INDUCE-seq: a novel tool for next-generation risk assessment

National Centre for the Replacement Refinement & Reduction of Animals in Research

Cardiff University / Prifysgol Caerdydd — School of Medicine / Ysgol Meddygaeth

Introduction

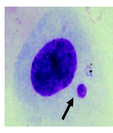
Rationale: INDUCE-seq was originally developed for the detection of double strand breaks (DSBs) induced by CRISPR-Cas9 nucleases. However, as an unbiased break detection method it can also be utilised for the detection of chemically-induced DSBs.

Aim: Develop INDUCE-seq for the quantitative detection of chemically-induced DSBs, with a focus on developing bioinformatic tools for characterising induced breaks and distinguishing these breaks from the background of endogenous breaks.

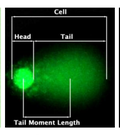
Approach: Treatment of cells with genotoxic chemicals, followed by break detection with INDUCE-seq. Analysis of break data as to determine break patterns associated with various chemicals. Extraction of latent information in order to elucidate the mechanisms of break induction.

Cell-based approaches

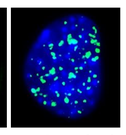
- Imaging/fluorescence-based
- Indirectly measures breaks
- Limited data output



Micronucleus test



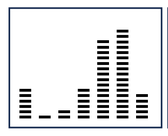
Comet assay



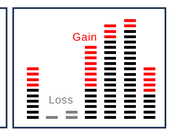
γH2AX foci imaging

Genomic techniques

- Sequencing-based
- Directly measures breaks
- Extensive data output



INDUCE-seq



Other NGS approaches

Next-generation risk assessment

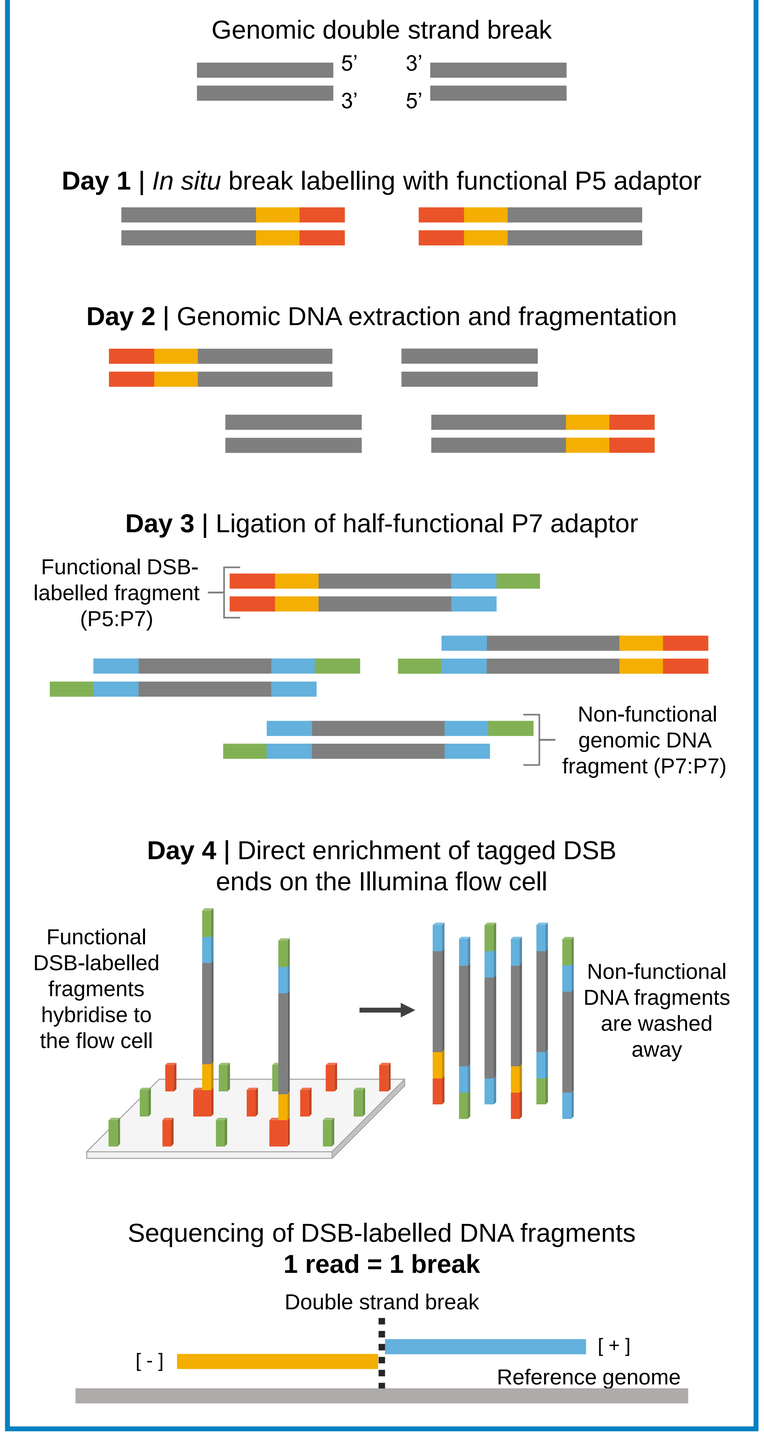
Integration of *in silico*, *in chemico* and *in vitro* approaches

Human-relevant

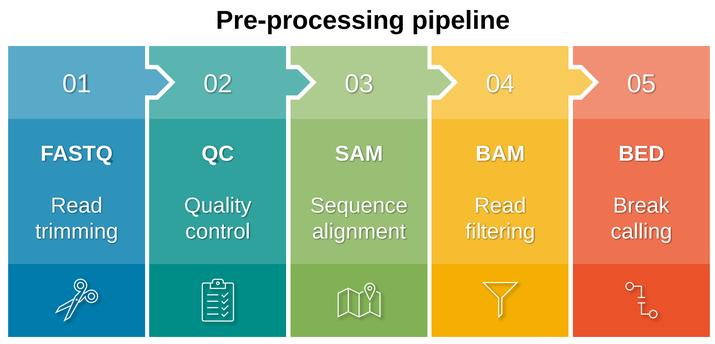
Exposure-led

Hypothesis-driven

INDUCE-seq methodology

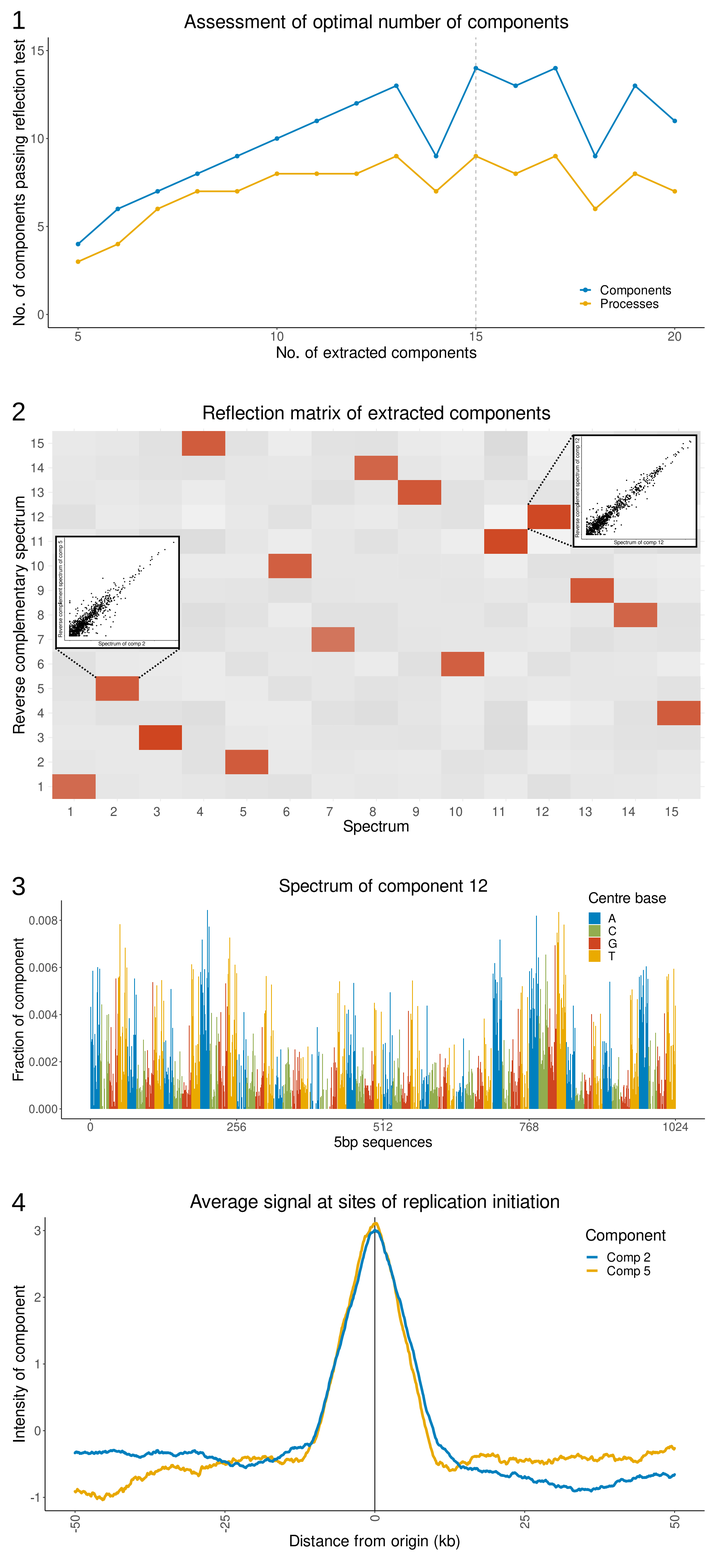


Data analysis



Latent features represent meaningful components extracted from break data and as such can be utilised to **classify breaks** based on the **mechanism** by which they were induced

Results



3Rs - Replacement

Development of **INDUCE-seq** for the quantitative detection of **chemically-induced breaks** in human cells

Provides additional genomic DNA damage to add to the various **omics methods** in development

Replace the use of rodents in industry sectors where animal safety studies are still used

These approaches represent the necessary tools for establishing **NGRA**

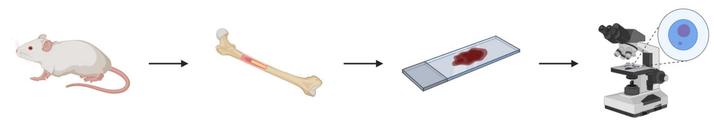
Most-widely used genotoxicity test is currently the **micronucleus assay**

The aim of NGRA is to increase acceptance of **non-animal data** in safety decision making

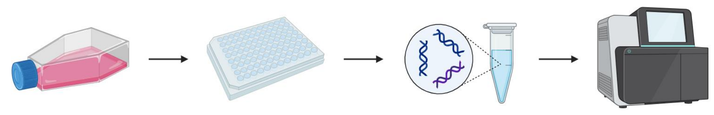
MN test involves **dosing rodents** with chemicals and sacrificing them to examine the bone marrow

INDUCE-seq is an alternative approach for **genotoxicity risk assessment studies** that utilises next-generation sequencing to directly measure DSBs

***In vivo* micronucleus assay** (historical approach)



INDUCE-seq (next-generation approach)



Discussion

INDUCE-seq is a novel genomic approach capable of unbiased genome-wide detection of chemically-induced breaks at a single-nucleotide resolution in human cells.

NMF has previously been used to extract signatures from catalogues of cancer mutations. These signatures have been associated with various mechanisms of DNA damage.

Volume-regularised NMF extracts a small number of signatures from an inventory of breaks and classifies the underlying processes as strand-independent or strand-dependent.

We hypothesise that chemicals with a direct mechanism of DNA damage will result in a new signature arising, associated with that chemical’s mechanism of action.

Conversely, chemicals with an indirect mechanism of DNA damage, disrupting endogenous processes, will change the distribution of pre-existing signatures.

Figure legends

Figure 1. The optimal number of components to extract by volume-regularised non-negative matrix factorisation (VRNMF). A range of components from 5 to 20 was evaluated, with 15 determined to be the optimal number to extract. Of these 15 components, 14 pass the reflection test, corresponding to 9 underlying processes. **Figure 2.** Reflection matrix of extracted components shows the correlation between components’ spectra and reverse complement spectra. Components with a spectrum that correlates with their own reverse complement are symmetrical. Components whose spectrum correlates with the reverse complement of another component are described as a pair of asymmetrical components. **Figure 3**. The complete spectrum of component 12. The spectrum shows the contribution of each of the 1024 5bp sequences to the overall profile of the signature. **Figure 4.** The average signal of components 2 and 5 relative to sites of replication initiation. The intensity of components 2 and 5 show an increase relative to sites of replication initiation.

References

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Unilever