Introduction to Bioinformatics

Introduction to Omics and Application in R

Haries Ramdhani. 2021.

Data Frames

Data frames:

- are tabular data objects
- can contain different types of data inside it
- contain vector of equal length

(Follow up on) Data Frames

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- are tabular data objects
- can contain different types of data inside it
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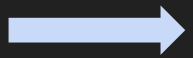
```
> data.frame(
      gender = c("Male", "Male","Female"),
      height = c(152, 171.5, 165),
      weight = c(81,93, 78),
      Age = c(42,38,26)
  )
```

	gender	height	weight	Age
1	Male	152.0	81	42
2	Male	171.5	93	38
3	Female	165.0	78	26

DPLYR

dplyr is a grammar of data manipulation, providing a consistent set of verbs that help you solve the most common data manipulation challenges

Previous subsets [ ]



- select()
- filter()
- group_by()
- summarize()
- mutate()

Installing and using DPLYR (and other packages)

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install.packages("name_of_the_packages")

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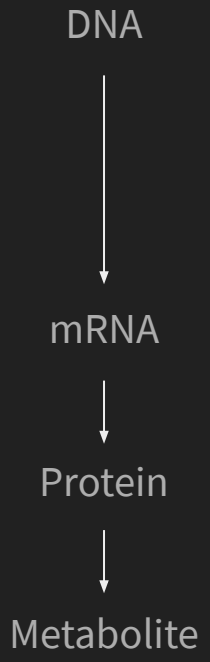
DPLYR

Common functions in DPLYR

- select() : select our intended column
- filter() : filter the data by some conditions
- group_by() : group the data by column
- summarize() : summarize the results of grouping
- mutate() : mutate a column, create new column with new logic

A Quick Glimpse on Omics



A Quick Glimpse on Omics

Genomics	DNA
Epigenomics	Epigenetic Modification
Transcriptomics	mRNA
Proteomics	Protein
Metabolomics	Metabolite

A Quick Glimpse on Omics

Genomics	DNA	Exploring Variants
Epigenomics	Epigenetic Modification	Quantifying Epigenetic Modification
Transcriptomics	mRNA	Gene Expression
Proteomics	Protein	Post Translational Modifications
Metabolomics	Metabolite	Determining phenotypes

A Quick Glimpse on Omics

Genomics	DNA
Epigenomics	Bisulfite treated DNA, Immunoprecipitation
Transcriptomics	mRNA -> cDNA
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A Quick Glimpse on Omics

Genomics	DNA	DNA-Seq (WGS, WES)
Epigenomics	Bisulfite treated DNA, Immunoprecipitation	BiSulfite Sequencing, EpicArray, ChipSEQ
Transcriptomics	mRNA -> cDNA	RNA-Seq, Expression Microarray
Proteomics	Protein	Protein Microarray, Spectometry
Metabolomics	Metabolite	Liquid Chromatography

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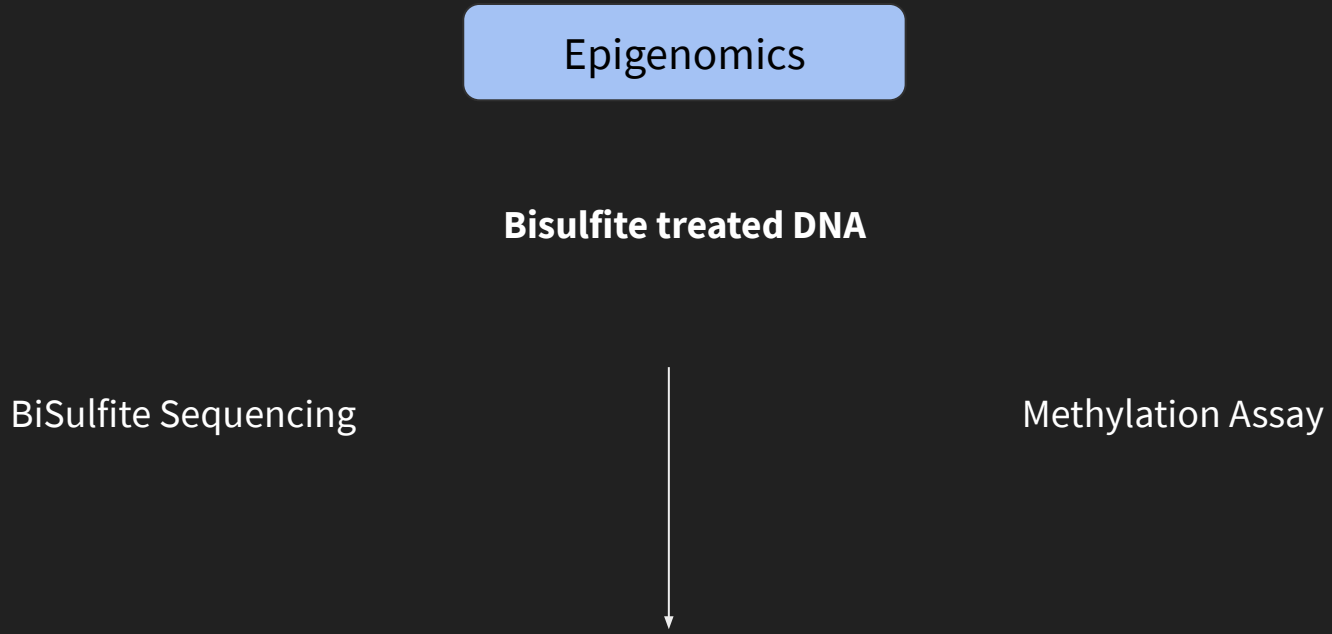
Epigenomics



Methylation Array Data

Samples

Array

Data

Methylation Array Data

- For each CpG sites, there are two things that we measure:
  - Unmethylated Intensity (U)
  - Methylated Intensity (M)

Maksimovic et al. 2020. A cross-package Bioconductor workflow for analysing methylation array data.

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  - M-value ($\log_2(M/U)$)
  - Beta- value (M / (M+ U))
- Beta values are generally preferable for describing the level of methylation at a locus or for graphical presentation because percentage methylation is easily interpretable. However, due to their distributional properties, M-values are more appropriate for statistical testing (Du et al. 2010)

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Methylation Array Data

- Illumina raw data files are usually either in plain text or binary format.
- The binary "IDAT" files (stands for "intensity data file") are generated by the scanner and can be parsed using R/BioConductor packages such as illuminaio)
- You can find the data for training in GEO Omnibus usually written under the ***Methylation profiling by array*** tag

Let’s Remember the Previous Lesson

Pipeline Summary

The pipeline allows you to choose between running either Bismark or bwa-meth / MethylDackel. Choose between workflows by using `--aligner bismark` (default, uses bowtie2 for alignment), `--aligner bismark_hisat` or `--aligner bwameth`.

Step	Bismark workflow	bwa-meth workflow
Generate Reference Genome Index *(optional)*	Bismark	bwa-meth
Raw data QC	FastQC	FastQC
Adapter sequence trimming	Trim Galore!	Trim Galore!
Align Reads	Bismark	bwa-meth
Deduplicate Alignments	Bismark	Picard MarkDuplicates
Extract methylation calls	Bismark	MethylDackel
Sample report	Bismark	-
Summary Report	Bismark	-
Alignment QC	Qualimap	Qualimap
Sample complexity	Preseq	Preseq
Project Report	MultiQC	MultiQC

For array data it’s a bit shorter

Main flow:

- Quality control
- Filtering
- Normalization
- Data exploration

Downstream Analysis:

- Probe wise differential methylation analysis
- Differential variability analysis
- GO analysis
- etc.

Loading the data

Before loading the data you have to import the needed libraries in R

- **Minfi** : provides tools for analyzing Illumina's Methylation arrays, specifically the 450k and EPIC
- **IlluminaHumanMethylation450kanno.ilmn12.hg19** : Annotation for Illumina's 450k methylation arrays
- **IlluminaHumanMethylation450kmanifest** : Annotation for Illumina's 450k methylation arrays
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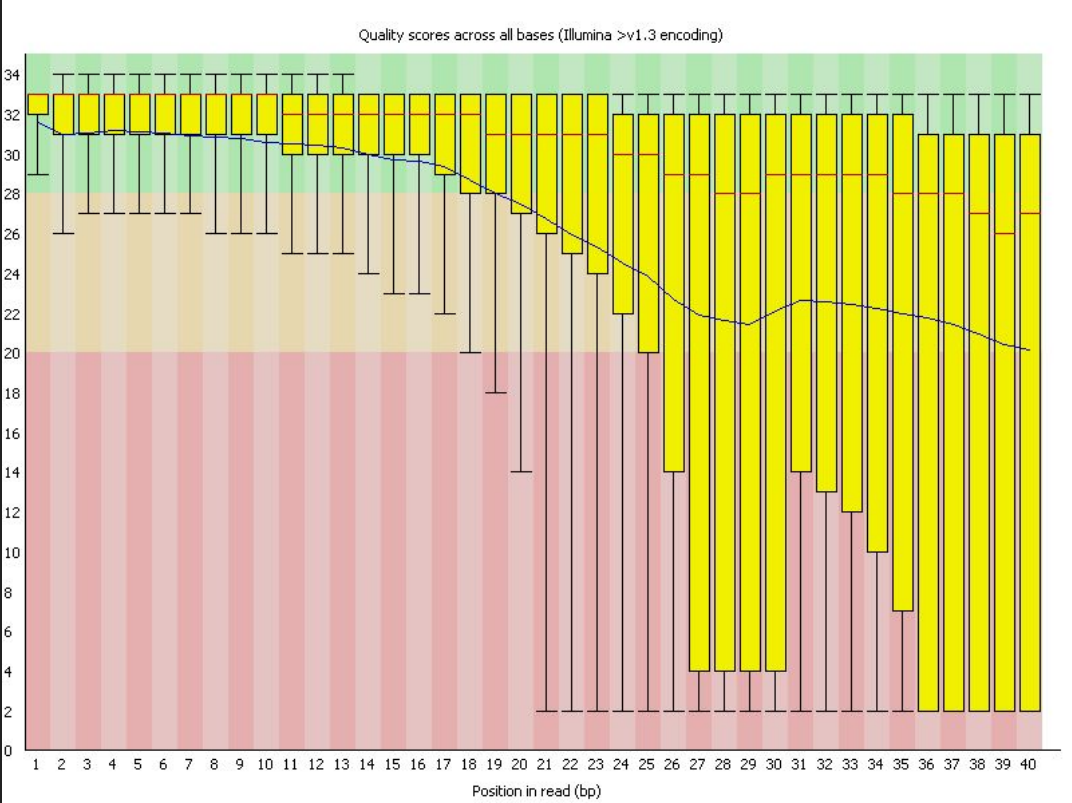
Quality Control in NGS



More information on interpreting:

https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon/lessons/qc_fastqc_assessment.html

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- The method used by minfi to calculate detection p-values compares the total signal (M+U) for each probe to the background signal level
- Poor quality samples can be easily excluded from the analysis using a detection p-value cutoff, for example >0.05

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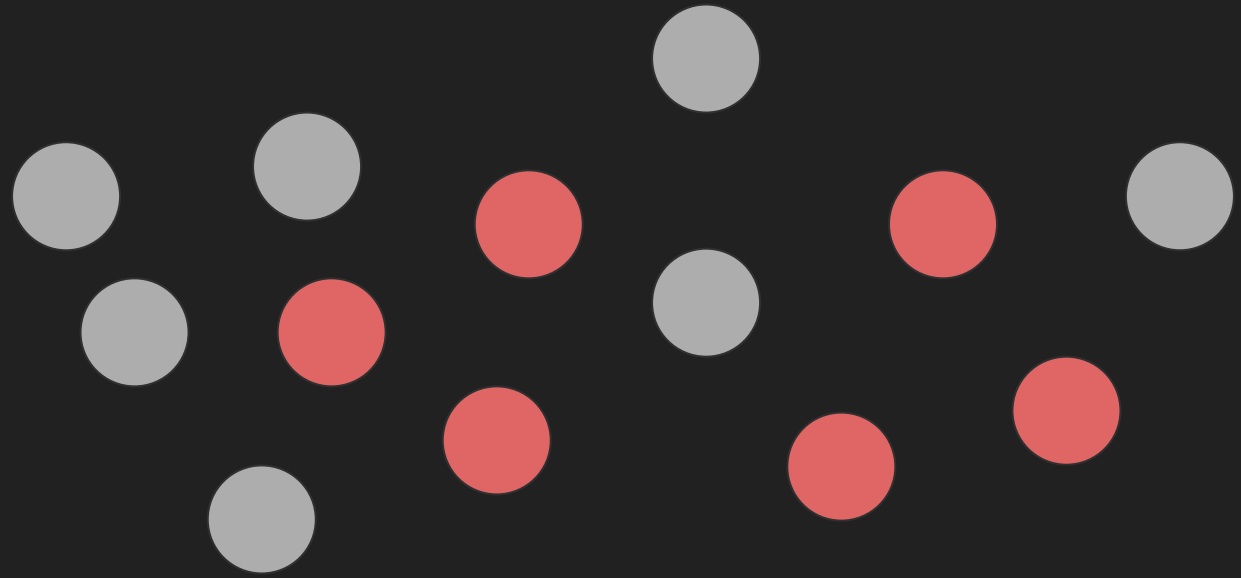

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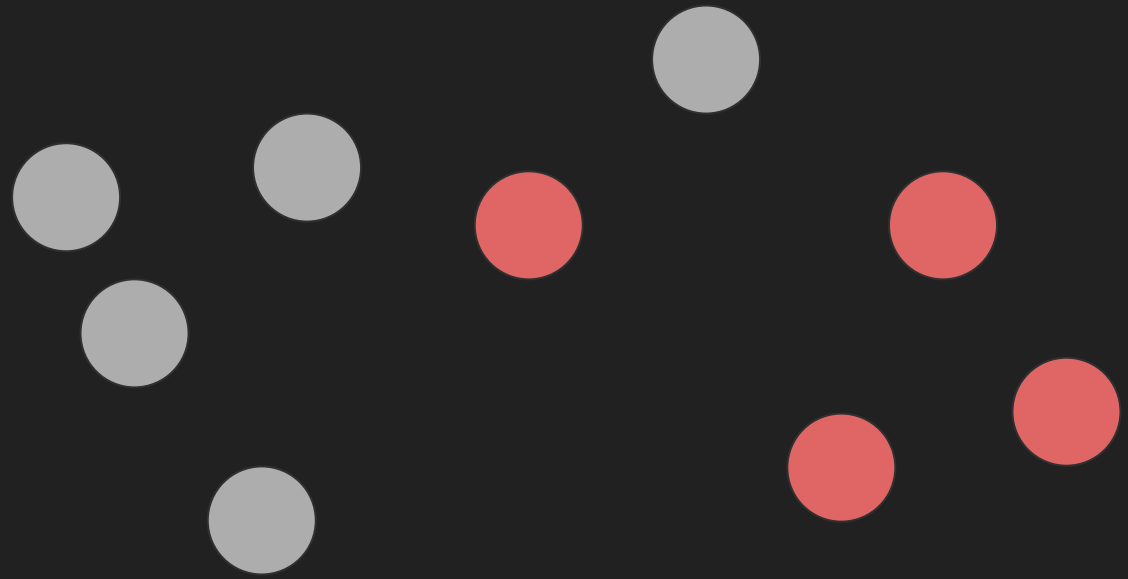

Normalisation

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- Many different types of normalisation have been developed for methylation arrays
- Several methods have been built into minfi and can be directly applied within its framework
- There is no BEST normalisation method, but a recent study by Fortin et al. (2014) has suggested that a good rule of thumb within the minfi framework to use:
  - preprocessFunnorm (for datasets with global methylation differences such as cancer/normal or vastly different tissue types,
  - whilst the preprocessQuantile function (Touleimat and Tost 2012) is more suited for datasets where you do not expect global differences between your samples, for example a single tissue.

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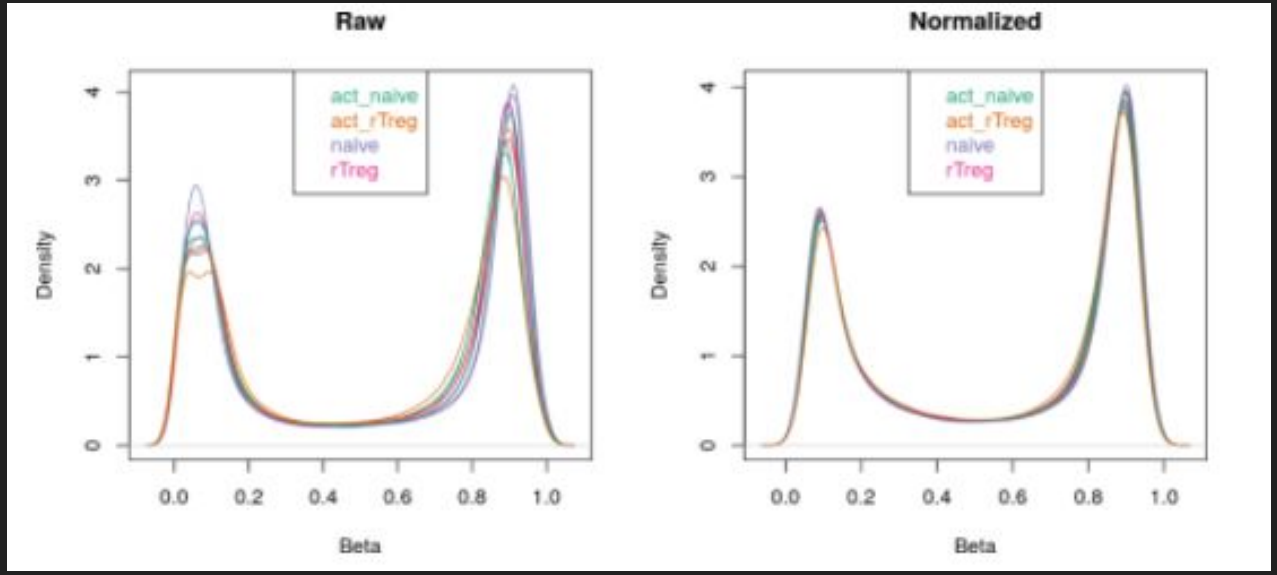

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Data Exploration

- We perform data exploration in case we want to explore more about our array data
- Maybe this way we can get new insights or new information regarding something that is not seen by our eyes (through data viz)
- Most common approach:
    - MDS (Multidimensional Scaling) : Look similarity between samples
        - Samples that are very similar should be clustered together

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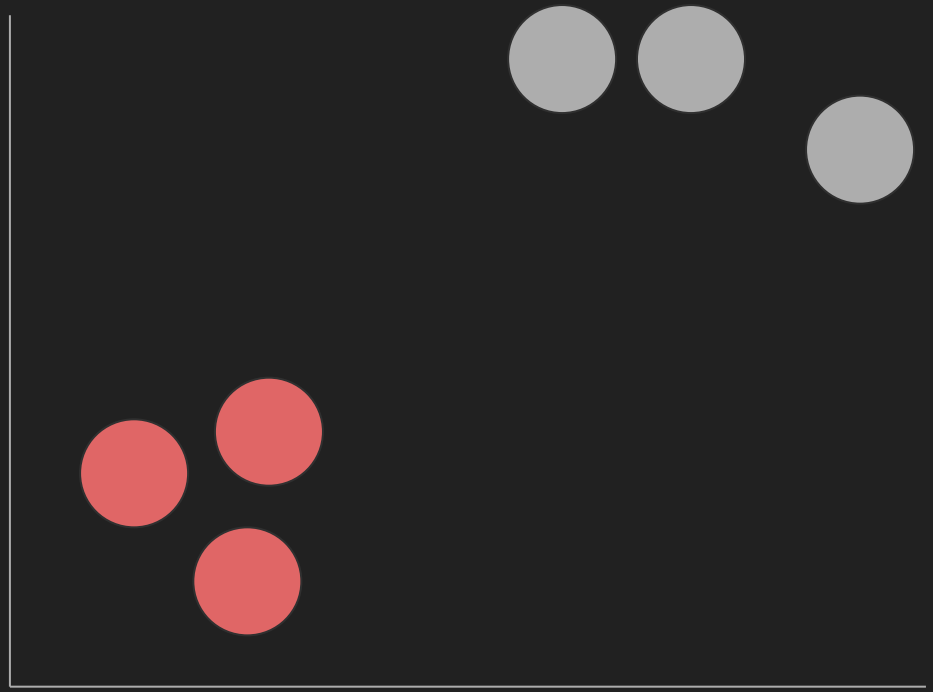

Data Exploration

Filtering

- Poor performing probes are generally filtered out prior to differential methylation analysis.
- As the signal from these probes is unreliable, by removing them we perform fewer statistical tests and thus incur a reduced multiple testing penalty.
- We filter out probes that have failed in one or more samples based on detection p-value.

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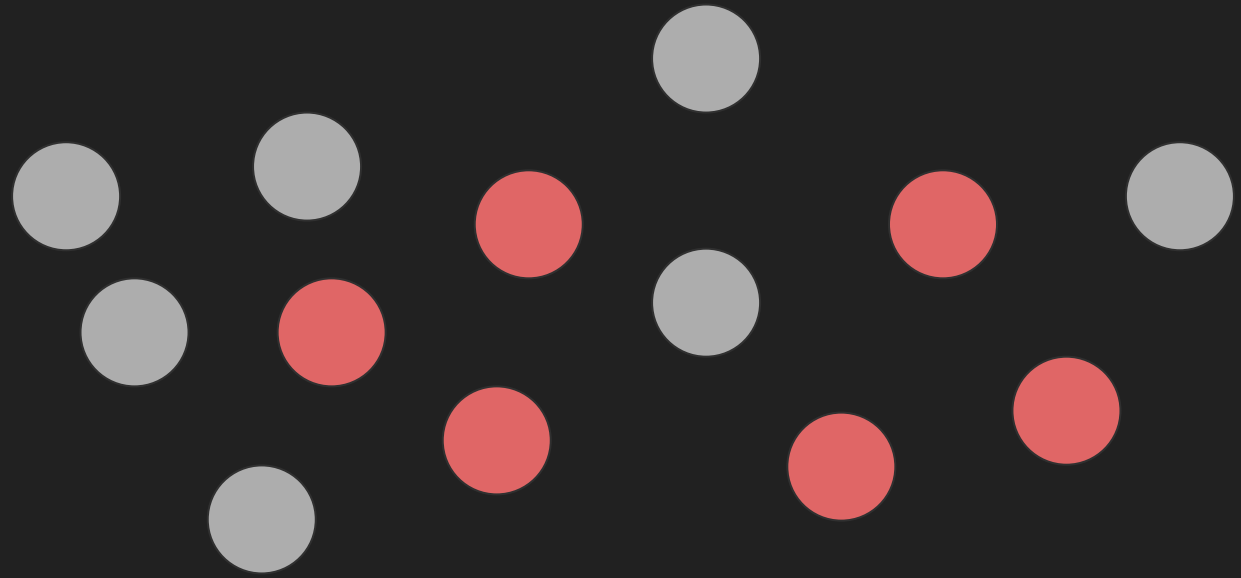

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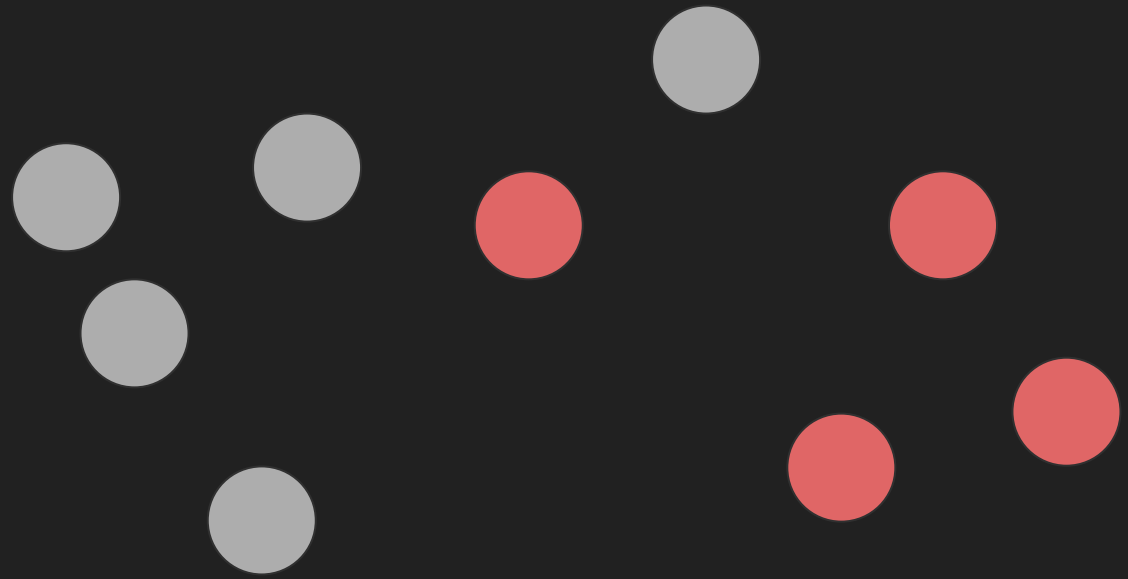

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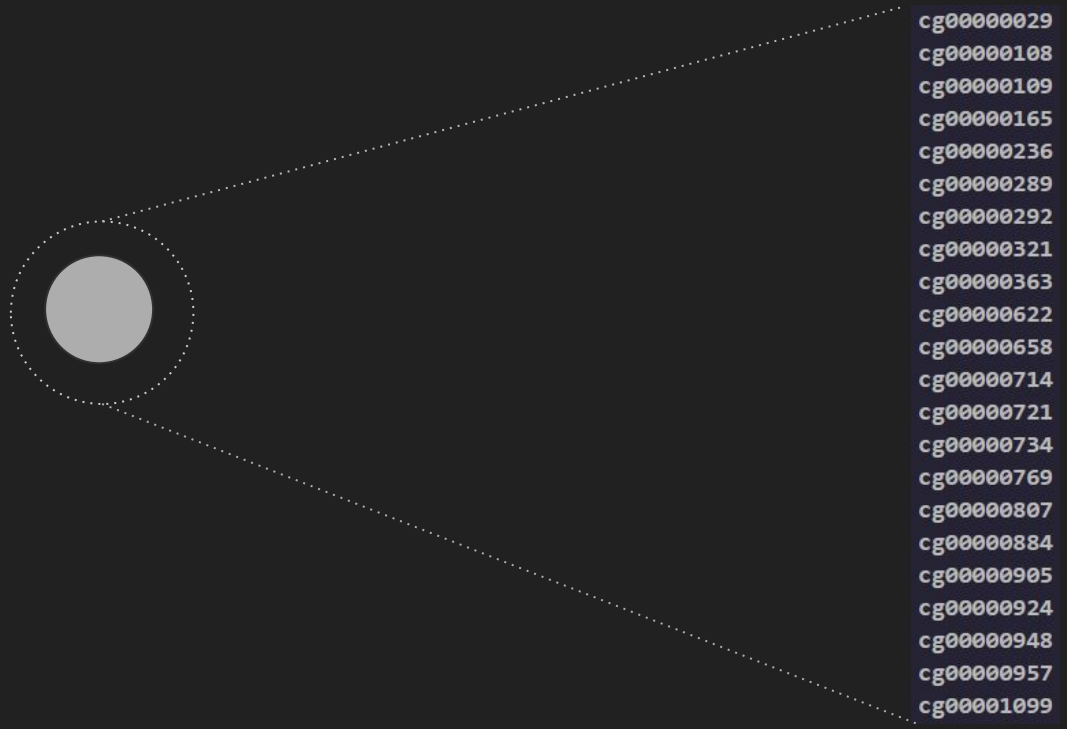
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