Single-cell eQTL Analysis:
Population Genomics Moving Closer To Precision Medicine

What is eQTL Analysis?

eQTL (expression Quantitative Trait Loci) analysis predicts the association between genetic variation and gene expression levels measured in hundreds of individuals. It aims at finding relationships between gene regulation and phenotypic traits (e.g., sex, age and disease status) (Figure 1). There are two categories of eQTLs, cis and trans. Cis-eQTLs are located close to the regulated gene (up to 1Mb) while trans-eQTLs are far from the regulated gene (>5Mb).

This analysis has been performed for years using bulk RNA-seq, correlating genetic variation with gene expression. The use of bulk RNAseq approaches allowed the identification of eQTLs with a sample-wide effect on gene expression, but what about cell-type specific effects? These remained hidden by the averaged gene expression measurements.

With the advent of single cell RNAseq it is now possible to discover the cell-type specific effects of genetic variations on gene expression.

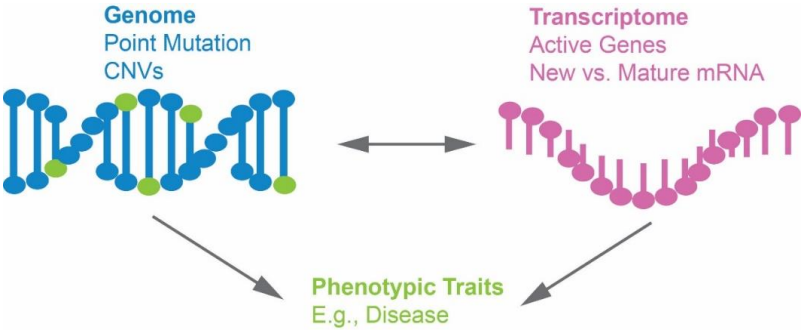

Figure 1 – eQTL Analysis reveals the interactions between genome and transcriptome, allowing for the prediction of the impact on phenotypic traits.

How can single-cell sequencing be used in eQTL Analysis?

scRNAseq generates information of gene expression at the single-cell level, unravelling the sample heterogeneity and expression variability among cells, while RNAseq only produces an averaged profile of the whole sample. It is now possible to identify the effects that the same SNP has in different cell types and at different biological contexts.

scRNAseq has several advantages over RNAseq:

- Ability to reveal complex and rare cell populations
- Unravel gene-gene and cell-cell regulatory relationships
- Track trajectories of different cell lineages

Cell-type specific identification of eQTLs was first demonstrated in 2013[1] measuring the expression of 92 genes in lymphoblastoid cell lines by parallel qPCR. The first real eQTL study at the single-cell level was completed in 2018[2] using single-cell sequencing of PBMCs. This work allowed for the identification of 22 cell-type specific *cis*-eQTLs.

Several more publications have been released demonstrating the power of scRNAseq in identifying cell-type specific *cis*- and *trans*-eQTLs and comparing them with bulk RNAseq GWAS data to better understand disease mechanisms.

Examples have been published for neurological disorders[4], chronic kidney disease[5], autoimmune diseases[3] and others. Moreover, a single-cell eQTL consortium, sc-eQTLGen[6], has been generated to leverage the emerging data resources in this field.

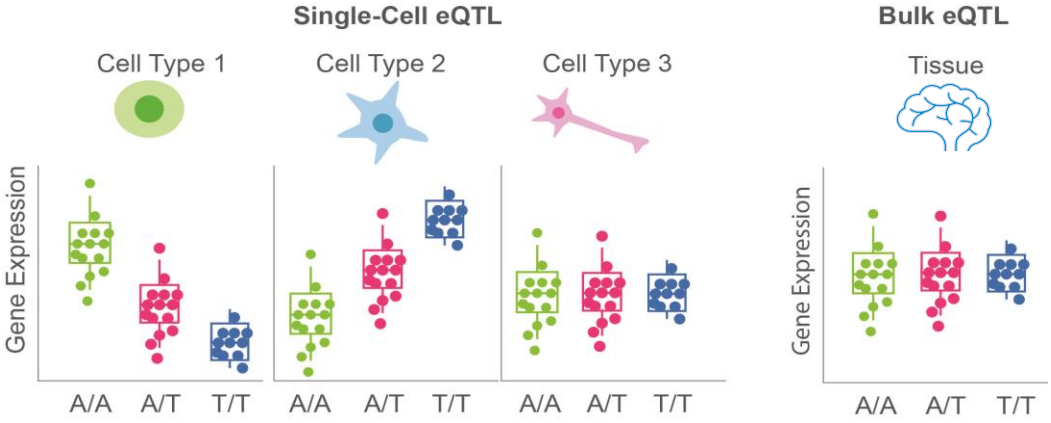

Figure 2 – Resolution differences between single-cell eQTL and bulk eQTL. Single-Cell eQTL allows to unravel genomic variations at the cell type level that might be lost at the tissue level, where the averaged expression prevents the inter-cell differences from being quantified.

How can Singleron help with the unravelling of cell-type specific eQTLs?

Singleron's extensive experience with single-cell data generation using our SCOPE-chip and single-cell kits makes the transition into large eQTL studies an easy one.

Our service lab can process over 660 different types of tissue and has extensive experience with clinical samples. As a result, we could easily develop an eQTL framework, where we work with our customers to design cutting-edge eQTL projects to answer their biological questions and unravel the link between genetic variation, gene regulation and phenotypic traits.

Due to the large phenotypic variation between individuals, eQTL studies must be conducted with tens to hundreds of individuals to ensure significant association between genetic polymorphisms and gene expression. In our framework, we recommend analyzing around 200 individuals, including a combination of control and experiment samples. This number allows the discovery of high confidence *cis*- and *trans*-eQTLs.

To conduct this research, two kinds of datasets need to be generated: scRNAseq and WGS, as the link between genetic variation and gene expression (cell type specific) needs to be established (Figure 2). In specific cases it might be possible to take advantage of previously published WGS cohorts.

scRNAseq data generates expression profiles of the different cells, that can be annotated with the donor clinical information. WGS data is used to identify SNPs and other genetic variations among the sample population. The combination of both datasets allows for the identification of general and cell-type specific eQTLs (Figure 3). This information can be compared prior to bulk RNAseq GWAS studies so that the SNPs identified in the dataset can be linked with disease associated loci. Unravelling the cell-type specific eQTLs allows for the understanding of the molecular mechanisms behind diseases or phenotypic traits but also to identify patient specific differences that will allow for better patient stratification and treatment choices.

For specific diseases such as immune disorders or cancers, the single-cell data could be combined with immune receptor information to further understand the diversity of the immune cells. This could be easily achieved with Singleron's GEXSCOPE® Single Cell V(D)J or sCircle® kits which combine full transcriptome information with immune receptor sequencing.

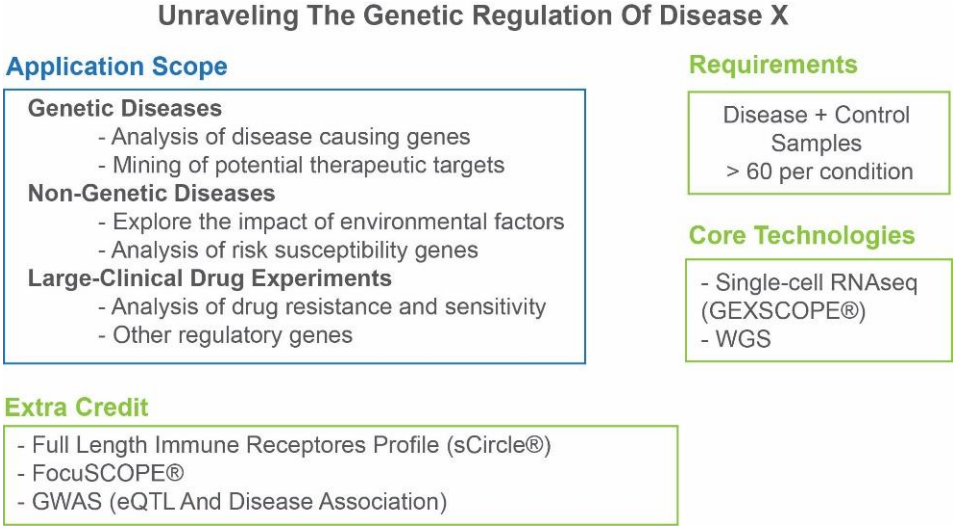

Figure 3 – eQTL Project Example. eQTL analysis can be applied to different types of disease to better understand its' regulation and what are the genes that impact disease progression and treatment response (Application Scope). These projects explore the impact of genetic variation. Considering the high variation that exists between individuals, single-cell eQTL analysis requires a high sample number in order to make significant predictions (Requirements). eQTL analysis focuses on the connection between gene expression and genetic alterations (Core Technologies), but the addition of extra layers of information such as immune profiling can add further information into disease regulation (Extra Credit).

eQTL Data Analysis Workflow

Bioinformatics experts at Singleron developed a single-cell eQTL analysis workflow that takes advantage of scRNAseq and WGS data to predict cell-type specific eQTLs. It utilizes multiple techniques and analysis pipelines that have been proven to generate valuable insights into understanding the role of gene regulation in phenotypic traits.

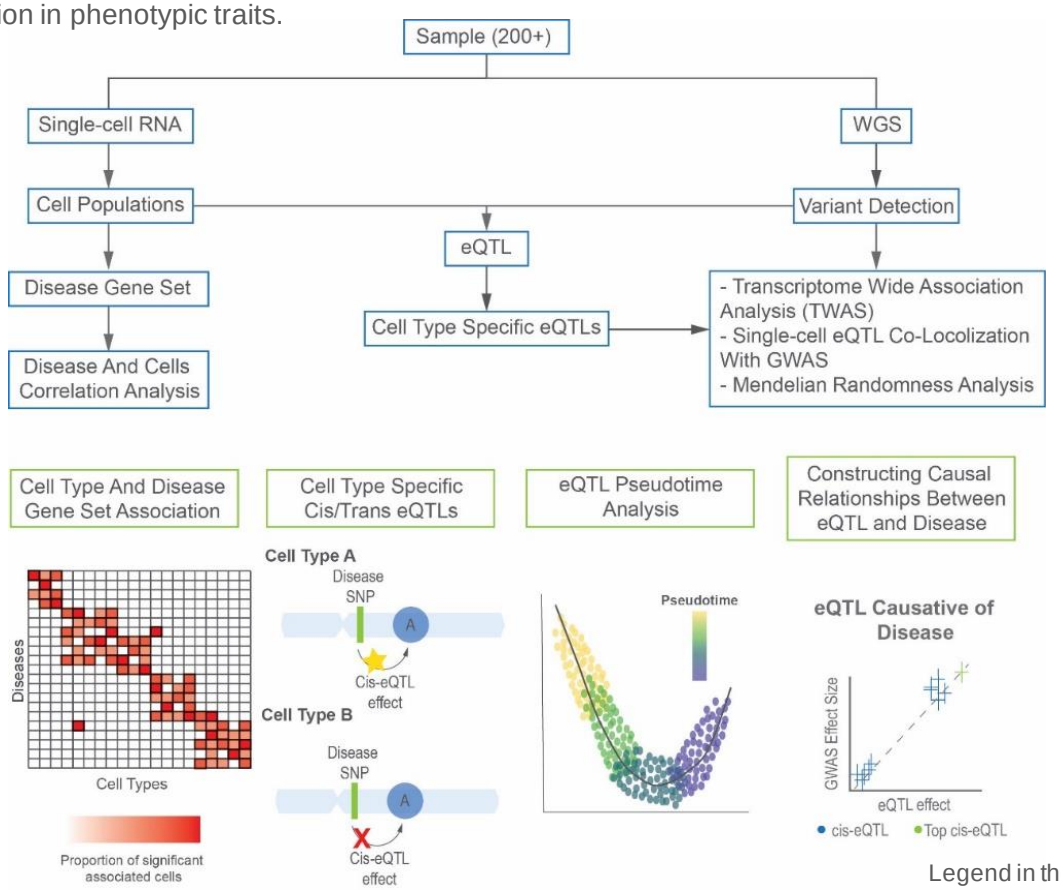

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Figure 4 – eQTL Projects Analysis. Schematic of the eQTL projects analysis offered at Singleron. The studies combine data from single-cell RNAseq and WGS to understand the regulation of expression by genome variants and correlation with disease traits. Examples of the analysis included in these projects are shown below (cell-type and disease gene set association, cell-type specific eQTLs, eQTL pseudotime and causal relationships between eQTL and diseases).

Singleron Service Workflow

Our unique tissue preservation buffer can keep tissues viable for 72h without affecting the transcriptome. This is particularly useful for processing large cohort of clinical samples including biopsies in a central lab with standard protocols.

Our service teams in Germany and Singapore have extensive single-cell expertise in both wet lab and data analysis and can support your project with a streamlined workflow.

Interested? Get A Free Consultation On Your Project With One Of Our Specialists

Call us
+49 221 16824777

Email us
info@singleronbio.com

Visit us
www.singleron.bio

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