NHS Blood and Transplant

University of BRISTOL

CRISPR/CAS9-mediated genome editing of the immortalised BEL-A2 erythroid cell line to create 'designer' reagent cells

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Reagent red blood cells

- Panels of reagent red blood cells of known phenotypes are used for detection and identification of antibodies
- Produced from blood donors – limited quantity and availability
- Sourcing rare phenotypes is difficult
- Need for continuous, unlimited supply of red blood cells with desired phenotypes

BioRad

Sustainable supply of selected RBC phenotypes?

Sustainable sources of *in vitro* produced red cells:

- Stem cell derived (hESCs, iPSC)
- CD34+ derived cells (adult peripheral or cord blood)
- Immortalised cell lines derived from patients with erythroleukaemias or human induced PSCs (HiDEP), CB progenitors (HUDEP) and embryonic stem cells

- BEL-A; the first human immortalised cell lines generated from adult erythroid cells

Selected phenotypes of *in vitro* produced red cells:

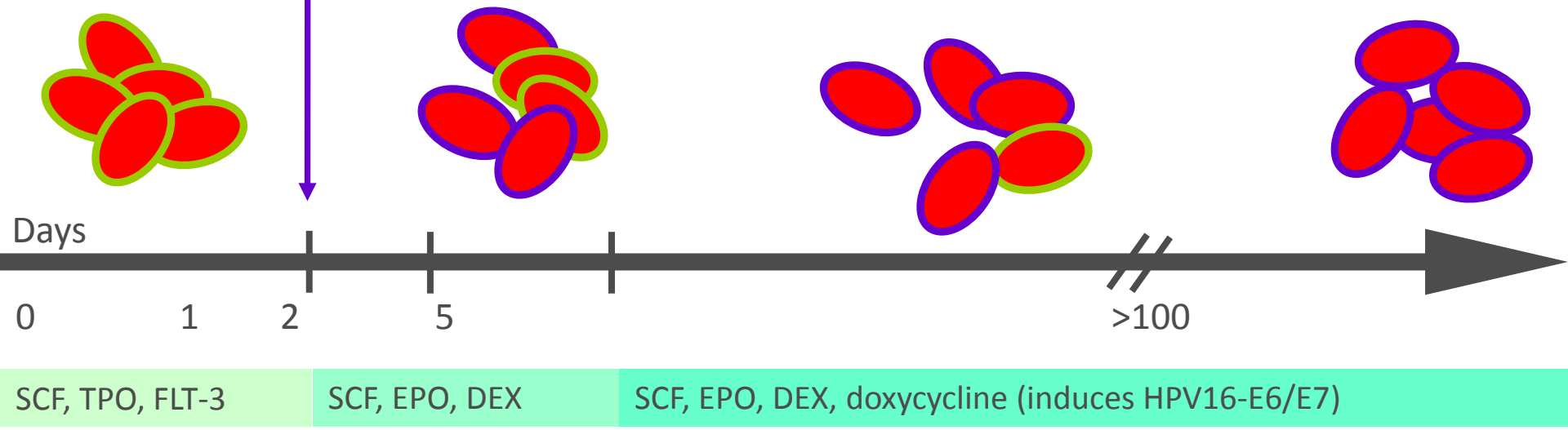
- Gene editing methods: zinc finger nucleases (ZFNs), transcription activator-like effector based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system

Bristol Erythroid cell Line from Adult progenitors (BEL-A)

Cell source: CD34+ cells (adult bone marrow)

Transduction of HPV16-E6/E7 with CSIV-TRE-RfA-UbC-KT lentiviral vector plasmid

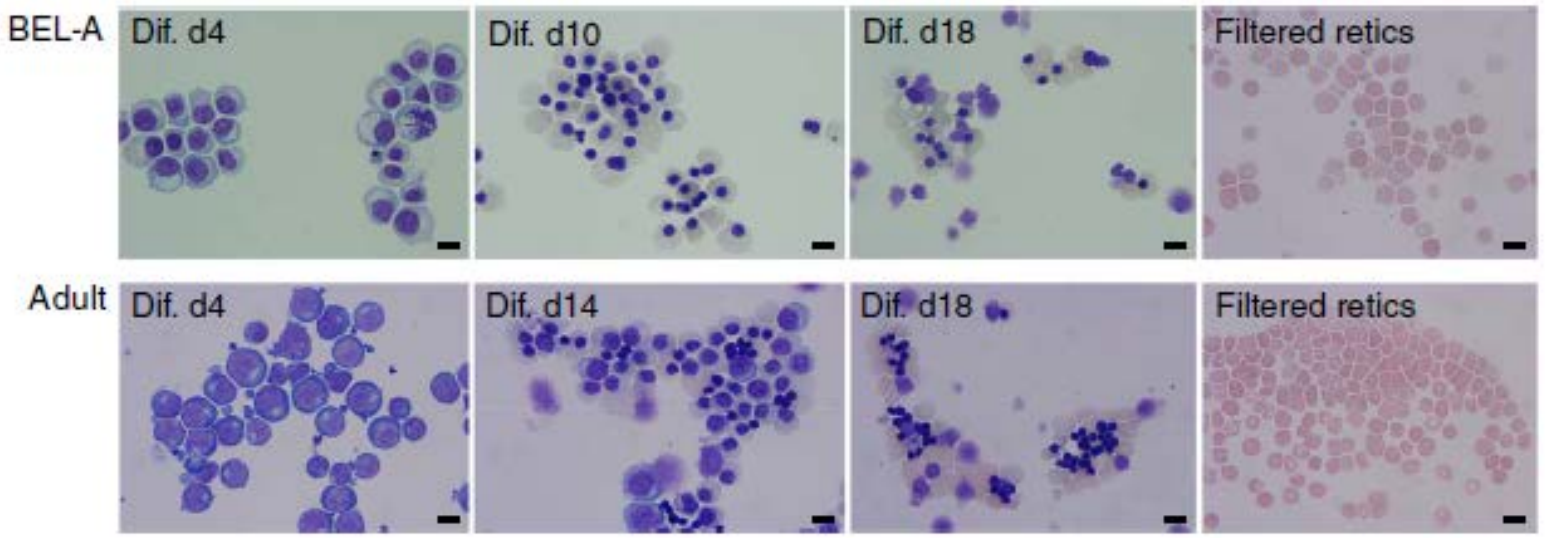
(genes required to efficiently immortalise a broad spectrum of cell types)



Trakarnsanga *et al*., 2017, Nat Commun 8: 14750.

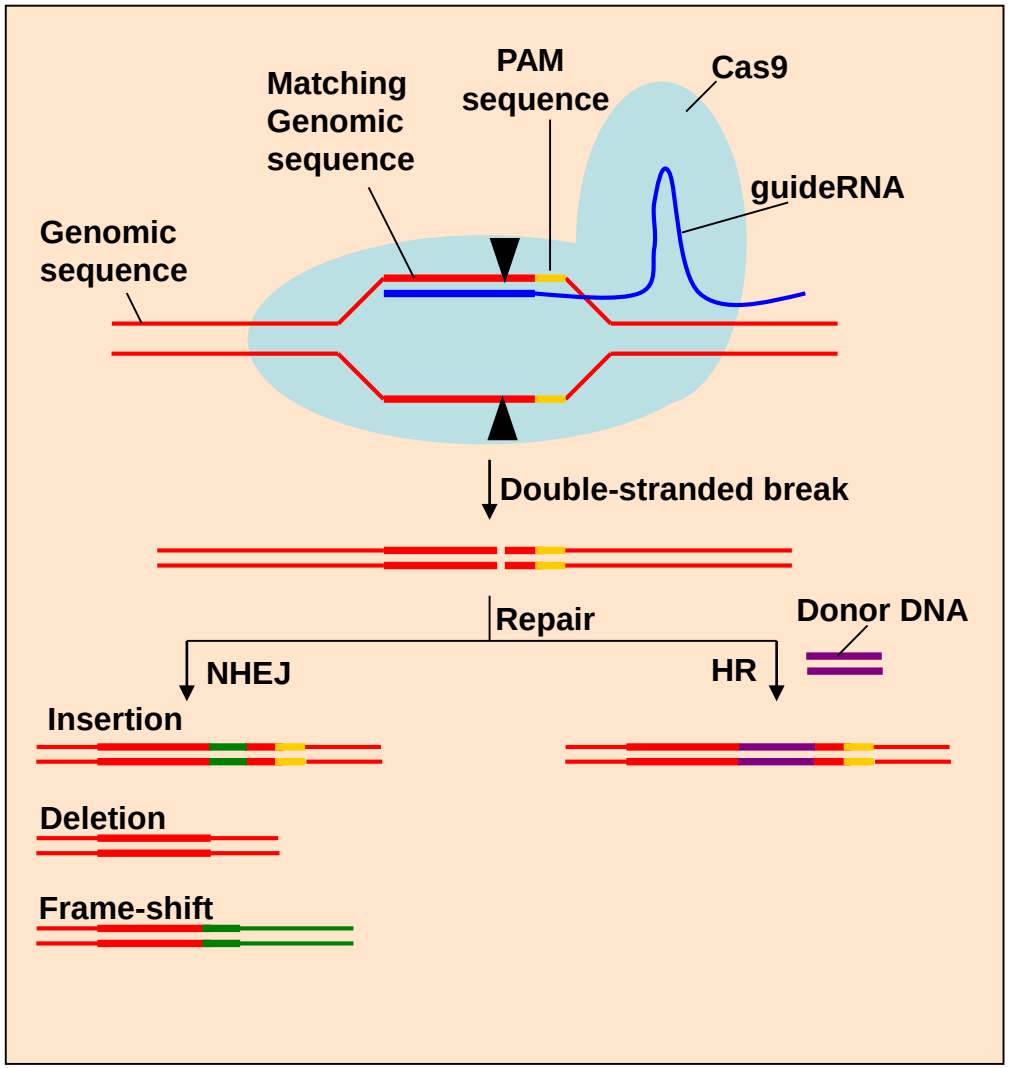
Erythroid differentiation of BEL-A2

- Proliferates continuously, maintained at early erythroid stage
- Normal expression of GPA, GPC, Rh, Kell, RhAG, band 3 etc
- Expresses adult β haemoglobin
- 20-30% enucleation in 3-stage differentiation media



Trakarnsanga *et al*., 2017

Genome-editing using the CRISPR/CAS9 system



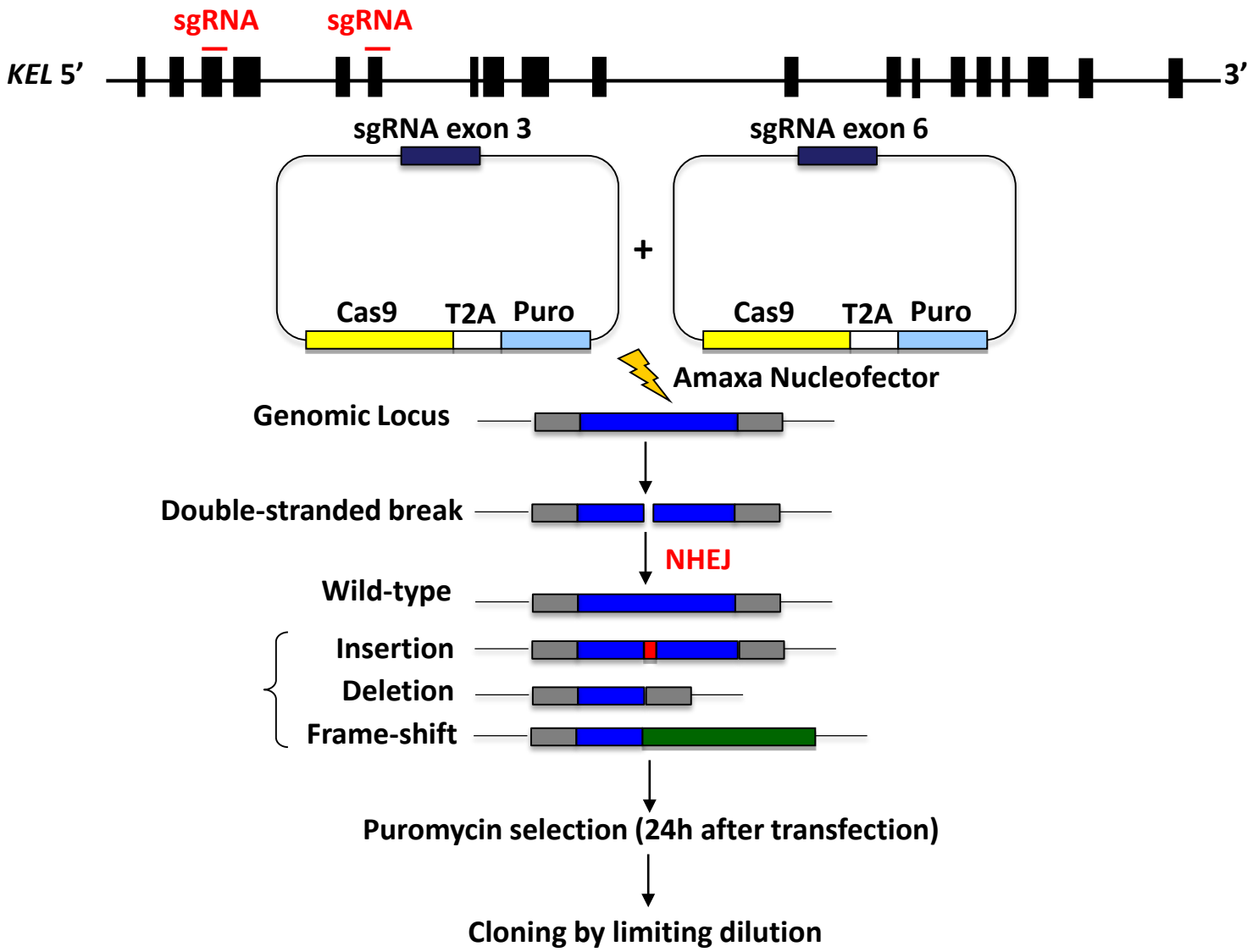
3 main components:

Cas9 – induces double-stranded break (DSB)

guideRNA – directs Cas9 to unique target sequence

DSBs fixed by **non-homologous end joining (NHEJ)** or **homologous recombination (HR)** (when repair template provided)

Strategy to knock-out *KEL* to generate Ko cells



KEL knock-out in BEL-A2 cells

Sequencing of *KEL* KO clone 23 exon 6

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WT              -----CTTCCTTAAACTTTAACCGAACGCTGAGACTTCTGATGAGTCAG-----
KEL KO clone 23 -----CTTCCTTAAACTT-------------AAACTTCTGATGAGTCAG-----
                     ************             * ****************
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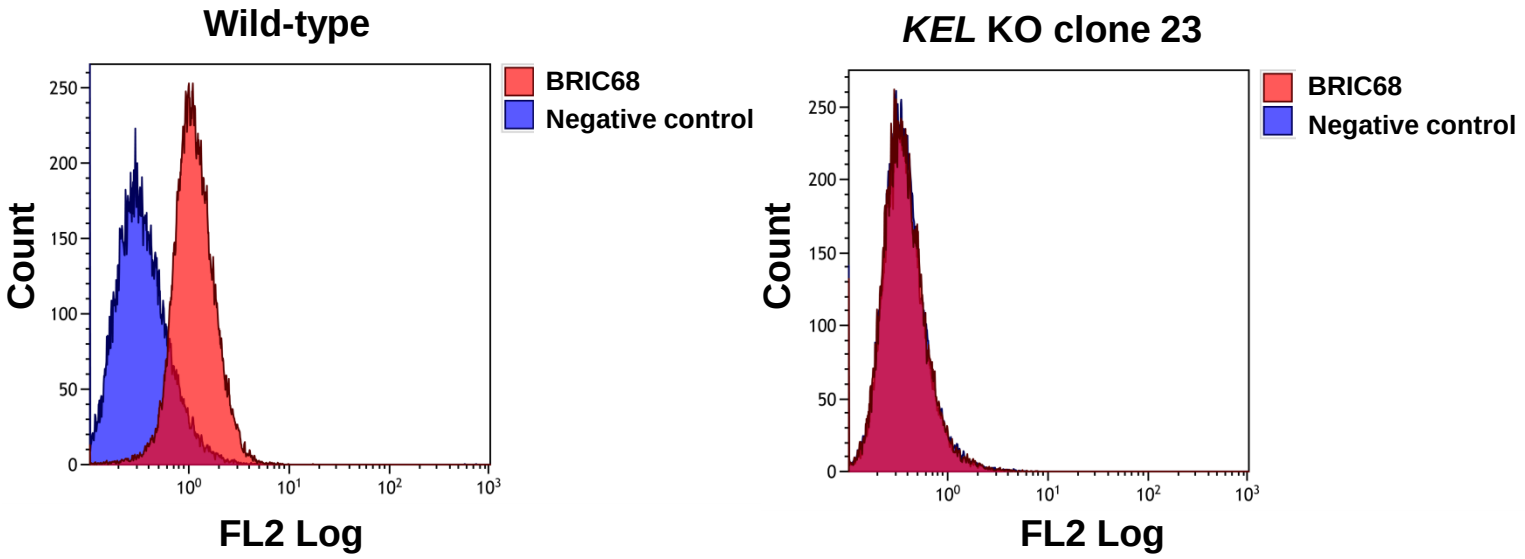
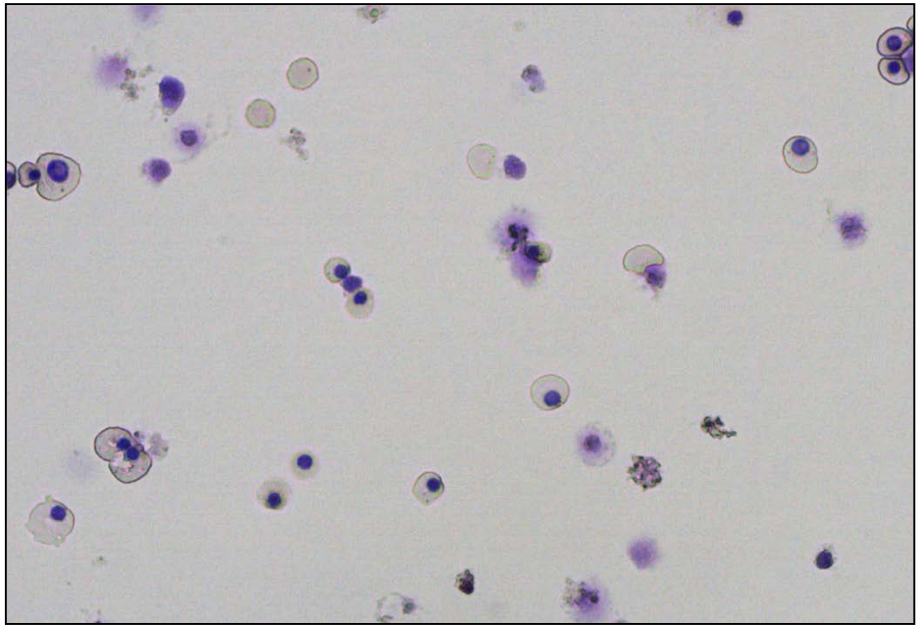
Flow assay using antibody against Kell glycoprotein (BRIC68)



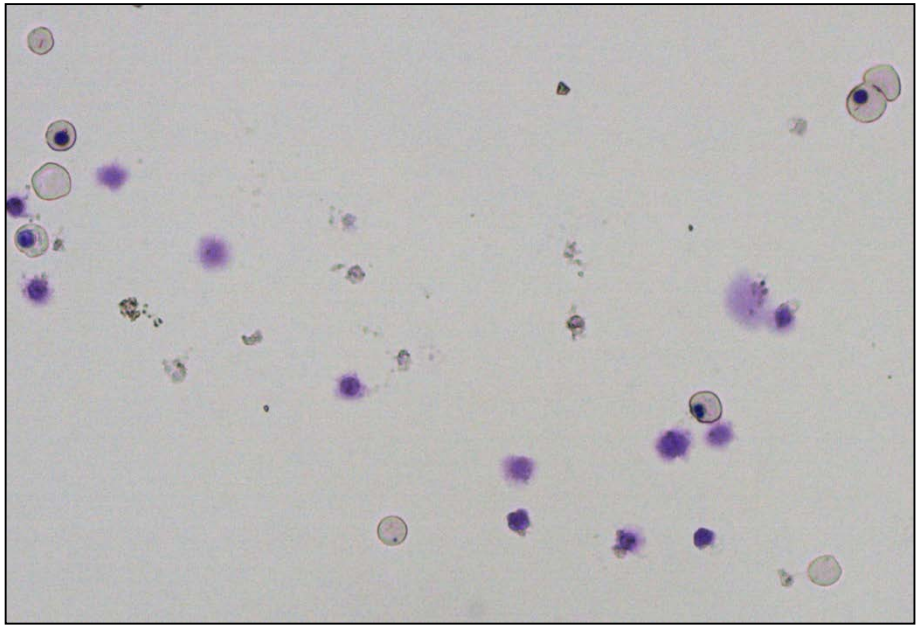
KEL knock-out in BEL-A2 cells

Cytospins (15 days in differentiation media)

Wild-type



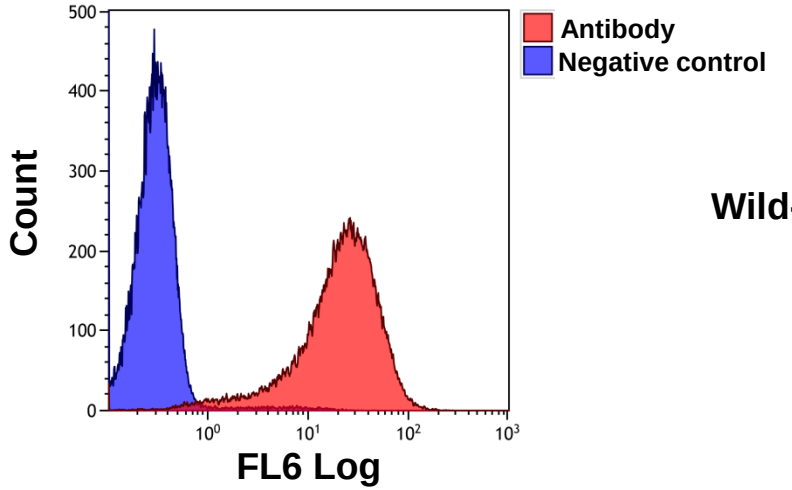
***KEL* KO clone 23**



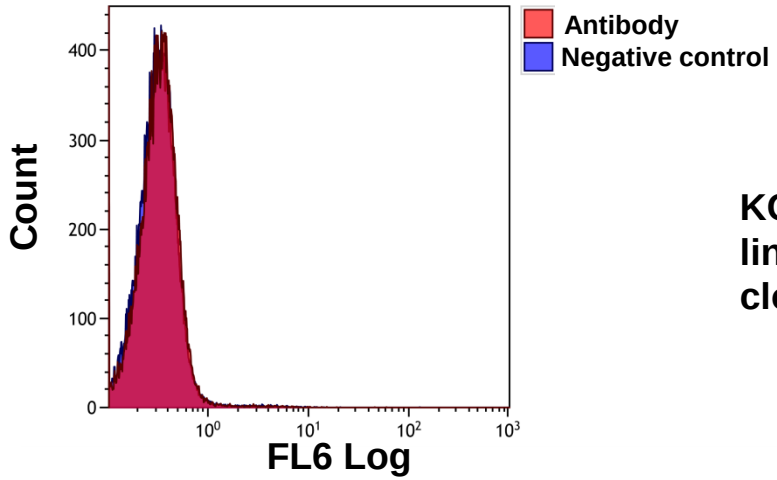
Knock-out cell line 2 in BEL-A2 cells

Flow assay using antibody against protein of interest

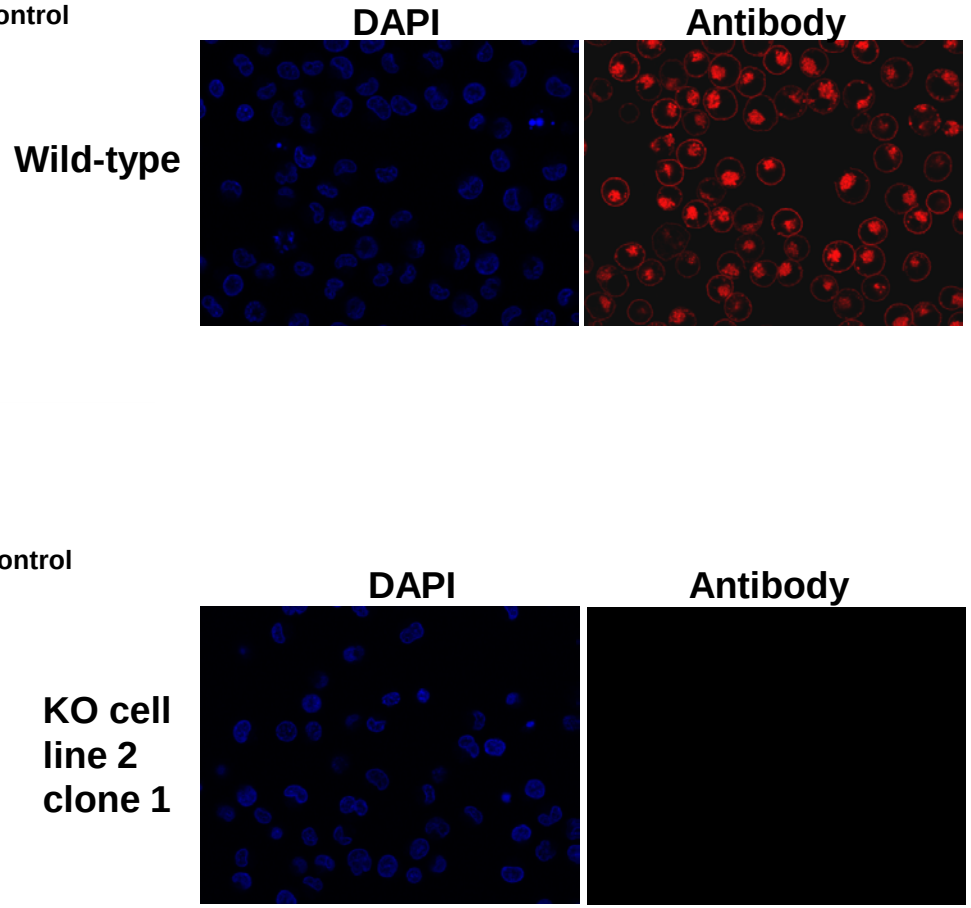
Wild-type



KO cell line 2 clone 1



Immunofluorescence assay using antibody against protein of interest



Conclusions

- CRISPR/CAS9 can be used on the BEL-A cell line to produce genetically-edited immortalised cell lines
- These transgenic cell lines can be differentiated down the erythroid pathway

Future work

- Improve transfection efficiency – lentiviral transduction to deliver CRISPR plasmids
- Improve rate of erythroid differentiation
- CRISPR/CAS9 gene editing via homologous recombination (in presence of donor repair template) - to ‘create’ desired allele

Potential use of ‘designer’ reagent cells

- Continuous, unlimited supply of ‘designer’ red blood cells with desired phenotypes, especially with rare phenotypes

  phenotypes with rare antigens [e.g., Di^a, Mur] and those lacking high frequency antigens [e.g., Lu(b-), k–]

- Diagnostics – in serological panels for detecting and identifying clinically important antibodies in the sera of patients
- Production of secreted recombinant antigens
- Use on automated diagnostic platforms as cells or membrane preparations

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Thank you