biobank uk
Research Analysis Platform

Enabled by DNAnexus®

Analyzing UK Biobank proteomics data on the UKB-RAP

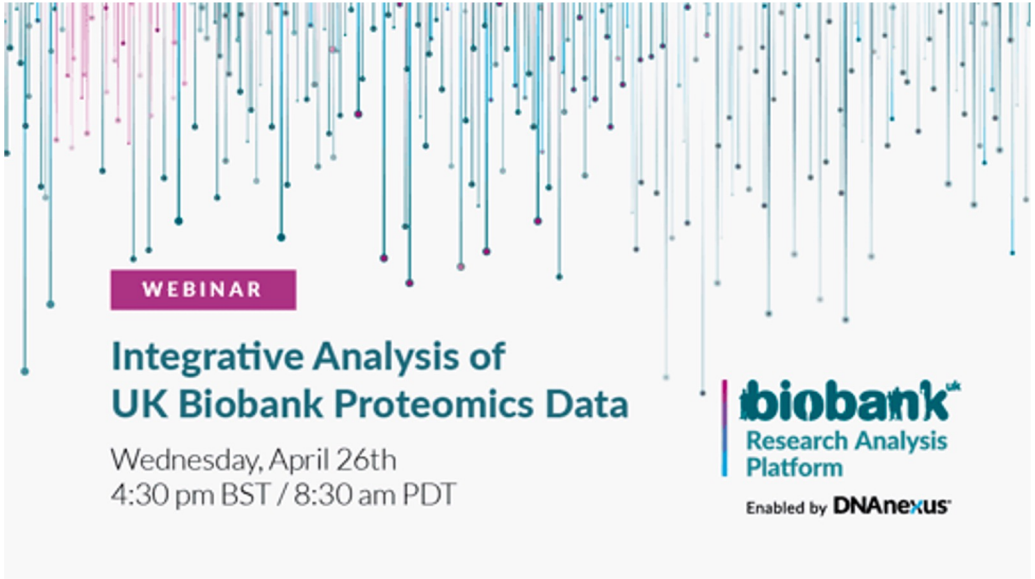
JUNE 2023

Speakers & Agenda

Alexandra Lee, PhD
Sr. Community Engagement/
Biomedical data scientist

1. New proteomics data on the UKB-RAP
2. How to access proteomics on UKB-RAP
3. Example: Differential expression analysis
4. Example: pQTL analysis

Previous proteomics webinar



https://www.youtube.com/watch?v=btOYvmgwZGA

Helpful resources

- UKB Research analysis platform overview - webinar
- Introduction to JupyterLab notebooks on RAP - webinar
- End to end target discovery with GWAS and PheWAS on the UKB research analysis platform - webinar

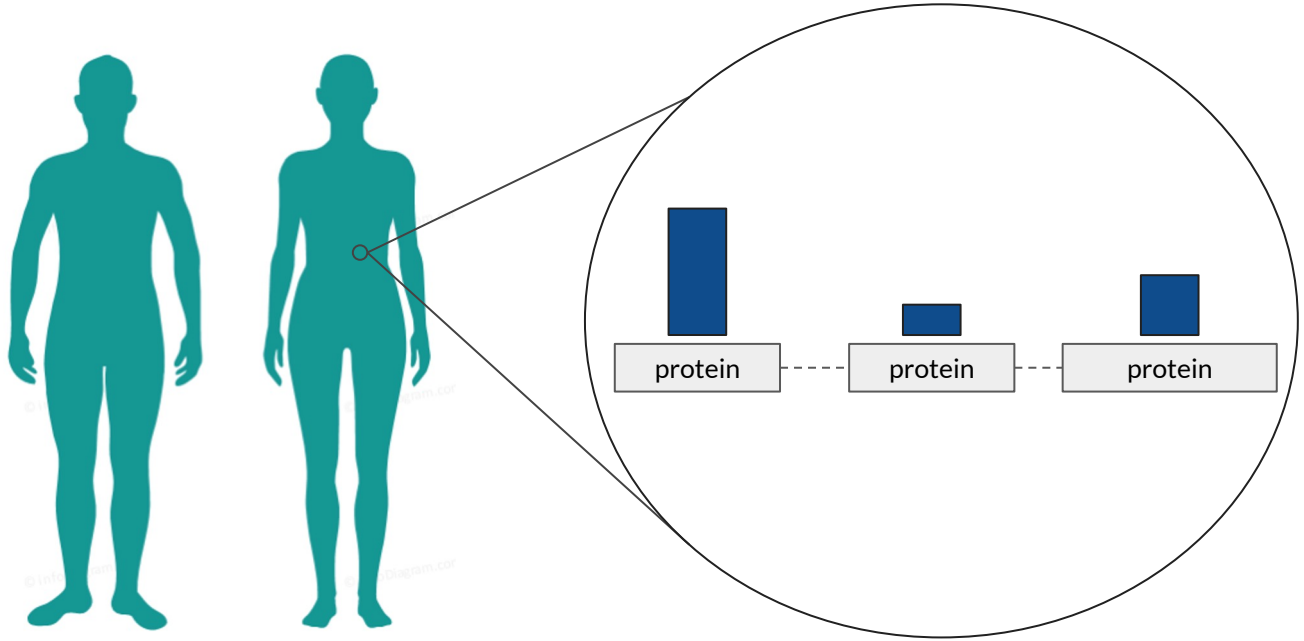
Learning Objectives

By the end of this session, you should be able to:

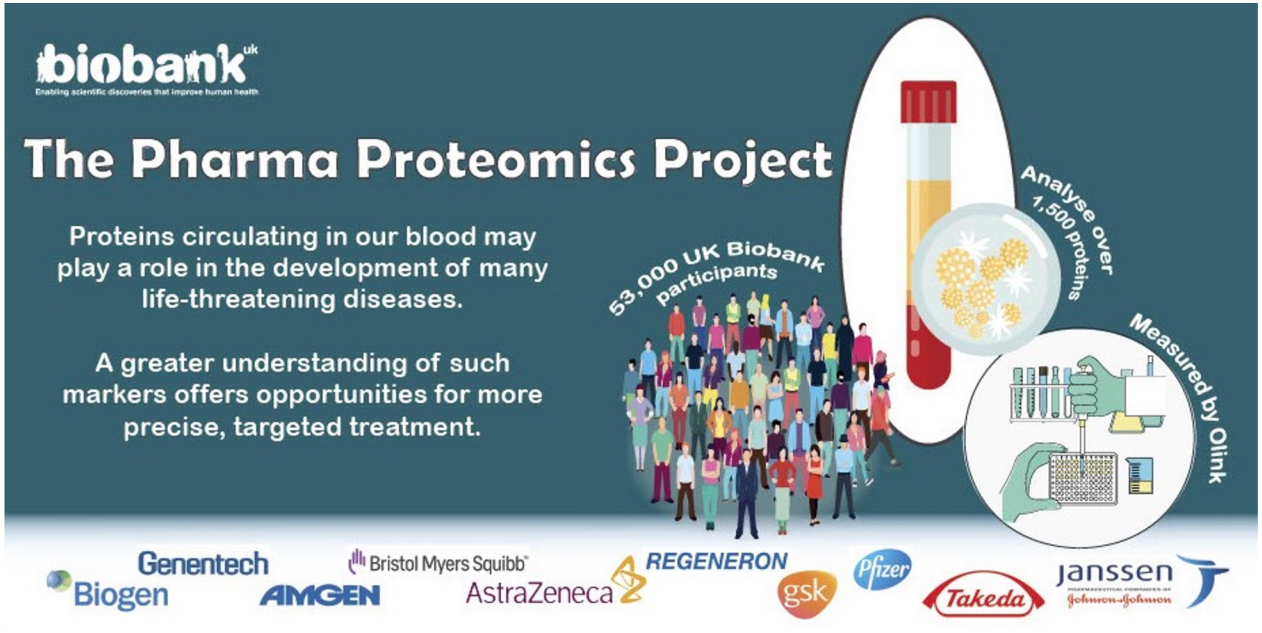
- **Articulate** the proteomics data available on the UKB-RAP
- **Apply** steps how to extract and access proteomic data on the UKB-RAP
- **Apply** steps how to download and access analysis code on the UKB-RAP
- **Execute** various proteomic analyses with the tools available on the UKB-RAP
- **Access** the UKB-RAP community forum and additional courses

1. About Proteomics

Proteomics gives a snapshot of an organism's state



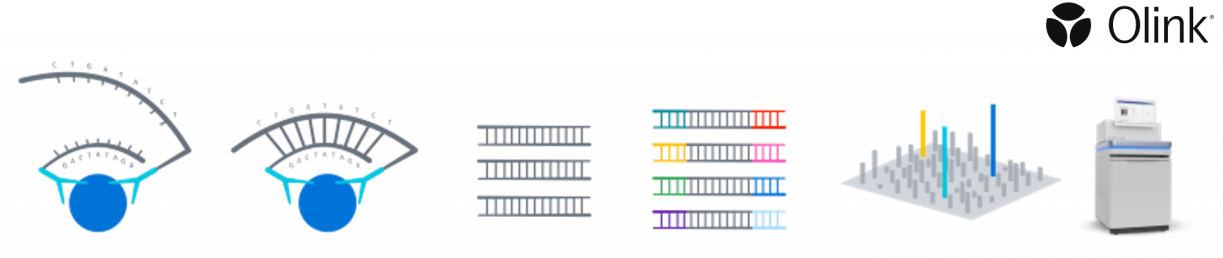
Proteomics data from UKB Pharma Proteomics Project (UKB-PPP)



- Measured by Olink
- ~53,000 participants
- ~1,500 proteins

https://www.biorxiv.org/content/10.1101/2022.06.17.496443v1.full.pdf

Olink technology



	ACAN;Aggrecan core protein	**ABHD14B**;Protein ABHD14B	**AARSD1**;Alanyl-tRNA editing protein Aarsd1	**ACTN4**;Alpha-actin-4
sample 1	7.6	4.3	7.2	2.3
sample 2	6.9	6.7	6.8	1.7
sample 3	3.2	9.2	3.0	0.2

White paper about Olink technology

Olink technology

~53,000 samples

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White paper about Olink technology

Olink technology

Protein panels (~1,500 proteins):

- Inflammation
- Oncology
- Cardiometabolic
- Neurology

	ACAN;Aggrecan core protein	**ABHD14B**;Protein ABHD14B	**AARSD1**;Alanyl-tRNA editing protein Aarsd1	**ACTN4**;Alpha-actin-4
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Normalized protein expression (NPX)

UKB-PPP preprint

2. How to access proteomics data on UKB RAP

Adding proteomics data to your project

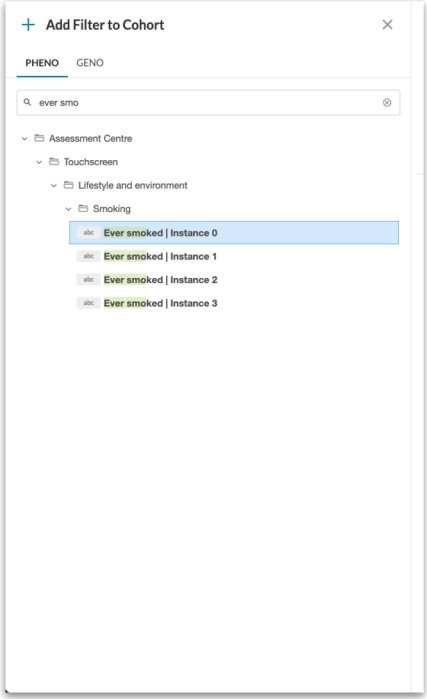
Note: Users will need to their UKB application be in Tier 2 or above in order to access this proteomics data

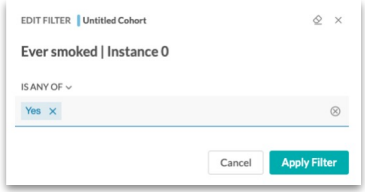
- Refresh data on existing project
- Create a new project and dispense proteomics data to this (*if refresh takes a while)

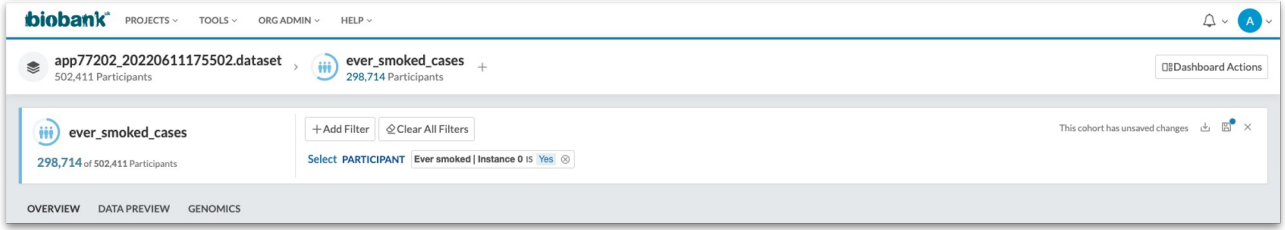
Collect data

1. Phenotype data
2. Protein expression data

Get phenotype data from Cohort Browser





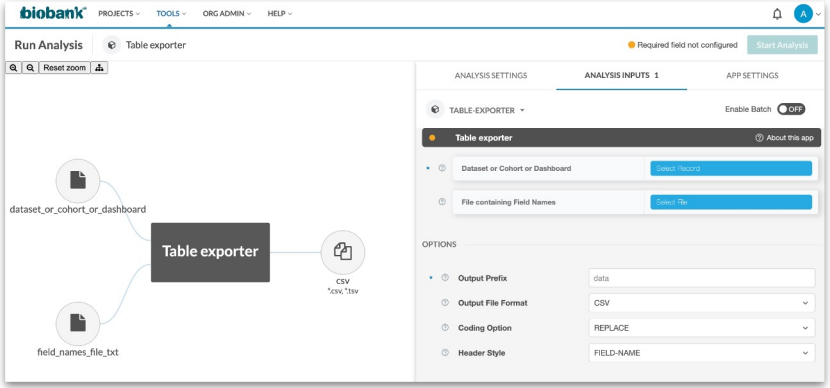



```
cohort_dataset = "project-XXX:record-XXX"
```

AD phenotype example
Ischemic disease example

Get protein expression data from Cohort Browser

Table Exporter App



dx extract_dataset

```
$ dx extract_dataset <dataset> --fields
“entity1.field1, entity1.field2,
entity2.field4”
```

Get protein expression data using Table Exporter app

TABLE-EXPORTER

Enable Batch OFF

Table exporter

About this app

Dataset or Cohort or Dashboard	ischaemic_cases
File containing Field Names	field_names.txt

OPTIONS

Output Prefix	
Output File Format	CSV
Coding Option	REPLACE
Header Style	FIELD-NAME

ADVANCED OPTIONS

Entity	olink_instance_0
Field Names	
Field Titles	

COHORT/DASHBOARD OPTIONS

Cohort Table Entity Names	
Cohort Table Entity Titles	

Get protein expression data using Table Exporter app

TABLE-EXPORTER

Enable Batch OFF

Table exporter

Dataset or Cohort or Dashboard: ischaemic_cases

File containing Field Names: field_names.txt

OPTIONS

Output Prefix

Output File Format: CSV

Coding Option: REPLACE

Header Style: FIELD-NAME

ADVANCED OPTIONS

Entity: olink_instance_0

Field Names

Field Titles

COHORT/DASHBOARD OPTIONS

Cohort Table Entity Names

Cohort Table Entity Titles

Phenotype dataset
*e.g. *.dataset*

Get protein expression data using Table Exporter app

TABLE-EXPORTER

Enable Batch OFF

Table exporter

About this app

Dataset or Cohort or Dashboard: ischaemic_cases

File containing Field Names: field_names.txt

List of field names
e.g. eid, aarsd1,...

OPTIONS

Output Prefix:

Output File Format: CSV

Coding Option: REPLACE

Header Style: FIELD-NAME

ADVANCED OPTIONS

Entity: olink_instance_0

Field Names:

Field Titles:

COHORT/DASHBOARD OPTIONS

Cohort Table Entity Names:

Cohort Table Entity Titles:

List of field names

Get protein expression data using Table Exporter app

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Enable Batch OFF

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Header Style	FIELD-NAME

ADVANCED OPTIONS

Entity	olink_instance_0
Field Names	
Field Titles	

entity table
e.g. olink_instance_#

COHORT/DASHBOARD OPTIONS

Cohort Table Entity Names	
Cohort Table Entity Titles	

Get protein expression data using dx extract_dataset

```
cmd = ['dx',
    'extract_dataset',
    cohort_dataset,
    '--fields',
    'olink_instance_0.eid, olink_instance_0.aarsd1,...',
    '--delimiter',
    ',',
    '--output',
    'filename.csv',
]
subprocess.check_call(cmd)
```

Get protein expression data using dx extract_dataset

```
cmd = ['dx',
    'extract_dataset',
    cohort_dataset,
    '--fields',
    'olink_instance_0.eid, olink_instance_0.aarsd1,...',
    '--delimiter',
    ',',
    '--output',
    'filename.csv',
]
subprocess.check_call(cmd)
```

Specify phenotype dataset identifier
`cohort_dataset = “project-XXX:record-XXX”`

Get protein expression data using dx extract_dataset

```
cmd = ['dx',
    'extract_dataset',
    cohort_dataset,
    '--fields',
    'olink_instance_0.eid, olink_instance_0.aarsd1,...',
    '--delimiter',
    ',',
    '--output',
    'filename.csv',
]
subprocess.check_call(cmd)
```

List of field names to extract
entity_table.field_name

Get list of field names for all proteins

```
cmd = ["dx", "extract_dataset", dataset, "-ddd", "--delimiter", ","]
subprocess.check_call(cmd)

data_dict_df = pd.read_csv("*data_dictionary.csv")
```

data_dictionary

	entity	name	title	units	coding_name
26	participant	p31	Sex	NaN	data_coding_9
27	participant	p34	Year of birth	years	NaN
23823	participant	p20107_i0	Illnesses of father | Instance 0	NaN	data_coding_1010

entity_dictionary

	entity	entity_title	entity_description	entity_label_plural	entity_label_singular
0	participant	Participant	NaN	Participants	Participant
1	death	Death Record	NaN	Death Records	Death Record
2	death_cause	Death Cause Record	NaN	Death Cause Records	Death Cause Record
3	hesin	Hospitalization Record	NaN	Hospitalization Records	Hospitalization Record

coding_dictionary

	coding_name	code	meaning	concept	display_order	parent_code
87816	data_coding_1010	13	Prostate cancer	NaN	1	NaN
87817	data_coding_1010	12	Severe depression	NaN	2	NaN
87818	data_coding_1010	11	Parkinson's disease	NaN	3	NaN

Get list of field names for all proteins

```
field_names = list(data_dict_df.loc[data_dict_df["entity"] == "olink_instance_0", "name"].values)
```

	entity	name	type
28093	gp_scripts	drug_name	string
28094	gp_scripts	quantity	string
28095	olink_instance_0	eid	string
28096	olink_instance_0	aarsd1	float
28097	olink_instance_0	abhd14b	float
28098	olink_instance_0	abl1	float
28099	olink_instance_0	acaa1	float
28100	olink_instance_0	acan	float
28101	olink_instance_0	ace2	float
28102	olink_instance_0	acox1	float

Get list of field names for all proteins

```
field_names_str = [f"olink_instance_0.{f}" for f in field_names]
field_names_query = ",".join(field_names_str)
```

Output

```
'olink_instance_0.eid,olink_instance_0.aarsd1,olink_instance_0.abhd14b,olink_instance_0.abl1,...
```

Get protein expression data using dx extract_dataset

```
cmd = ['dx',
    'extract_dataset',
    cohort_dataset,
    '--fields',
    'olink_instance_0.eid, olink_instance_0.aarsd1,...',
    '--delimiter',
    ',',
    '--output',
    'filename.csv',
]
subprocess.check_call(cmd)
```

Write result to filename.csv

Notebook to extract proteomics data

Sample protein expression data

~1,500 proteins

~53,000 samples

	ACAN	ABHD14B	AARSD1	ACTN4
sample 1	7.6	4.3	7.2	2.3
sample 2	6.9	6.7	6.8	1.7
sample 3	3.2	9.2	3.0	

npx values

Three protein expression datasets

Protein dataset	No. samples	Description	Entity Table
1	~53,000	Randomly selected from 500K UKB participants, pre-selected	`olink_instance_0`
2	~1,000	COVID-19 imaging first visit	`olink_instance_2`
3	~1,000	COVID-19 imaging second visit	`olink_instance_3`

Three protein expression datasets

Protein dataset	No. samples	Description	Entity Table
1	~53,000	Randomly selected from 500K UKB participants, pre-selected	`olink_instance_0`
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Three protein expression datasets

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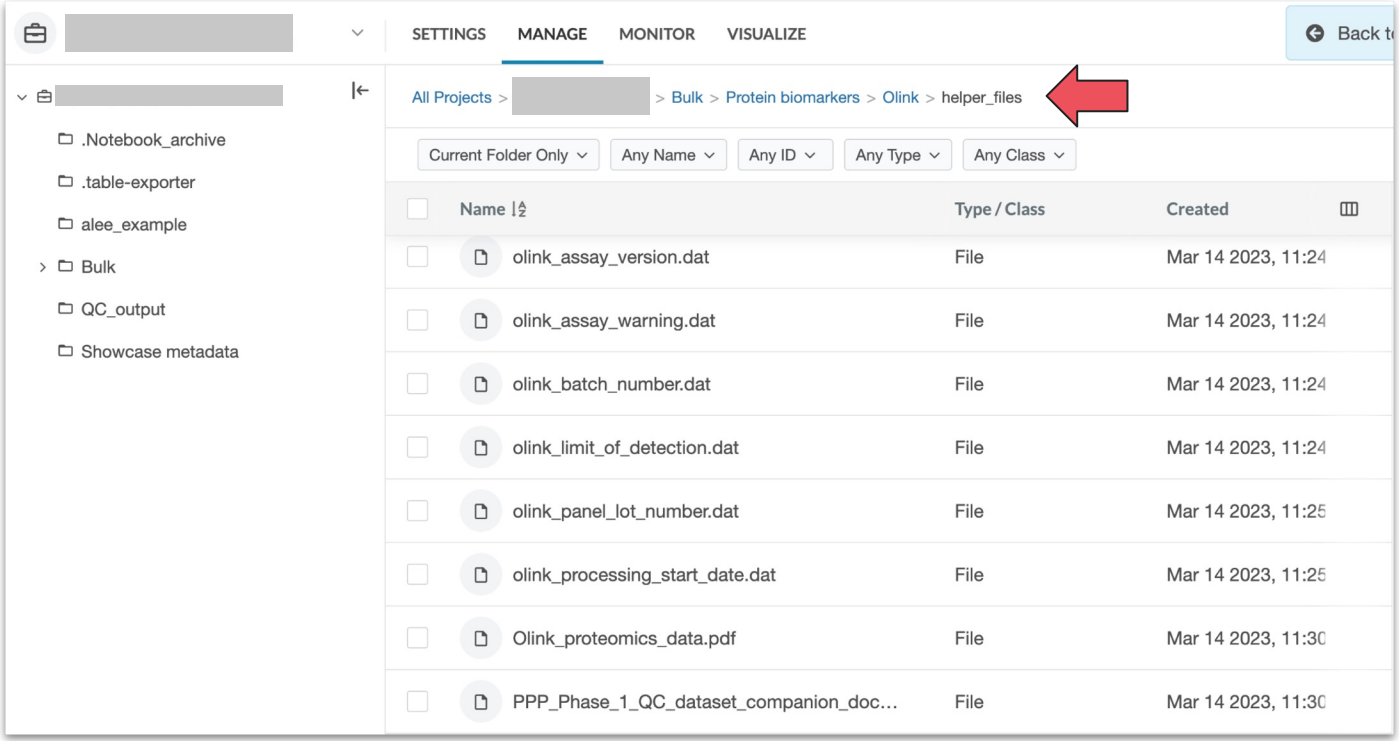
Metadata available in Bulk folder



Metadata available in Bulk folder

SETTINGS MANAGE MONITOR VISUALIZE Back t

- .Notebook_archive
- .table-exporter
- alee_example
- Bulk
- QC_output
- Showcase metadata

All Projects > [redacted] > Bulk > Protein biomarkers > Olink > helper_files

Current Folder Only | Any Name | Any ID | Any Type | Any Class

Name	Type / Class	Created
olink_assay_version.dat	File	Mar 14 2023, 11:24
olink_assay_warning.dat	File	Mar 14 2023, 11:24
olink_batch_number.dat		Mar 14 2023, 11:24
olink_limit_of_detection.dat		Mar 14 2023, 11:24
olink_panel_lot_number.dat	File	Mar 14 2023, 11:25
olink_processing_start_date.dat	File	Mar 14 2023, 11:25
Olink_proteomics_data.pdf	F	
PPP_Phase_1_QC_dataset_companion_doc…		

Limit of detection

PDF of QC steps performed

3. How to access analysis code on UKB-RAP

Code is available on github!

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dnanexus / UKB_RAP Public

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Code

UKB_RAP / proteomics /

https://github.com/dnanexus/UKB_RAP/tree/main/proteomics

Add the analysis scripts to the UKB-RAP

Steps to perform on your local machine:

1. Clone the repository:
2. Navigate into the cloned repository:
3. Login to UKB-RAP:
4. Upload analysis scripts to the platform:

```
$git clone https://github.com/dnanexus/UKB_RAP.git
$cd UKB_RAP
$dx login
$dx upload -r <proteomics> –destination <path on the UKB-RAP>
```

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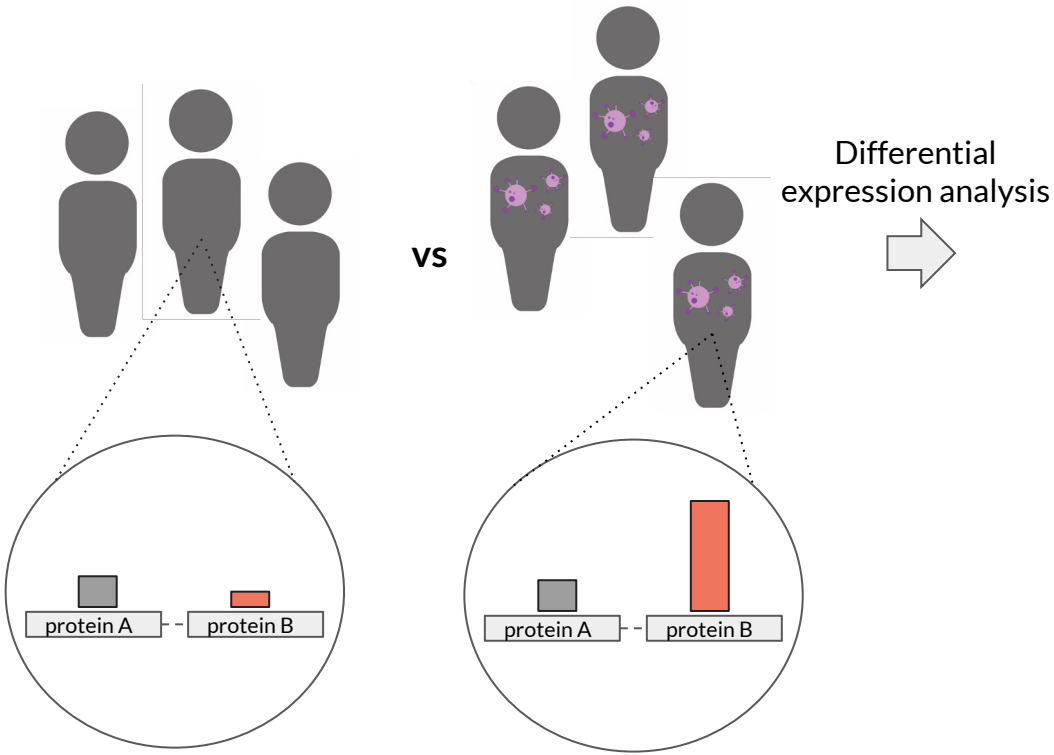
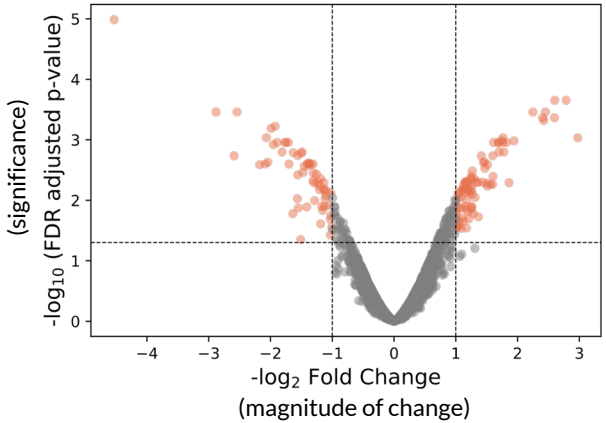
$cd UKB_RAP
$dx login
$dx upload -r <proteomics> –destination <path on the UKB-RAP>
```

4. Example: Differential expression analysis

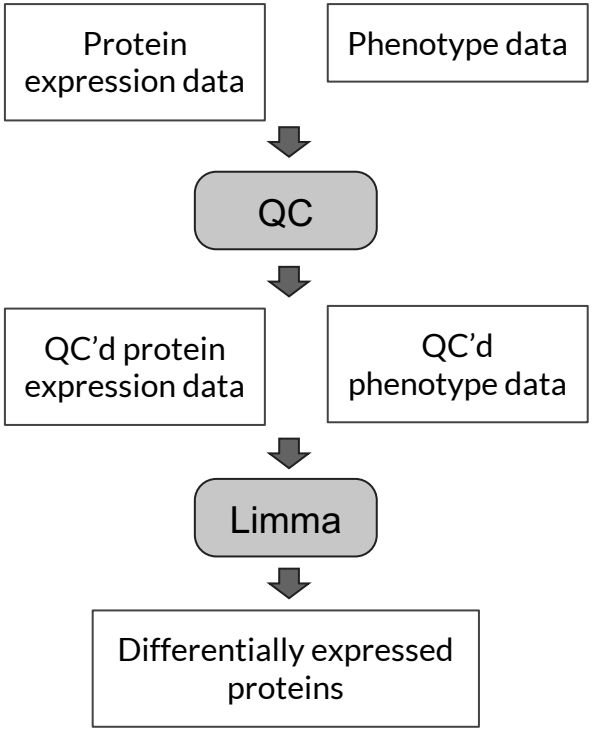
Differential expression analysis used to study mechanisms of disease



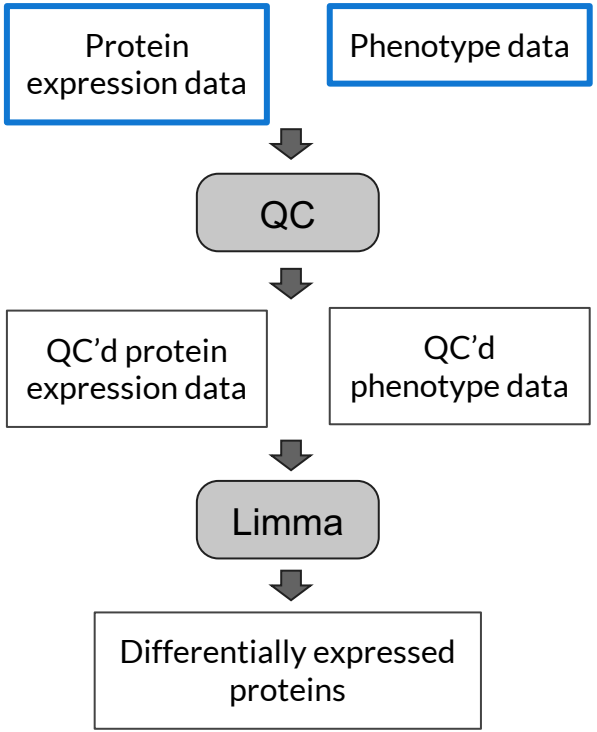
Differential expression analysis



Approach

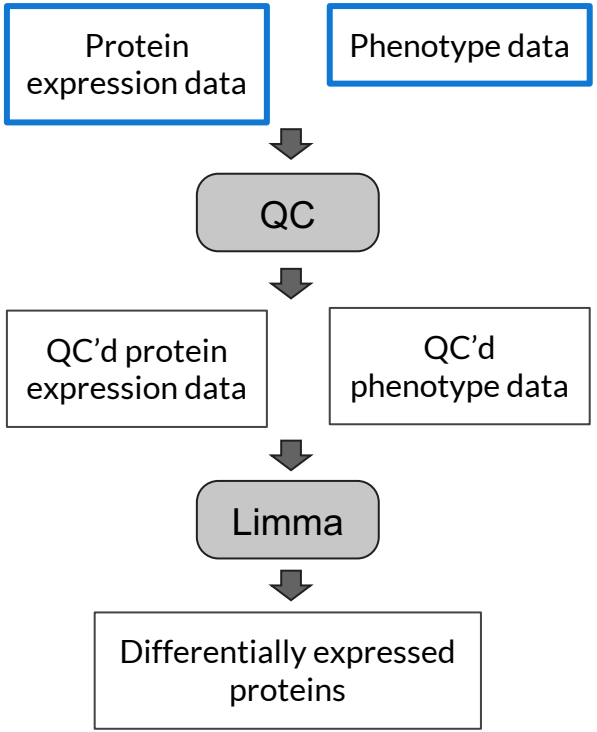


Approach



NOTE: *We are using public proteomic data, not UKB data, for demonstration purposes*

Collect input data

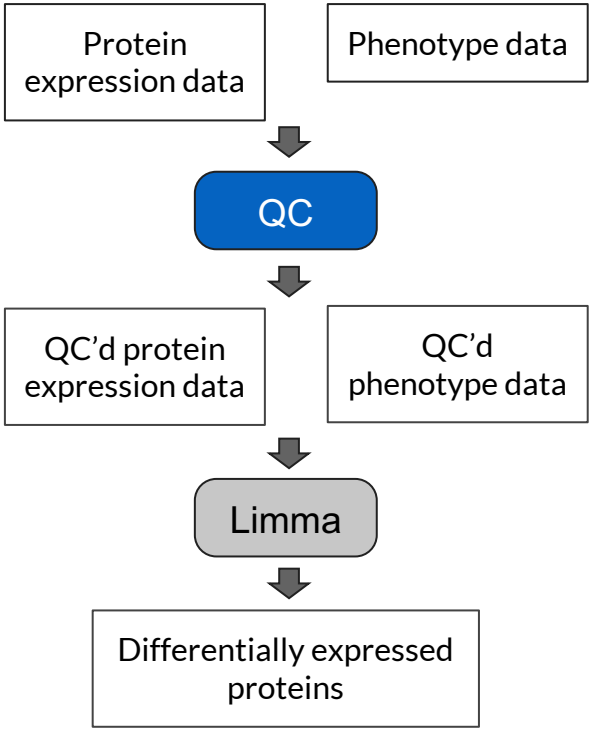


Plasma_Sample	CA1	ICAM1	CHL1	TGFBI	ENG
H0529.3	7.62107	6.79971	4.73174	9.33471	3.12445
H0441.1	6.96085	6.98459	4.31338	9.06819	3.31576
H0558.3	7.16983	7.04907	4.72713	8.92804	3.16308
H0499.2	7.59577	6.80282	4.51559	9.17979	3.19292
H0468.3	7.25945	6.91728	4.84307	9.91809	3.47692

Plasma_Sample	PIDN	Age_at_Baseline	Sex	Outcome
H0529.3	9677	90+	Male	MCI_Decline_AD
H0441.1	9974	90+	Female	MCI_Stable_AD
H0558.3	9681	90+	Female	MCI_Decline_AD
H0499.2	9502	88	Male	MCI_Stable_AD
H0468.3	9635	87	Female	MCI_Stable_AD

Input data from *Kivisakk et al*

QC input data



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H0499.2	7.59577	6.80282	4.51559	9.17979	3.19292
H0468.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

- Removing missing, outlier data
- Normalize/scale data

Plasma_Sample	PIDN	Age_at_Baseline	Sex	Outcome
H0529.3	9677	90+	Male	MCI_Decline_AD
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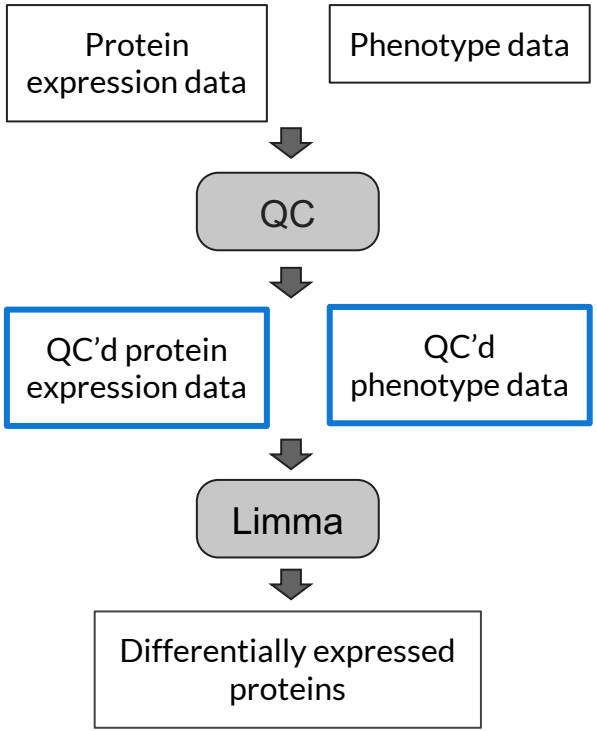
H0499.2	9502	88	Male	MCI_Stable_AD
H0468.3	9635	87	Female	MCI_Stable_AD

Get QC'd input data



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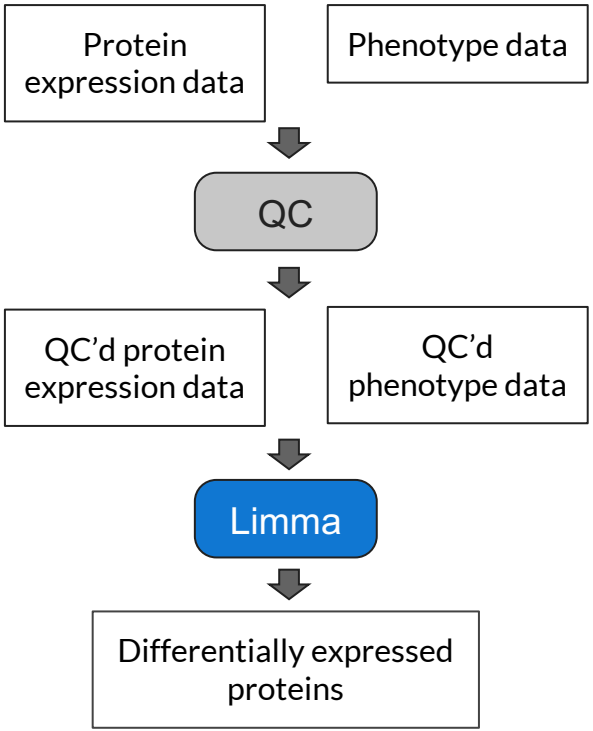
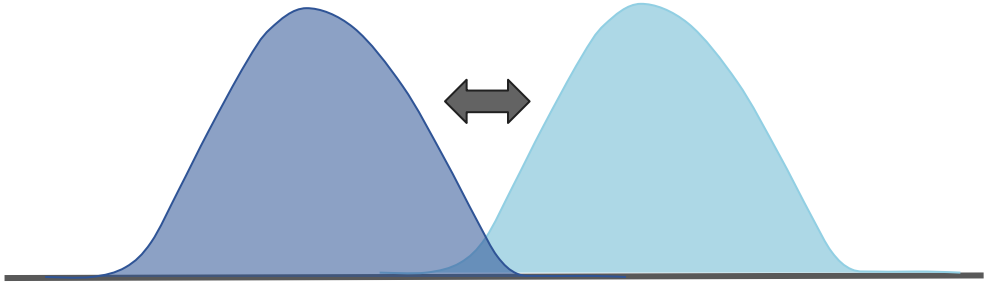
H0499.2	9502	88	Male	MCI_Stable_AD
H0468.3	9635	87	Female	MCI_Stable_AD

Perform differential expression analysis using Limma

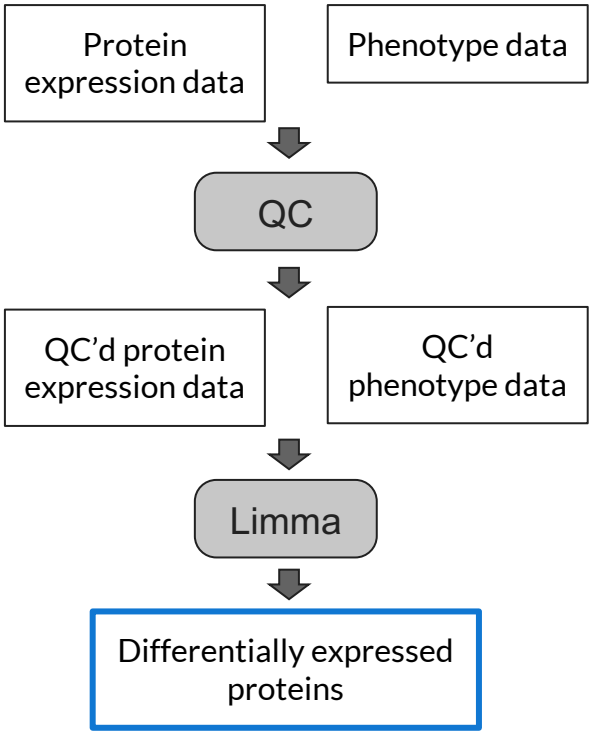


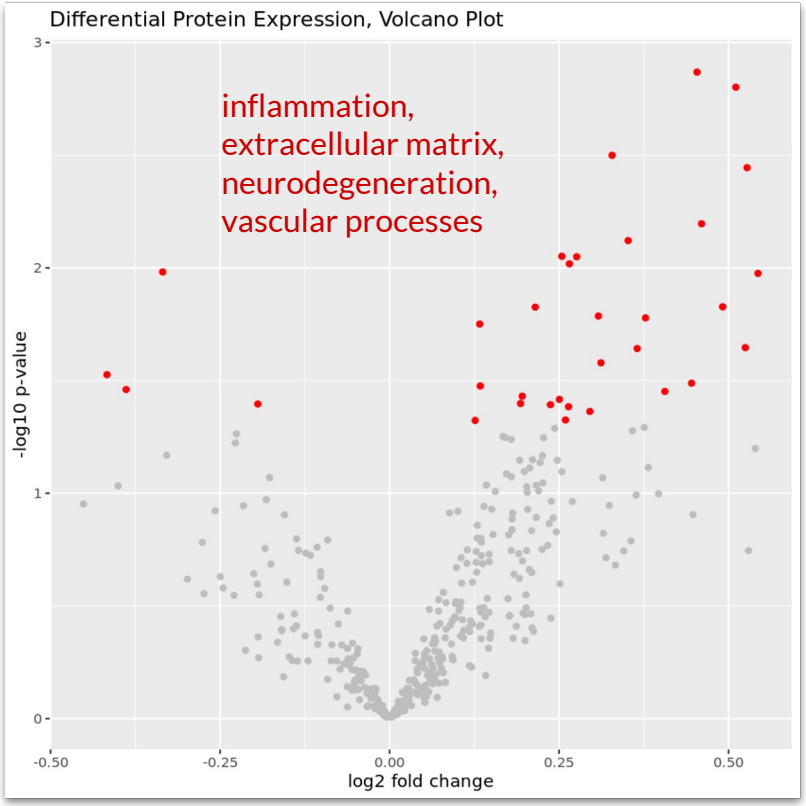


protein X expression in stable group

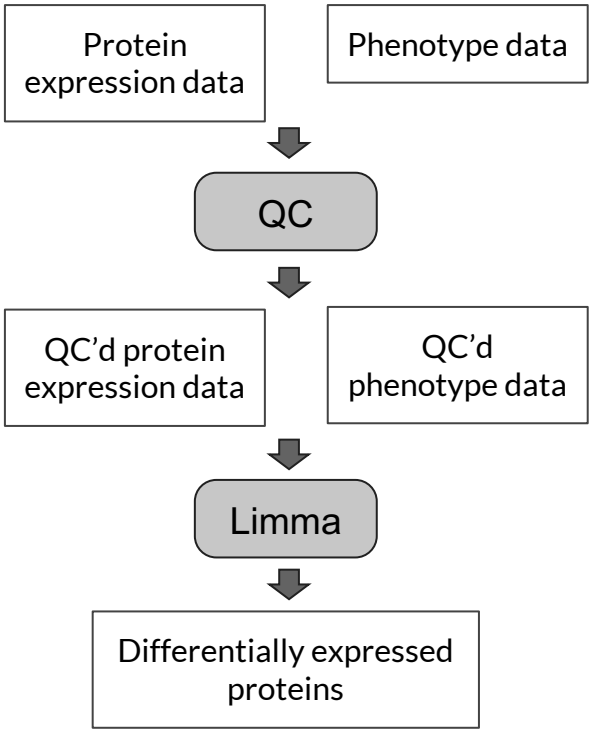
protein X expression in decline group

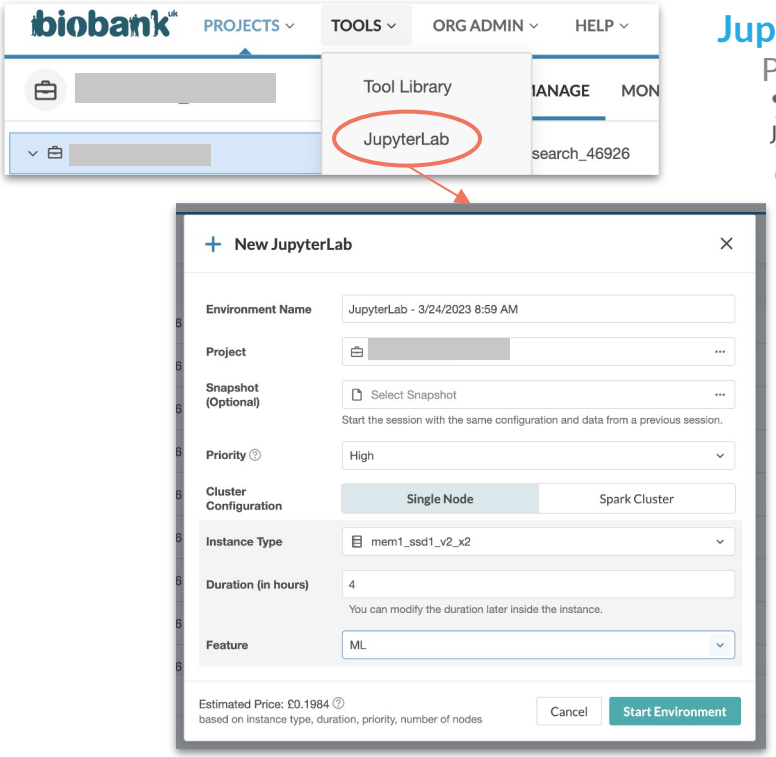
Found differentially expressed proteins





Run analysis using JupyterLab

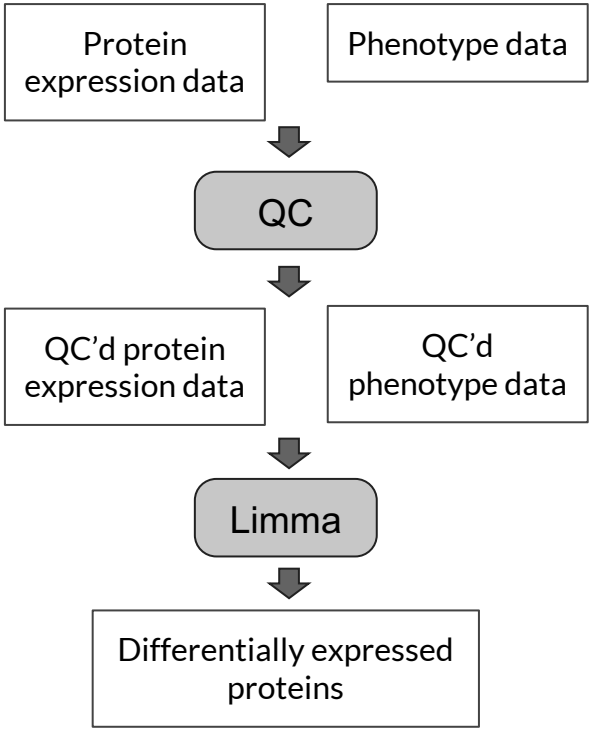


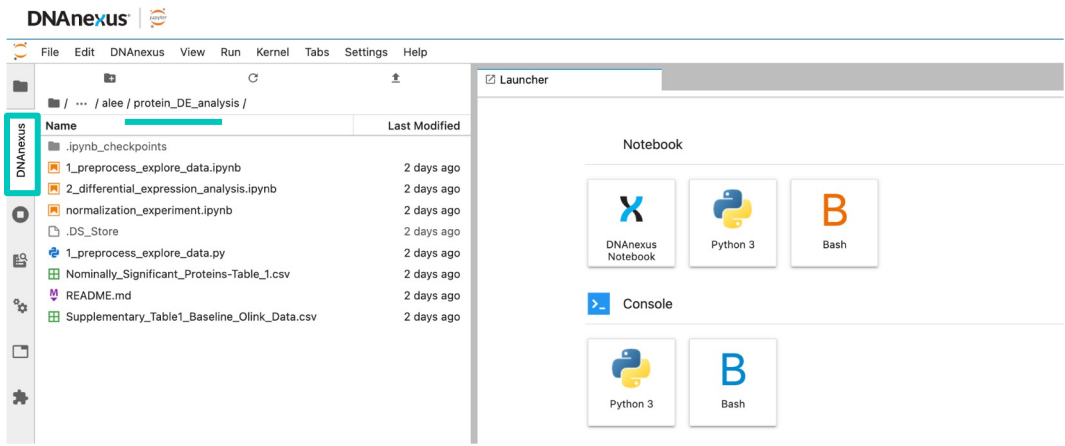


JupyterLab
Python

JupyterLab webinar

Run analysis using JupyterLab





Resources

	Link	Configuration	Runtime & cost
Code to explore input data	https://github.com/dnanexus/UKB_RAP/blob/main/proteomics/protein_DE_analysis/1_preprocess_explore_data.ipynb	**Kernel:** ML **Priority**: normal **Recommended instance**: mem1_ssd1_v2_x2	**Runtime**: ~ 1min **Cost**: ~£ 0.082
Code to perform differential expression	https://github.com/dnanexus/UKB_RAP/blob/main/proteomics/protein_DE_analysis/2_differential_expression_analysis.ipynb	**Kernel**: PYTHON_R **Priority**: normal **Recommended instance**: mem1_ssd1_v2_x2	**Runtime**: ~ 5 min **Cost**: ~£0.015
Input data publication	https://academic.oup.com/braincomms/article/4/4/fcac155/6608340?login=false#366642284		

5 minute break
Course evaluation

5. Example: pQTL analysis

pQTL identify SNPs that influence changes in protein expression

SNP array

```
10010021001000100211110002
00010011020010200101010201
01020201010001010101001020
10010021001000100111110002
00010011020010200101010201
```

pQTL

protein expression

7.6	4.3	…	7.1
6.9	6.7	…	6.5
3.2	9.2	…	9.2
2.9	9.9	…	3.4
8.9	4.6	…	5.3

GWAS identify SNPs that influence trait

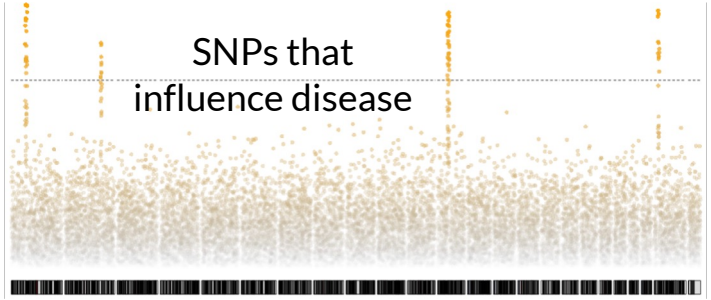
SNP array

```
10010021001000100211110002
00010011020010200101010201
01020201010001010101001020
10010021001000100111110002
00010011020010200101010201
```

disease

```
1
1
1
0
0
```

GWAS



Input genotype

Phenotype

Result

GWAS identify SNPs that influence trait

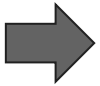
SNP array

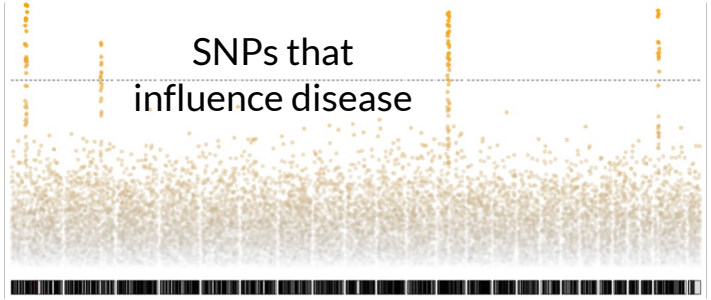
```
10010021001000100211110002
00010011020010200101010201
01020201010001010101001020
10010021001000100111110002
00010011020010200101010201
```

disease

```
1
1
1
0
0
```

GWAS





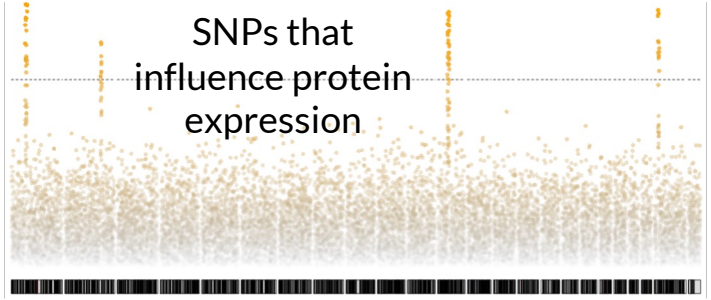
SNP array

```
10010021001000100211110002
00010011020010200101010201
01020201010001010101001020
10010021001000100111110002
00010011020010200101010201
```

protein expression

7.6	4.3	…	7.1
6.9	6.7	…	6.5
3.2	9.2	…	9.2
2.9	9.9	…	3.4
8.9	4.6	…	5.3

pQTL



Input genotype

Phenotype

Result

GWAS identify SNPs that influence trait

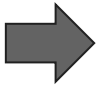
SNP array

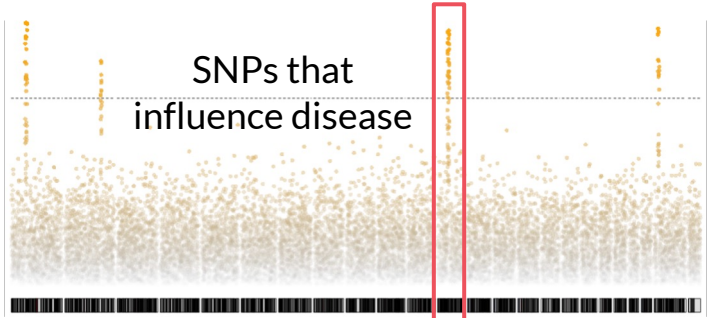
```
1001002100100010021111000 2
0001001102001020010101020 1
0102020101000101010100102 0
1001002100100010011111000 2
0001001102001020010101020 1
```

disease

```
1
1
1
0
0
```

GWAS





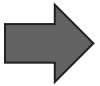
SNP array

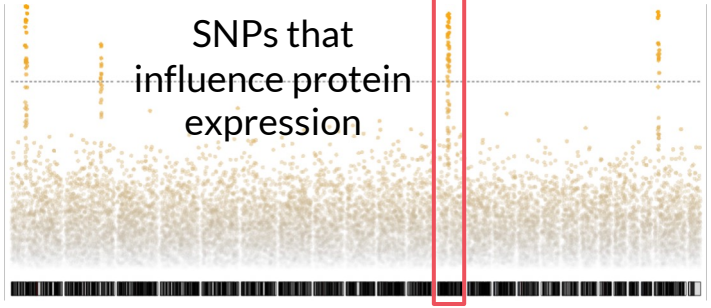
```
1001002100100010021111000 2
0001001102001020010101020 1
0102020101000101010100102 0
1001002100100010011111000 2
0001001102001020010101020 1
```

protein expression

7.6	4.3	…	7.1
6.9	6.7	…	6.5
3.2	9.2	…	9.2
2.9	9.9	…	3.4
8.9	4.6	…	5.3

pQTL





Input genotype

Phenotype

Result

About REGENIE

First step - calculate Polygenic Risk Score (PRS) for background association correction

Phenotype + Array data → PRS (for each chromosome)

REGENIE paper
End-to-end Target Discovery webinar

About REGENIE

First step - calculate Polygenic Risk Score (PRS) for background association correction

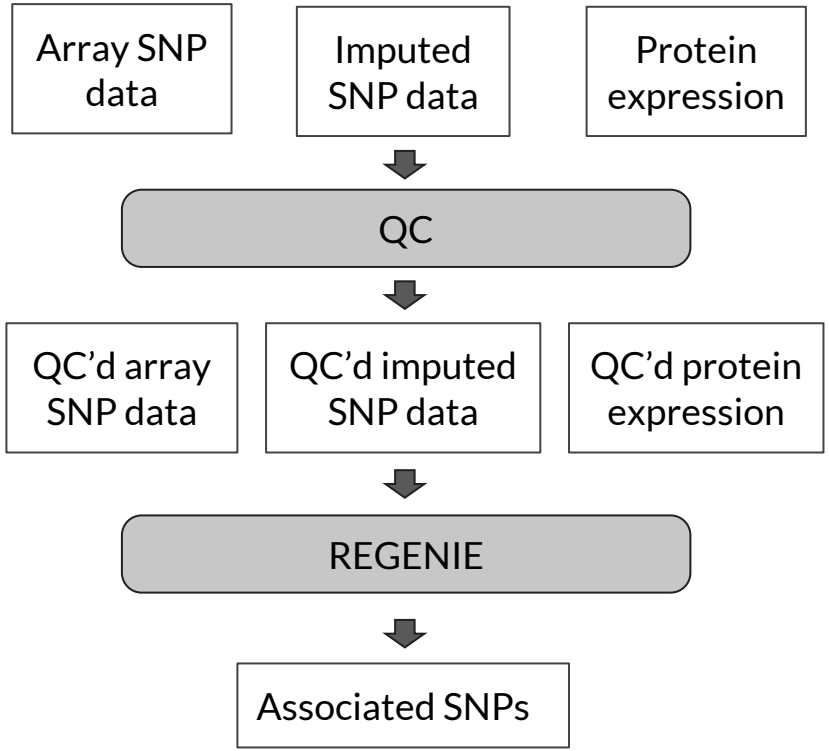
Phenotype + Array data → PRS (for each chromosome)

Second step - test variant-phenotype association

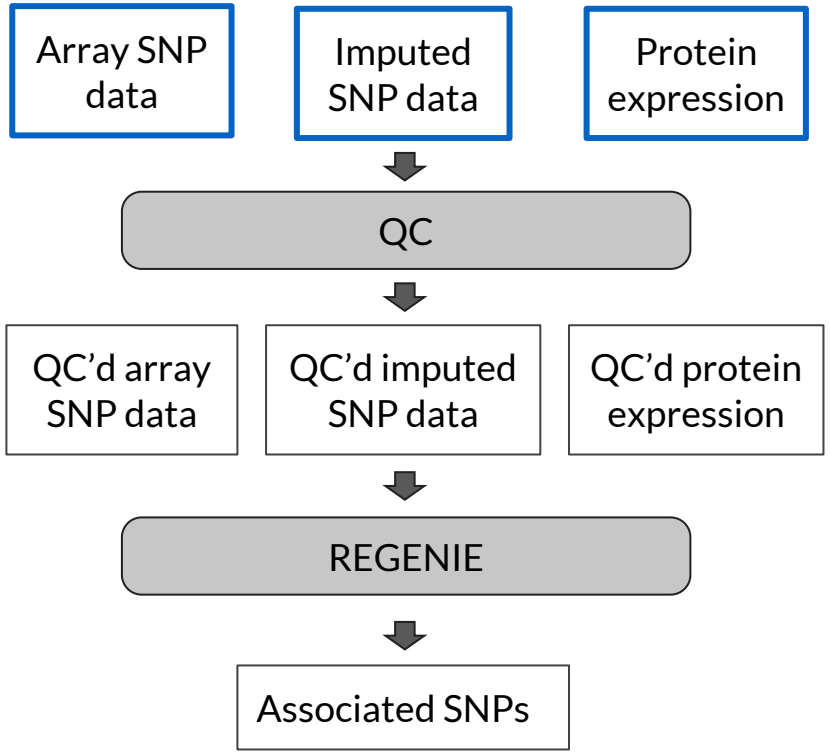
Phenotype + Imputed data + PRS (use as covariate) → Variant effect

REGENIE paper
End-to-end Target Discovery webinar

Approach

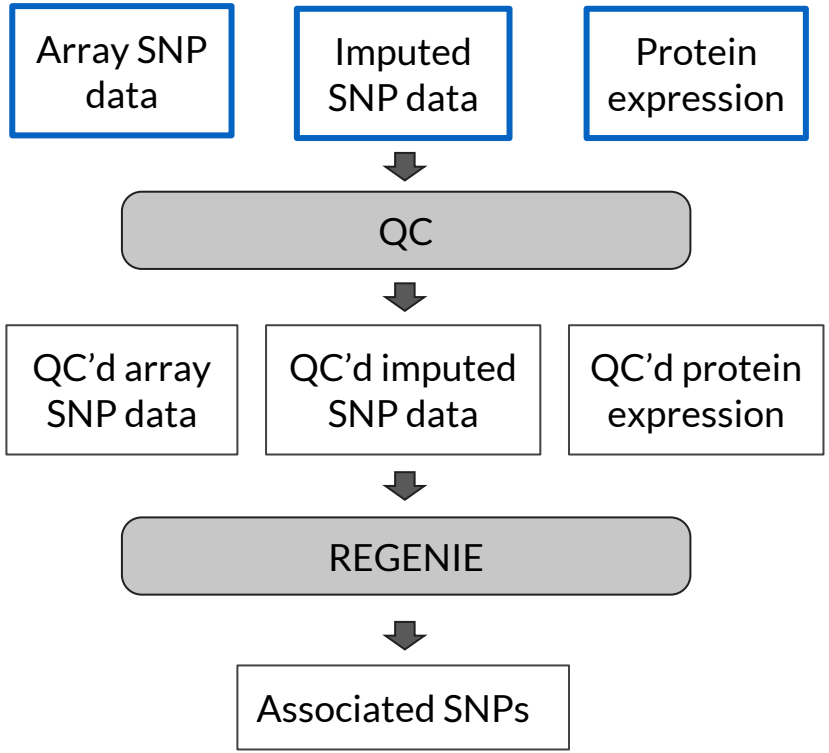


Simulate input data

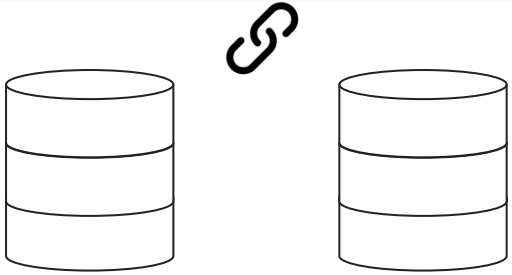


NOTE: *We are using public proteomic data, not UKB data, for demonstration purposes*

Matched genotype and protein expression data



FID	IID	CA1	ICAM1	CHL1	TGFBI	ENG
2894753	2894753	7.62107	6.79971	4.73174	9.33471	3.12445
2352368	2352368	6.96085	6.98459	4.31338	9.06819	3.31576
1483346	1483346	7.16983	7.04907	4.72713	8.92804	3.16308
2352196	2352196	7.45724	6.89523	4.57029	9.27165	3.06199
4886500	4886500	7.81354	6.71708	4.93904	9.51350	3.66898

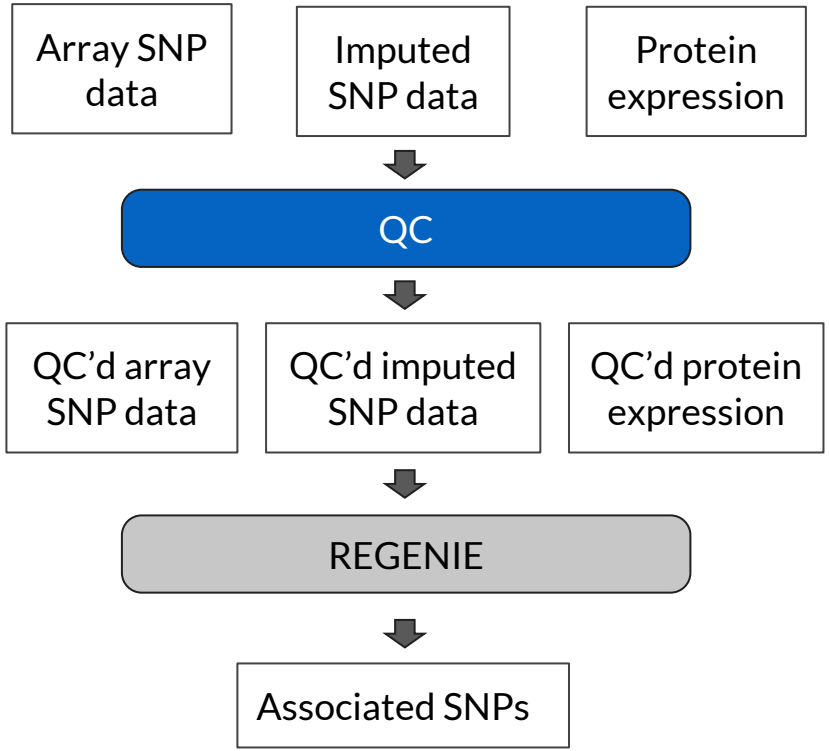


UKB array SNP data

UKB imputed SNP data

Notebook to simulate data

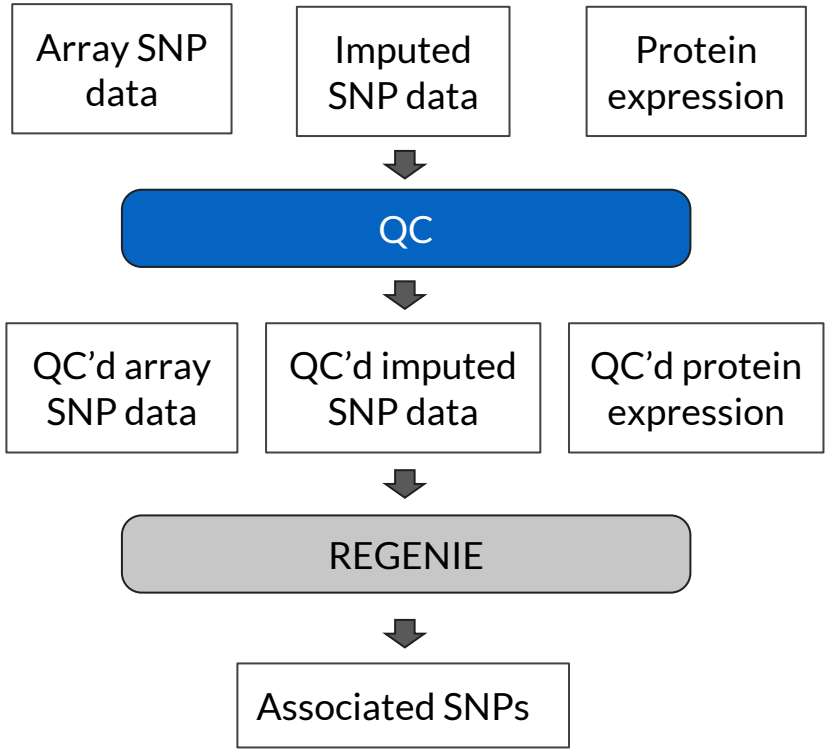
QC input data



FID	IID	CA1	ICAM1	CHL1	TGFBI	ENG
2894753	2894753	7.62107	6.79971	4.73174	9.33471	3.12445
2352368	2352368	6.96085	6.98459	4.31338	9.06819	3.31576
1483346	1483346	7.16983	7.04907	4.72713	8.92804	3.16308
2352196	2352196	7.45724	6.89523	4.57029	9.27165	3.06199
4886500	4886500	7.01354	6.71708	4.93904	9.51350	3.66898

- Filter samples to remove possible confounders
- Removing missing or low quality variants and proteins

QC input data



FID	IID	CA1	ICAM1	CHL1	TGFBI	ENG
2894753	2894753	7.62107	6.79971	4.73174	9.33471	3.12445
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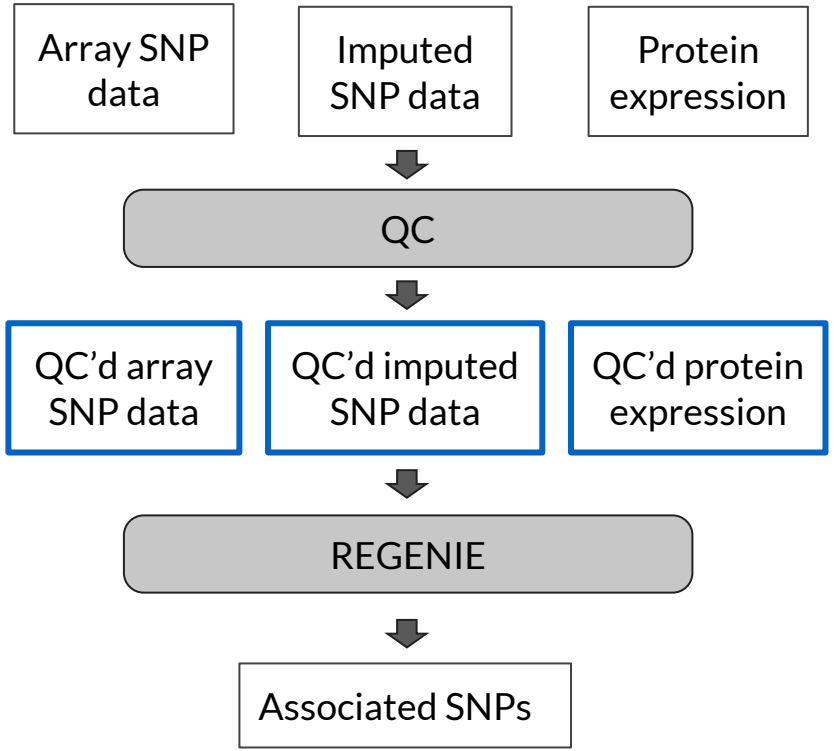
1483346	1483346	7.16983	7.04907	4.72713	8.92804	3.16308
2352196	2352196	7.45724	6.89523	4.57029	9.27165	3.06199
4886500	4886500	7.81354	6.71706	4.93904	9.51350	3.66898

- Sample QC steps
- Array variant QC steps
- Imputed variant QC steps

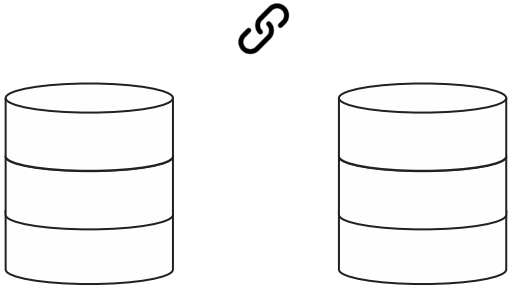
UKB array SNP data

UKB imputed SNP data

Get QC'd input data



FID	IID	CA1	ICAM1	CHL1	TGFBI	ENG
2894753	2894753	7.62107	6.79971	4.73174	9.33471	3.12445
2352368	2352368	6.96085	6.98459	4.31338	9.06819	3.31576
1483346	1483346	7.16983	7.04907	4.72713	8.92804	3.16308
2352196	2352196	7.45724	6.89523	4.57029	9.27165	3.06199
4886500	4886500	7.81354	6.71708	4.93904	9.51350	3.66898

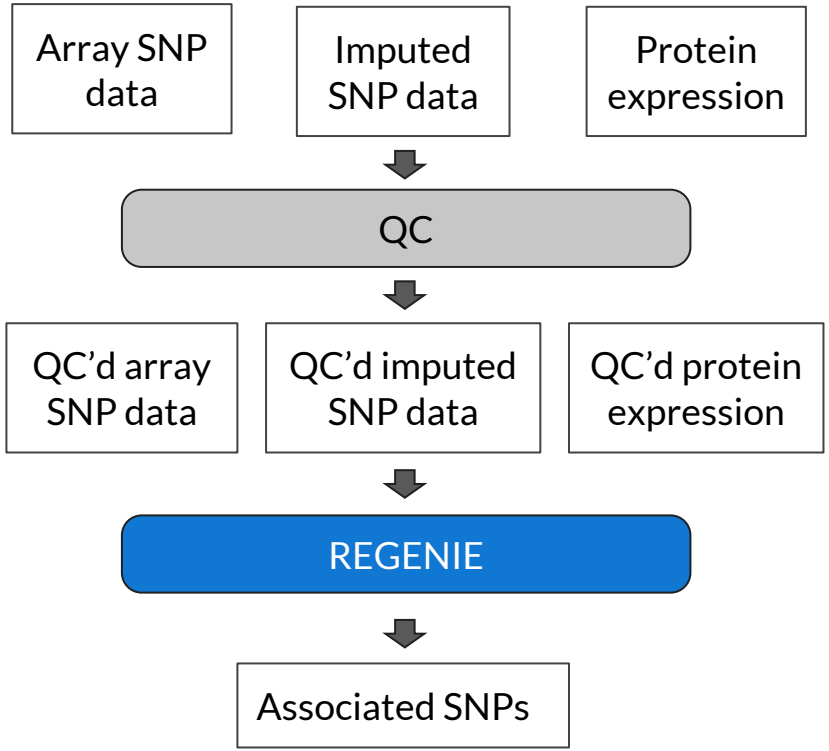


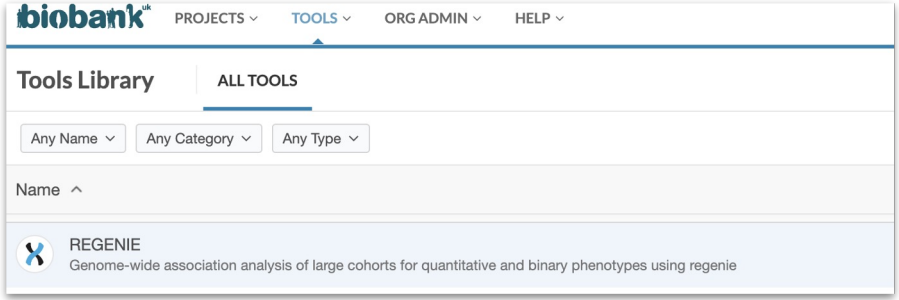
UKB array SNP data

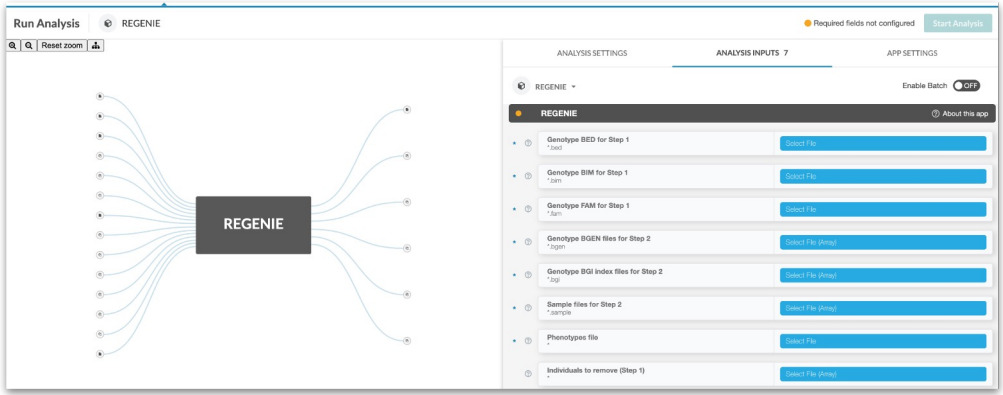
UKB imputed SNP data

- 100,100 samples
- ~500,000 variants
- 200 proteins

Run GWAS







REGENIE GWAS analysis settings

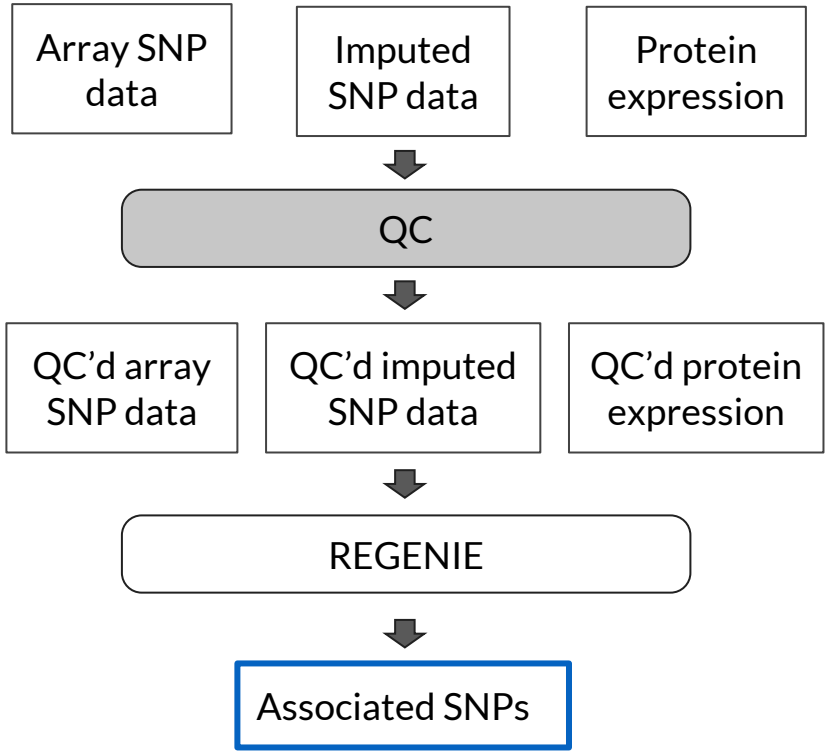
Step 1

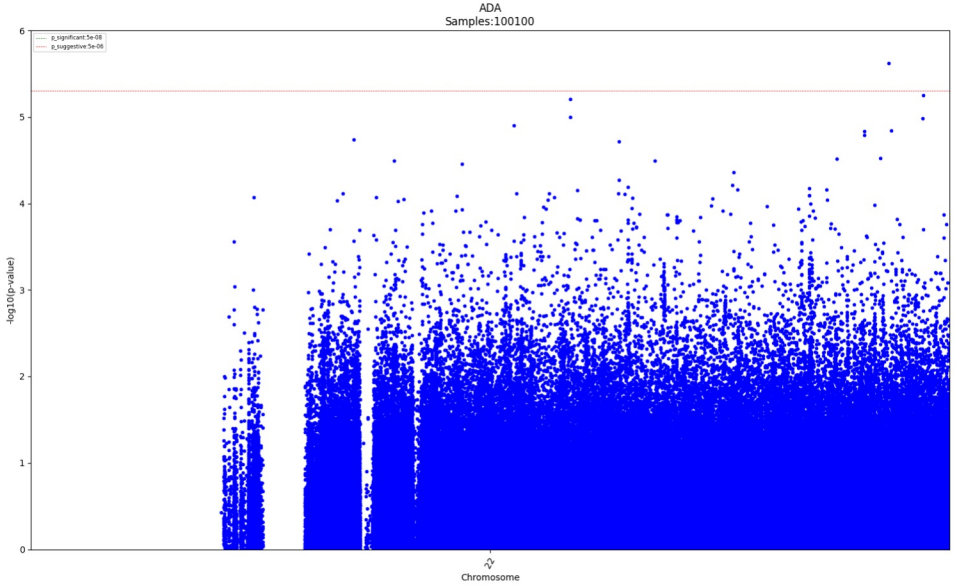
- **Phenotype File:** `<.phe file containing protein expression>`
- **QC'D genotype Files (SNP array):** `<.[bed, bim, fam] QC'd genotype files after liftover>`
- **Variant IDs to extract:** `<.snplist file containing list of QC'd array SNPs>`

Step 2

- **Phenotype File:** `<.phe file containing protein expression>`
- **Sample ID File:** `<.phe file containing protein expression>`
- **BGEN, BGI, SAMPLE genotype Files (Imputed SNP):** `/Bulk/Imputation/ukb21008_c22_b0_v1.[bgen, bgi, sample]`
- **Variant IDs to extract:** `<.snplist file containing list of QC'd imputed SNPs>`

Found significantly associated SNPs





Resources

	Link	Configuration	Runtime & cost
Code to create simulated protein expression data	https://github.com/dnanexus/UKB_RAP/blob/main/proteomics/protein_pQTL/1_simulate_input_data.ipynb	**Kernel**: PYTHON_R **Priority**: normal **Recommended instance**: mem1_ssd1_v2_x2	**Runtime**: ~ 1min **Cost**: ~£ 0.0069
QC steps from end-to-end webinar	https://github.com/dnanexus/UKB_RAP/tree/main/end_to_end_gwas_phewas		
Steps to run REGENIE	https://github.com/dnanexus/UKB_RAP/blob/main/proteomics/protein_pQTL/README.md		**Runtime**: ~ 19 hours **Cost**: ~£1.04
REGENIE publication	https://www.nature.com/articles/s41588-021-00870-7		

Conclusion

- Researchers can use UKB-RAP to analyze proteomic data
- Proteomic data can be extracted via the Cohort Browser
- Differential expression analysis can be done using custom code in JupyterLab
- pQTL analysis can be done using REGENIE app following end-to-end tutorial steps

Helpful resources

- Integrative analysis of UKB proteomics data - webinar
- UKB Research analysis platform overview - webinar
- Introduction to JupyterLab notebooks on RAP - webinar
- End to end target discovery with GWAS and PheWAS on the UKB research analysis platform - webinar

Upcoming events

- **Webinar: Dementia and Multimorbidity in Late-Life disease: Longitudinal and Multimodal Data Science Approaches**
  - When: Late June TBA
  - Registration TBA
- Subscribe
- All webinar recordings

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Acknowledgements

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Arkarachai Fungtammasan, PhD
Scientific Community Manager/ Principal Scientist

UKB-RAP team
Ben Busby, PhD
Ted Laderas, PhD
SciProd team

Thank you! Questions?