Profiling genetic and epigenetic changes, at read-level, after cellular rejuvenation

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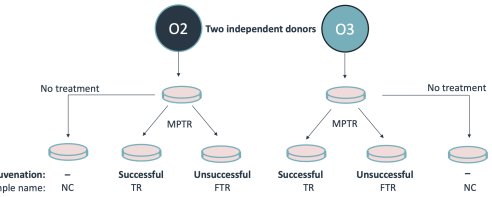
1. Introduction

In addition to the genetic alphabet A, C, G and T, DNA contains epigenetic information in the form of methylated cytosines**.** Genetics and epigenetics, and their interaction, are of fundamental importance for biomedical and clinical research and practice.

At the cellular level, **ageing** is associated with reduced function, altered gene expression and a **perturbed epigenome** (Horvath 2013). Recent work has demonstrated that the epigenome and some elements of function are rejuvenated by the maturation phase of iPSC reprogramming, without loss of cell identity.

Here we apply the Gill et al (2022) **"maturation phase transient reprogramming" (MPTR)**, where reprogramming factors (OSKM) are expressed until the rejuvenation point and then withdrawn. To investigate **genetic and epigenetic changes** after transient reprogramming, we use the novel **5-Letter sequencing*** technology.



*See poster P530 for details on 5-Letter sequencing by Cambridge Epigenetix

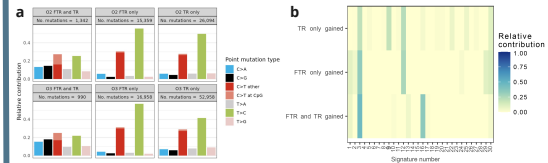
2. MPTR introduces differential levels of modC

Maturation phase transient reprogramming introduces epigenetic changes. (a) Analysis of differentially methylated regions (DMRs) between negative controls (NC) and samples that were successfully rejuvenated (TR) or that failed to rejuvenate (FTR). DMRs were identified using a -1/+1 cutoff on the corrected t statistic, corresponding to the top/bottom ~2% for TR samples and top/bottom ~7% for FTR samples. **(b)** Genes closest to CpG islands most hypo- or hyper-methylated in TR/FTR samples compared to NC.

Communications

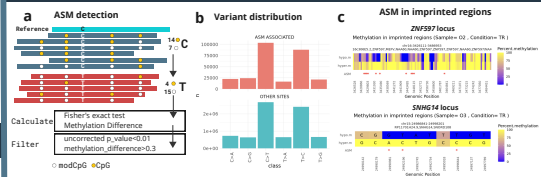
Horvath 2013: "*DNA methylation age of human tissues and cell types*", Horvath, 2013;14(10):R115. doi: 10.1186/gb-2013-14-10-r115.
Gill et al 2022 "*Multi-omic rejuvenation of human cells by maturation phase transient reprogramming*", Gill et al., 2022 Apr 8;11:e71624. doi: 10.7554/eLife.71624.
BioRxiv paper: "*Accurate simultaneous sequencing of genetic and epigenetic bases in DNA*", Füllgrabe and Gosal et al., DOI: 10.1101/2022.07.08.499285. (Peer-reviewed paper in press)
Poster P530: *"Accurate and simultaneous sequencing of genetics and epigenetics in DNA"*, Creed et al.
Poster P520: *"Joint genetic and epigenetic sequencing technology leads to improved genetics compared to existing methylation calling methods"*, Lumby et al.

3. MPTR introduces genetic variation



Variants introduced by MPTR (a) Number and type of somatic mutations detected in successfully rejuvenated cells (TR), cells that failed (FTR) or in both (FTR and TR) relative to controls. Successfully reprogrammed cells accumulated more mutations than cells that failed, which may be caused by *de novo* mutation or selection of rare cell populations. **(b)** COSMIC mutational signature analysis of the mutations detailed in (a). Mutations unique to rejuvenated cells (TR) enrich in signature 9, which is associated with active DNA demethylation involving the enzyme activity-induced deaminase (AID).

4. Detection of Allele-Specific Methylation (ASM)



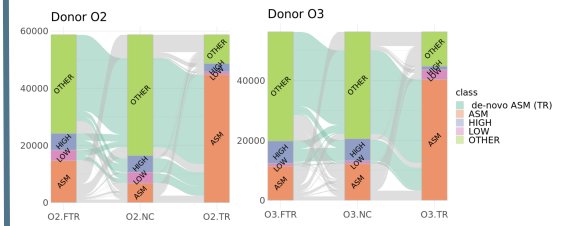
ASM detection with 5-Letter sequencing. (a) Schematics of ASM detection. CpG/mCpG associated with each variant are counted **(b)** A large number of C>T variants, which would be missed without 5-letter sequencing, is associated with allele-specific methylation. **(c)** Examples of allele-specific methylation on different alleles within imprinted loci connected with Prader-Willi Syndrome (*SNHG14*), or Russell-Silver syndrome (*ZNF597*). Red asterisks indicate alleles significantly associated with ASM.

7. Conclusions

Here, we use CEGX 5-letter sequencing technology to ask how MPTR (transient reprogramming) affects genomic sequence and allele specific methylation profiles.

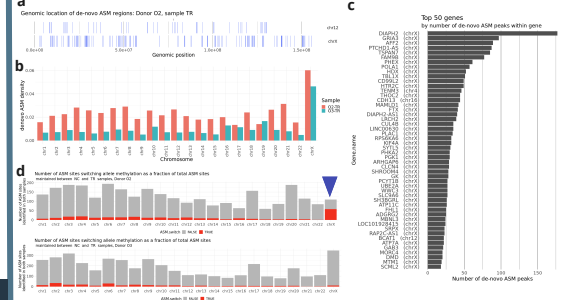
- Using the high accuracy sequence calls generated by 5-letter sequencing, we show that MPTR increases the number of detectable somatic mutations relative to controls. These could be caused by mutagenic processes, such as AID driven demethylation during the process as suggested by signature analysis, or due to selection bottlenecks.
- We call allele specific methylation (ASM) levels genome wide after MPTR. We find that ASM is increased following MPTR relative to controls, but not substantially so at canonically imprinted sites.
- Excitingly, we find that *de novo* ASM following MPTR is enriched on the X chromosome. We speculate that this indicates improved X chromosome silencing, which is known to deteriorate with age.

5. Successful MPTR is associated with increased ASM



Sankey representation of methylation status changes between untreated controls (NC), successfully rejuvenated cells (TR), and cells that failed rejuvenation (FTR). Successful MPTR is associated with increased ASM in both donors. The heterozygous sites gaining ASM in TR samples are largely associated with intermediate methylation in NC samples ("OTHER") or to a smaller extent with fully methylated ("HIGH") or demethylated ("LOW") sites. The majority of these sites are not converted to ASM in FTR samples.

6. MPTR-associated ASM on chromosome X



MPTR induces epigenetic changes on chromosome X. (a) Localisation of genomic windows (vertical blue lines) showing *de-novo* ASM in TR samples on chromosome X and a chromosome of comparable length (chr12) for donor O2; **(b)** Density of *de novo* ASM in TR samples from different donors, calculated as the length of windows displaying *de novo* ASM divided by the total length of the chromosome. **(c)** Top 50 genes by number of *de novo* ASM windows within the gene body in both O2 and O3 donors. Most genes are located on chromosome X. **(d)** In donor O2 (but not in donor O3), approximately half of the ASM sites that retained their ASM status during MPTR switched the methylation from one allele to the other.

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