Analyses of whole-genome CRISPR/Cas9 screens identify genetic dependencies in *NRAS*-mutant melanoma

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INTRODUCTION

- Melanoma is the third most common cancer in New Zealand.
- Approximately 20% of melanoma patients harbour *NRAS* mutations.
- Currently, there are no effective treatments available for *NRAS*-mutant melanomas.
- Therefore, this project aims to identify genetic dependencies in *NRAS*-mutant melanoma, based on the genetic concept of induced essentiality, which will potentially uncover novel drug targets for treating *NRAS*-mutant melanoma.

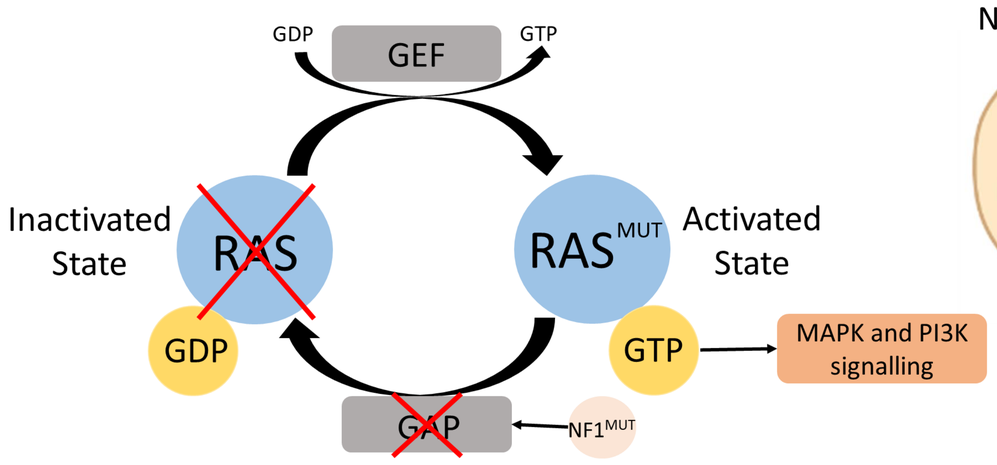


Figure 1 Accumulation of active, GTP-bound *NRAS* leads to unchecked cell proliferation and migration through downstream activation of MAPK and PI3K signalling pathways.

Jacob, J. et al. (2012) *Cancer* **118**: 4014-4023.

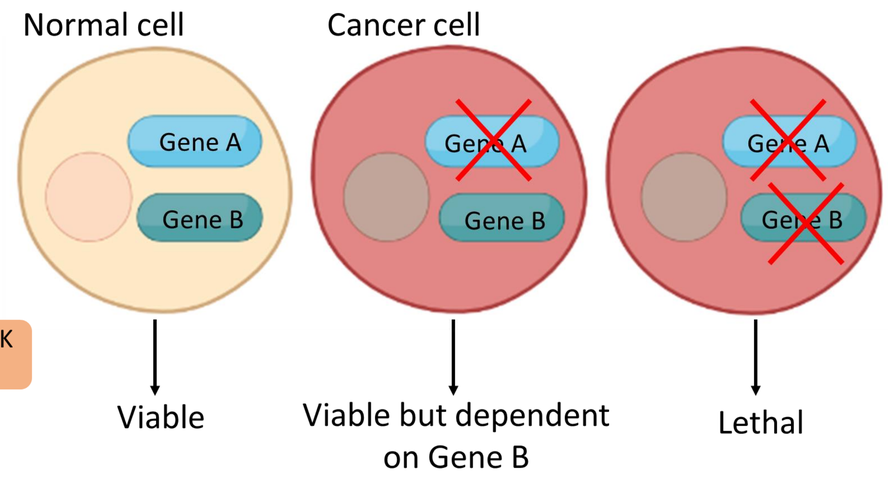
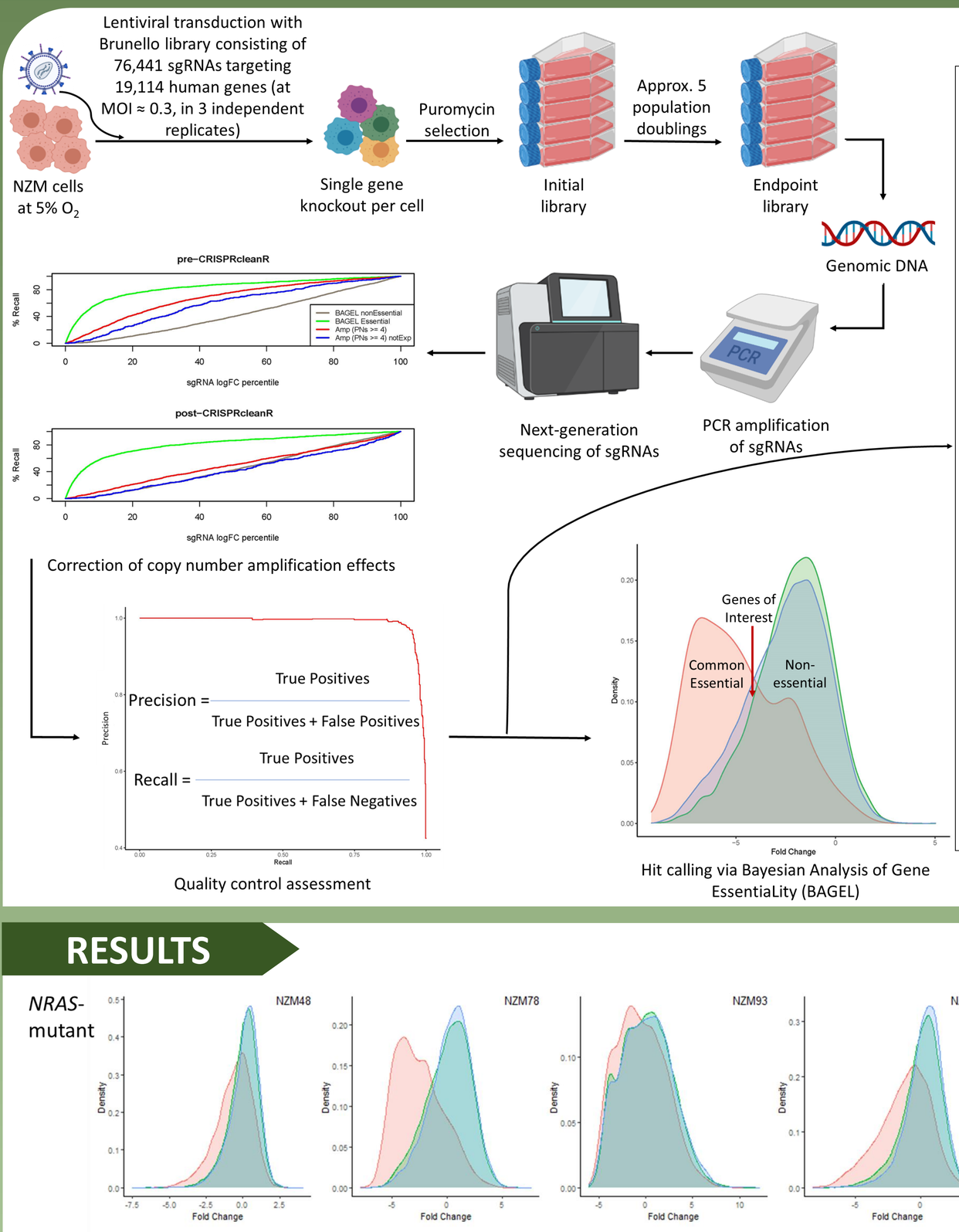


Figure 2 The genetic concept of **induced essentiality**, where abnormalities or deletions in one of the genes create dependency on the other gene for cell survival. We aim to identify genes that if targeted, will selectively kill cells that harbour *NRAS* mutations, while sparing cells lacking such mutation.

Ashworth, A. et al. (2011) *Cell* **145**: 30-38.

METHOD



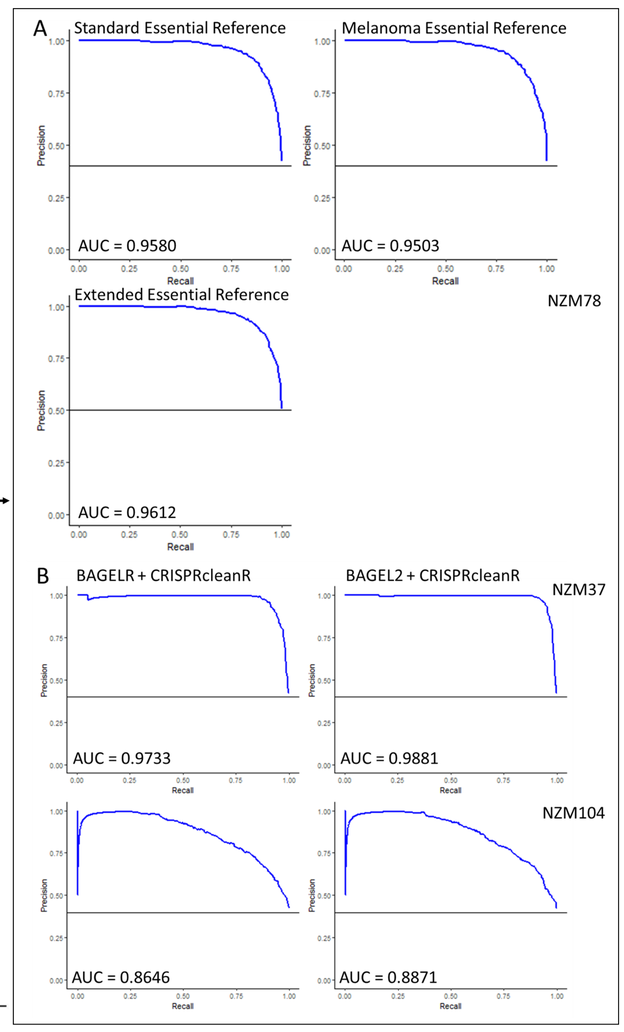


Figure 3 Summary of workflow. Genome-wide dropout screens were conducted in the New Zealand Melanoma (NZM) cell lines with the Brunello library, in multiple replicates to account for any variations due to transductions. Following copy number correction and quality control assessment, **BAGEL** was used to estimate the essentiality profile of genes by calculating the Bayes factor (BF), which is the likelihood of each gene in respect of a reference set of essential and non-essential genes. **A**, combining standard common essential genes with genes that are commonly essential to melanoma cell lines (n=57) provides higher precision and recall. **B**, **BAGELR** and **BAGEL2** are recent models developed based on the BAGEL pipeline, BAGEL2 computes a broader range of gene-level BF, which improves the performance at detecting essential genes.

Behan, F. et al. (2019) *Nature* **568**: 511-516.
Hart & Moffat. (2016) *BMC Bioinformatics* **17**: 164.

RESULTS

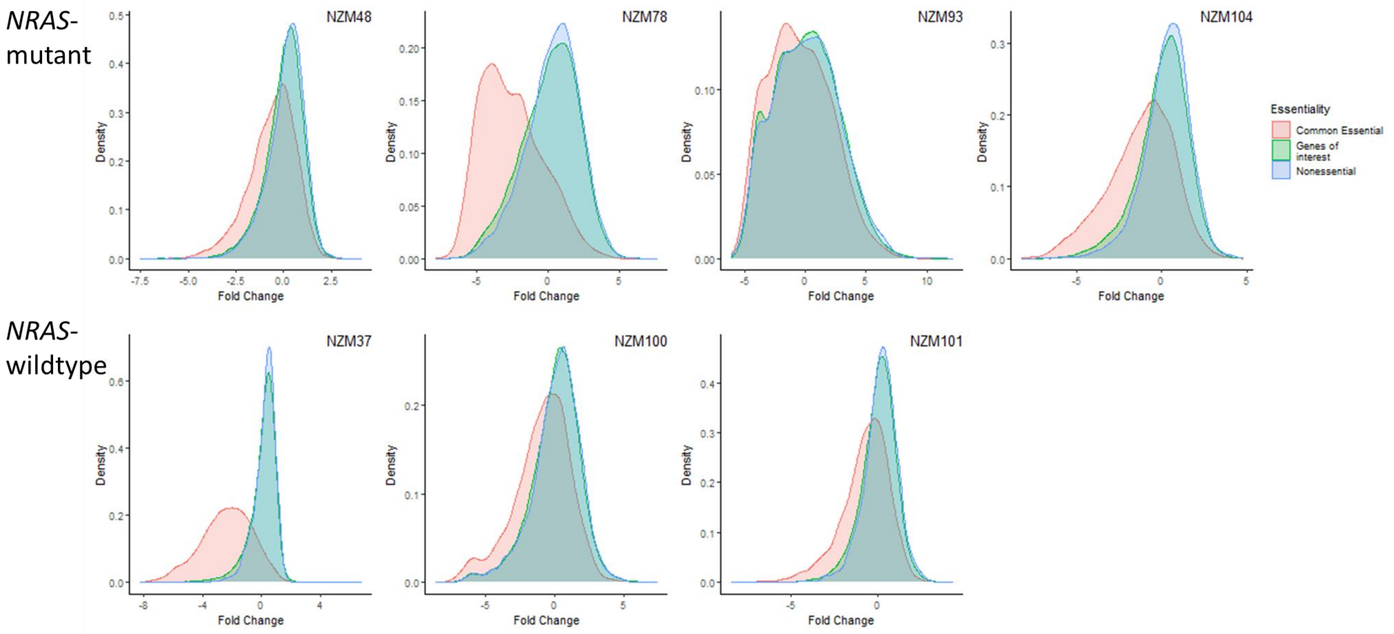


Figure 4 Distributions of common essential genes (red), nonessential genes (blue), and candidate essential genes to each cell line (green). Currently, 7 NZM cell lines have been successfully transduced and sequenced. NZM37 and NZM78 have shown distinct separation between the distributions of common essential and nonessential genes, whereas the other cell lines appeared to have more overlapped distributions. Increased overlapping area could affect the ability in detecting genes that are essential uniquely to each cell line. However, a consistent pattern was observed across all replicates within each cell line, these variations are most likely not due to transductions, but characteristics of each cell line.

CONCLUSION and FUTURE DIRECTIONS

- We have developed an optimised bioinformatic analysis incorporating CRISPRCleanR, BAGEL2 and an extended essential gene set to identify genes deleterious to the fitness of *NRAS*-mutant melanoma cells.
- Whole-genome CRISPR/Cas9-mediated knockout were performed in 10 NZM cell lines, of these, 7 cell lines have been sequenced.
- We have identified genes that are essential uniquely to the sequenced NZM cell lines.
- Once whole-genome knockout screens are conducted in at least 6 *NRAS*-mutant and 6 *NRAS*-wildtype NZM cell lines, genes that are essential to either all or most of the *NRAS*-mutant cell lines, while being nonessential to either all or most of the *NRAS*-wildtype cell lines, will be considered as candidate genes, which will be validated by individual gene knockout studies and competitive growth assays.
- The identification of genetic dependencies alongside *NRAS* mutations may provide potential new drug targets for the development of therapeutic strategies for the treatment of *NRAS*-mutant melanoma.

The following software are used for analysis, R (v4.1.0), BAGEL, and R/Bioconductor packages (CRISPRcleanR, BAGELR and ReactomePA). Figures created with BioRender.com.