪cells"""))
display(md(f"""#### Cells are identified by barcode (e.g.: {', '.join(adata.obs.
↪index[:3])}, etc)"""))
display(md(f"""#### The number -1, -2, etc, represent the chromium well"""))
display(md(f"""#### A chromium well refers to the microfluidic chambers in the␣
↪10x Genomics Chromium Controller that are used to encapsulate single cells␣
↪and barcoded beads into individual droplets, enabling high-throughput␣
↪single-cell genomics. These wells play a key role in isolating single cells,␣
↪capturing their RNA, and associating it with unique barcodes for sequencing.
↪"""))
```

Raw data has 36755 genes and 103805 cells

Cells are identified by barcode (e.g.: AAACCCACAACAAGAT-1, AAACCCACAACGGCTC-1, AAACCCACACAGAAGC-1, etc)

The number -1, -2, etc, represent the chromium well

A chromium well refers to the microfluidic chambers in the 10x Genomics Chromium Controller that are used to encapsulate single cells and barcoded beads into individual droplets, enabling high-throughput single-cell genomics. These wells play a key role in isolating single cells, capturing their RNA, and associating it with unique barcodes for sequencing.

[6]:
```
display(md("##### Gene Expression: Represent the transcriptomic profile of the␣
↪cells (the genes that are being expressed)"))
display(md("##### CRISPR Guide Capture: Which sgRNAs (and therefore which␣
↪genes) were targeted in each cell."))
display(adata.var)
display(adata.var.feature_types.value_counts().reset_index())
```

Gene Expression: Represent the transcriptomic profile of the cells (the genes that are being expressed)

CRISPR Guide Capture: Which sgRNAs (and therefore which genes) were targeted in each cell.

```
                   gene_ids         feature_types
MIR1302-2HG  ENSG00000243485       Gene Expression
FAM138A      ENSG00000237613       Gene Expression
OR4F5        ENSG00000186092       Gene Expression
AL627309.1   ENSG00000238009       Gene Expression
AL627309.3   ENSG00000239945       Gene Expression
…                      …                    …
TRIM21-2            TRIM21-2  CRISPR Guide Capture
VAV1-1                VAV1-1  CRISPR Guide Capture
VAV1-2                VAV1-2  CRISPR Guide Capture
WT1-1                  WT1-1  CRISPR Guide Capture
WT1-2                  WT1-2  CRISPR Guide Capture

[36755 rows x 2 columns]
```

```
          feature_types  count
0       Gene Expression  36601
1  CRISPR Guide Capture    154
```

3.0.2 Cell Metadata

From the CRISPR Guide Capture columns we can get the cell metada reference: 01_build_metadata_table_for_guide_calls

[7]:
```
df_cell_metadata = pd.read_csv('../data/cell_metadata.txt', sep='\t')
df_cell_metadata['well'] = df_cell_metadata['cell_barcode'].apply(lambda x:␣
 ↪x[-1:])
df_cell_metadata = df_cell_metadata.set_index('cell_barcode')
df_cell_metadata
```

[7]:
```
                   condition     crispr  guide_id    gene well
cell_barcode
GGGAGATAGACCGTTT-1    Nostim  perturbed  ABCB10-1  ABCB10    1
GACGCTGCATTGTCGA-1    Nostim  perturbed  ABCB10-1  ABCB10    1
TTAATCCTCGTGCACG-1    Nostim  perturbed  ABCB10-1  ABCB10    1
ACACGCGTCGACCTAA-1    Nostim  perturbed  ABCB10-1  ABCB10    1
CATCCACCATCGATGT-1    Nostim  perturbed  ABCB10-1  ABCB10    1
…                        …          …        …       …   …
GTTGTCCGTGGTTTAC-8      Stim  perturbed     WT1-2     WT1    8
GTCTAGAAGGCACTCC-8      Stim  perturbed     WT1-2     WT1    8
TCCTAATCATACACCA-8      Stim  perturbed     WT1-2     WT1    8
AGACCCGGTATTGACC-8      Stim  perturbed     WT1-2     WT1    8
AGTGTTGTCATTTACC-8      Stim  perturbed     WT1-2     WT1    8

[60657 rows x 5 columns]
```

[8]:
```python
display(md("#### Perturbed vs No TARGET"))
display(df_cell_metadata['crispr'].value_counts().reset_index())
display(md('# ~56,000 cels'))
```

Perturbed vs No TARGET

```
      crispr  count
0  perturbed  56774
1         NT   3883
```

4 ~56,000 cels

[9]:
```python
display(md("#### Total cells per guide id"))
display(df_cell_metadata[['guide_id']].value_counts().reset_index())
display(md('# ~150,000 sgRNAs'))
# Un sgRNA activa solo una porción del gen
```

Total cells per guide id

```
       guide_id  count
0     TRAF3IP2-1   1056
1         LAT2-2    983
2         EMP3-1    931
3         CD27-1    849
4     TNFRSF1B-2    797
..           ...    ...
149      PRDM1-2     20
150       IRX4-2     15
151       DEF6-2     11
152       IRX4-1      2
153       TCF7-1      1

[154 rows x 2 columns]
```

5 ~150,000 sgRNAs

[10]:
```python
display(md("#### Total cells per gene"))
display(df_cell_metadata['gene'].value_counts().reset_index())
display(md('# ~70 genes'))
```

Total cells per gene

```
        gene  count
0  NO-TARGET   3883
1       EMP3   1588
2   TRAF3IP2   1564
3       CD27   1455
```

```
4    TNFRSF1B   1344
..        …     …
69       EOMES    262
70       HELZ2    128
71        TCF7    115
72       PRDM1     40
73        IRX4     17

[74 rows x 2 columns]
```

6 ~70 genes

[11]:
```python
display(md("#### Total cells per gene per guide_id"))
genes = df_cell_metadata['gene'].unique()[:4]
'WT1'
all_genes_count = []
for gene in genes:
    gene_count_df = df_cell_metadata[df_cell_metadata['gene'] == gene].
 ↪value_counts('guide_id').reset_index()
    gene_count_df = add_top_column(gene_count_df, gene)
    all_genes_count.append(gene_count_df)
pd.concat(all_genes_count, axis=1)
```

Total cells per gene per guide_id

[11]:

	ABCB10		AKAP12		ALX4		APOBEC3C	
	guide_id	count	guide_id	count	guide_id	count	guide_id	count
0	ABCB10-2	208	AKAP12-1	255	ALX4-1	380	APOBEC3C-1	486
1	ABCB10-1	189	AKAP12-2	104	ALX4-2	132	APOBEC3C-2	168

[12]:
```python
display(md("#### Total cells per well"))
df_cell_metadata.value_counts('well').reset_index()
```

Total cells per well

[12]:

	well	count
0	2	7838
1	3	7750
2	1	7742
3	5	7642
4	7	7502
5	4	7491
6	6	7430
7	8	7262

[13]:
```python
# sgRNAs are uniformly distributed per chromium well
sgRNAs = df_cell_metadata['guide_id'].unique()[:10]
sgRNAcounts = pd.DataFrame([{'well': i} for i in range(8)])
```

```
for sgRNA in sgRNAs:
    sgRNAcounts[sgRNA] = df_cell_metadata[df_cell_metadata['guide_id'] ==␣
 ↪sgRNA].value_counts('well').reset_index().sort_values('well')['count']
sgRNAcounts.set_index('well')
```

[13]:

well	ABCB10-1	ABCB10-2	AKAP12-1	AKAP12-2	ALX4-1	ALX4-2	APOBEC3C-1
0	29	37	45	19	62	26	71
1	28	34	39	18	51	20	70
2	24	33	34	14	48	19	62
3	24	27	33	12	47	16	62
4	24	26	30	12	47	15	59
5	20	22	26	12	44	14	57
6	20	15	24	10	41	12	54
7	20	14	24	7	40	10	51

well	APOBEC3C-2	APOBEC3D-1	APOBEC3D-2
0	27	40	57
1	26	34	50
2	25	31	47
3	21	30	45
4	19	28	43
5	18	28	42
6	18	27	41
7	14	25	34

6.0.1 Merge and clean data

[14]:
```
# Merge cell metadata with matrix
adata.obs = adata.obs.merge(df_cell_metadata, left_index=True,␣
 ↪right_index=True, how='left')
adata.obs
```

[14]:

	condition	crispr	guide_id	gene	well
AAACCCACAACAAGAT-1	Nostim	perturbed	PLCG2-2	PLCG2	1
AAACCCACAACGGCTC-1	Nostim	perturbed	HELZ2-1	HELZ2	1
AAACCCACACAGAAGC-1	NaN	NaN	NaN	NaN	NaN
AAACCCACACCCTGTT-1	Nostim	perturbed	OTUD7B-1	OTUD7B	1
AAACCCACACTATGTG-1	Nostim	perturbed	CD247-1	CD247	1
…	…	…	…	…	…
TTTGTTGGTCCAGCCA-8	NaN	NaN	NaN	NaN	NaN
TTTGTTGGTCCCACGA-8	NaN	NaN	NaN	NaN	NaN
TTTGTTGGTGAGTGAC-8	Stim	perturbed	IL2RB-1	IL2RB	8
TTTGTTGGTGCAAGAC-8	NaN	NaN	NaN	NaN	NaN
TTTGTTGTCTTCGCTG-8	NaN	NaN	NaN	NaN	NaN

```
[103805 rows x 5 columns]
```

[32]:
```python
genes = df_cell_metadata['gene'].unique()
set(genes) - set(adata.var.index)
```

[32]:
```
{'NO-TARGET'}
```

[41]:
```python
[g for g in adata.var.index if 'targ' in g.lower()]
```

[41]:
```
['NO-TARGET-1',
 'NO-TARGET-2',
 'NO-TARGET-3',
 'NO-TARGET-4',
 'NO-TARGET-5',
 'NO-TARGET-6',
 'NO-TARGET-7',
 'NO-TARGET-8']
```

[52]:
```python
display(md("#### Data merged"))
adata_filtered = adata[~adata.obs['guide_id'].isna(), adata.var.index.
 ↪isin(genes)]
display(adata_filtered)
display(md("#### Around 4000 cells have crispr NT"))
```

Data merged

```
View of AnnData object with n_obs × n_vars = 60657 × 73
    obs: 'condition', 'crispr', 'guide_id', 'gene', 'well'
    var: 'gene_ids', 'feature_types'
```

Around 4000 cells have crispr NT

[45]:
```python
sc.write('../data_out/matrix_filtered.h5ad', adata_filtered)
```

[54]:
```python
# Around 4000 cells have crispr NT
(adata_filtered.obs['crispr'] == 'NT').sum()
```

[54]:
```
3883
```