

Investigating the roles of divergent histone tails using gene editing in *Trypanosoma brucei*



University of Dundee



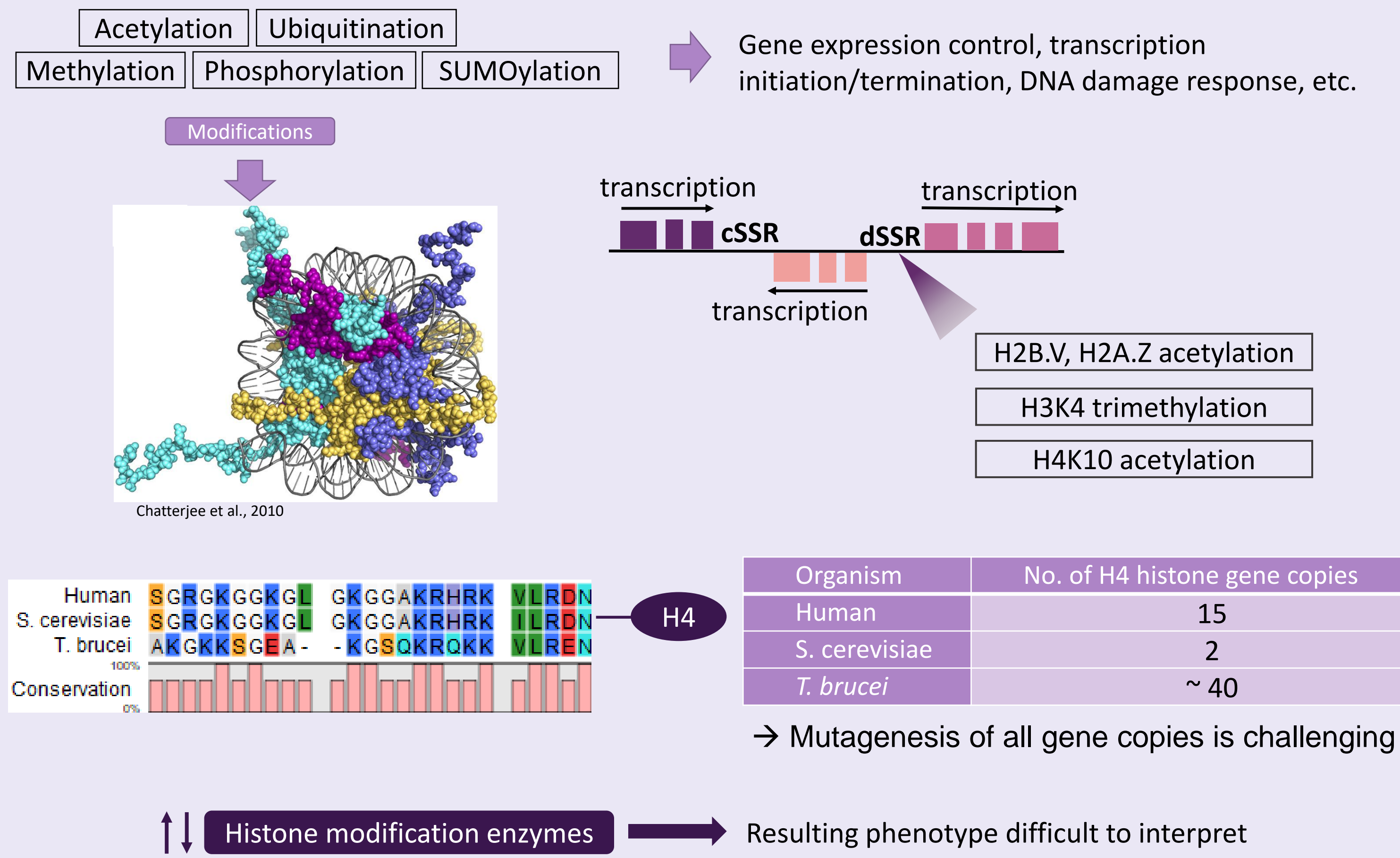
wellcome centre anti-infectives research

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Introduction

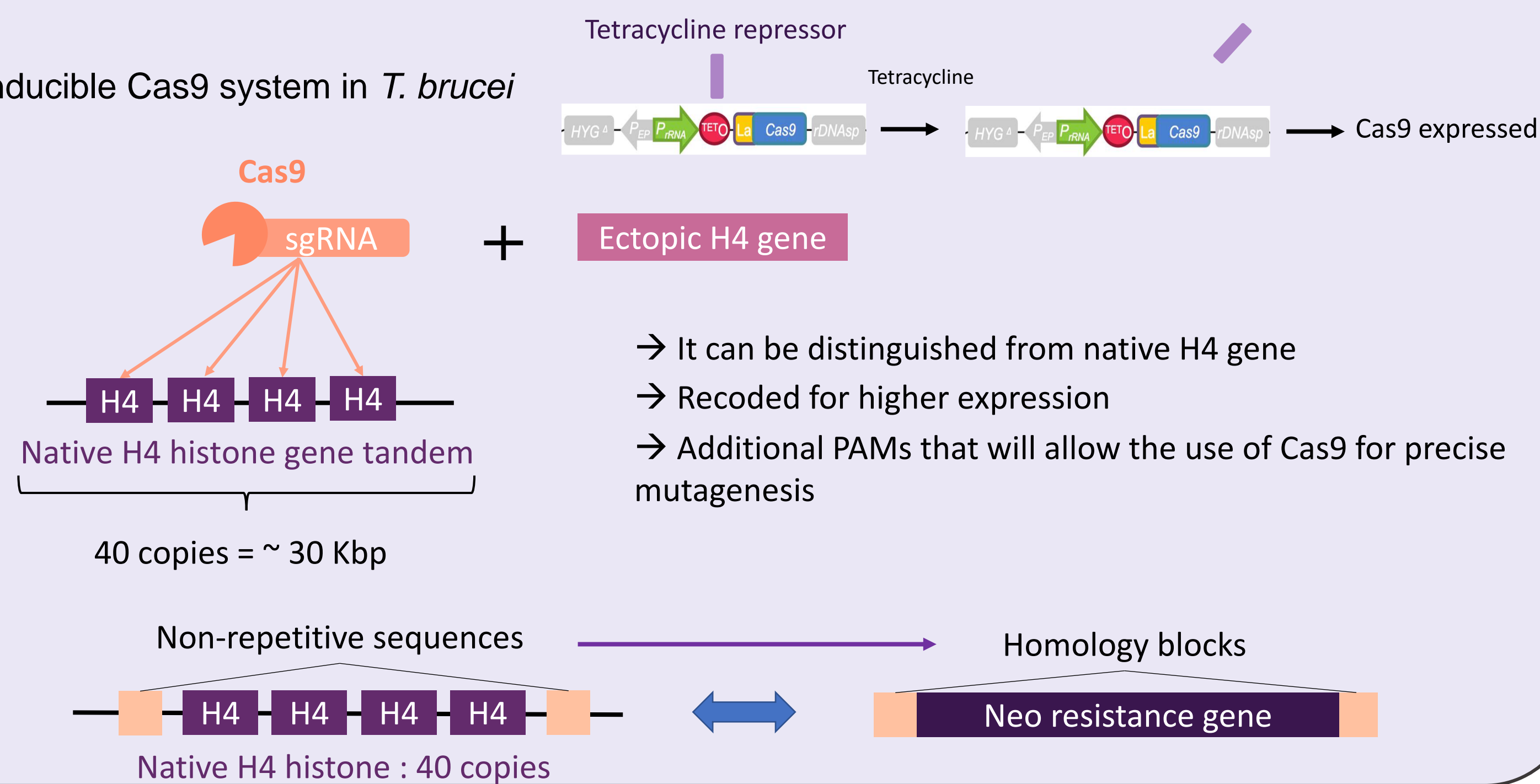


Aim: generate a *T. brucei* cell line with a single copy of one core histone gene

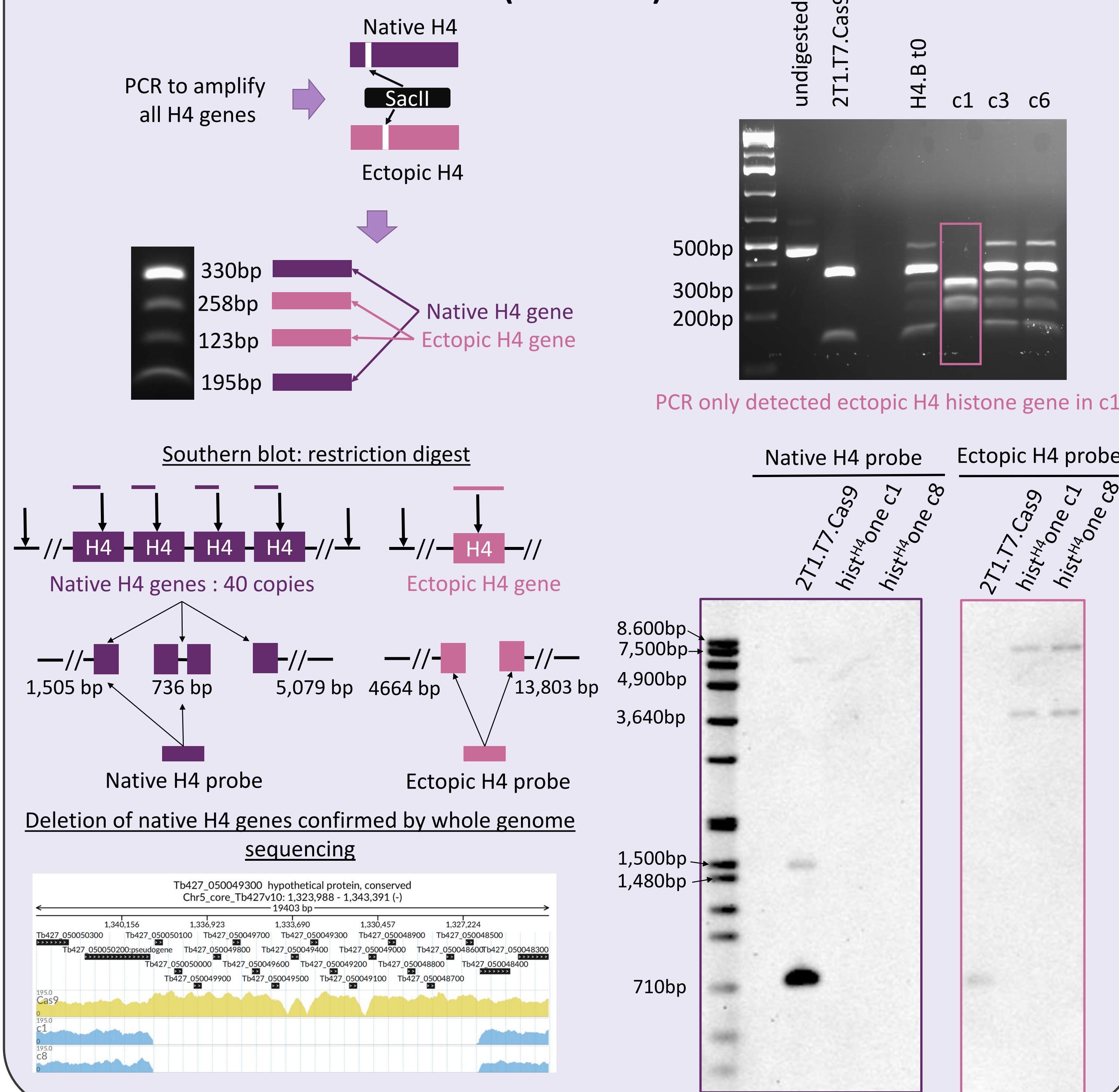
- To enable precise mutagenesis of histone gene residues
- Insight into histone tail modification roles and better understanding of epigenetic mechanisms of DNA metabolism control

1 Strategy for establishing cell line with a single H4 gene

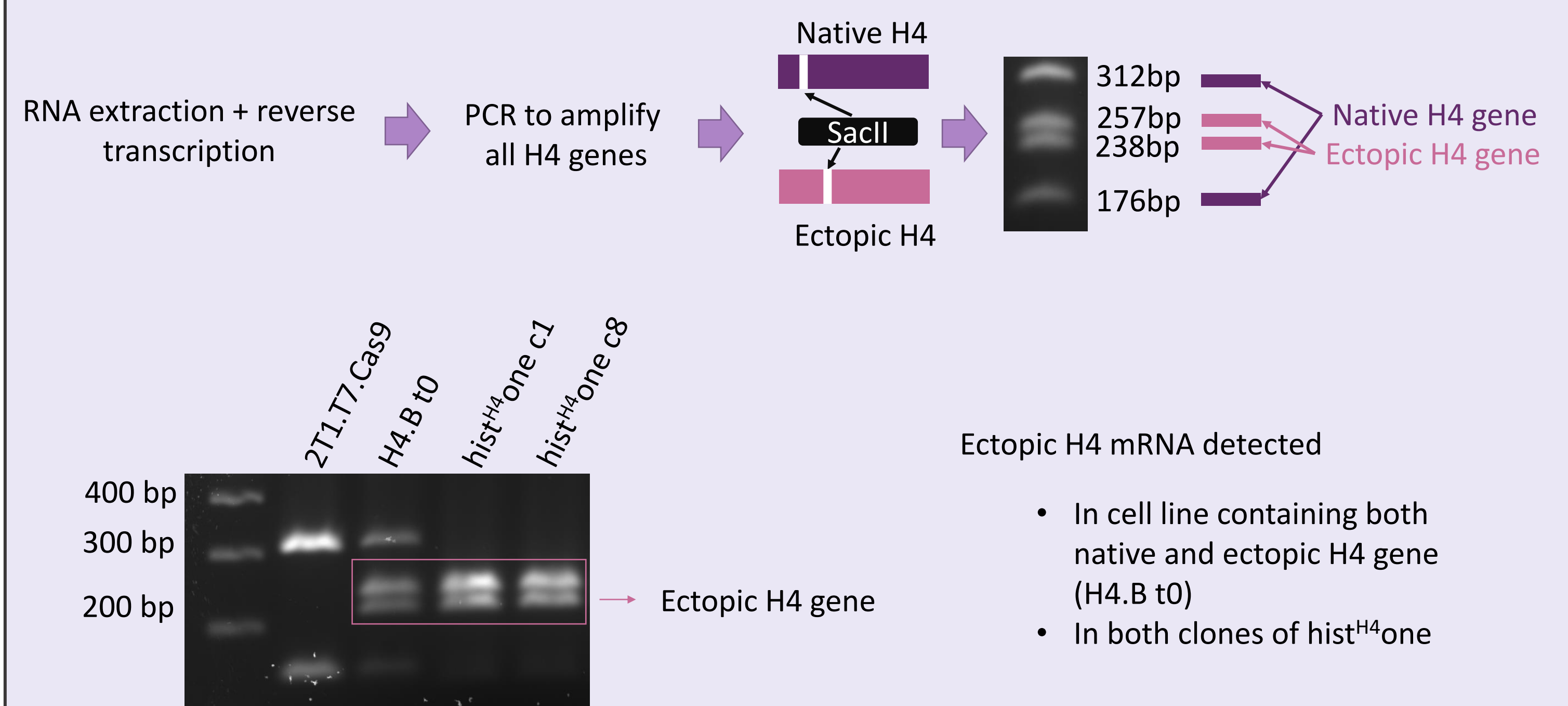
- Inducible Cas9 system in *T. brucei*



2 Cell line with a single H4 histone gene was successfully generated (*hist^{H4}one*)



3 Ectopic H4 histone gene is expressed



4 Site saturation mutagenesis to investigate the role of H4 histone residues and their mutations

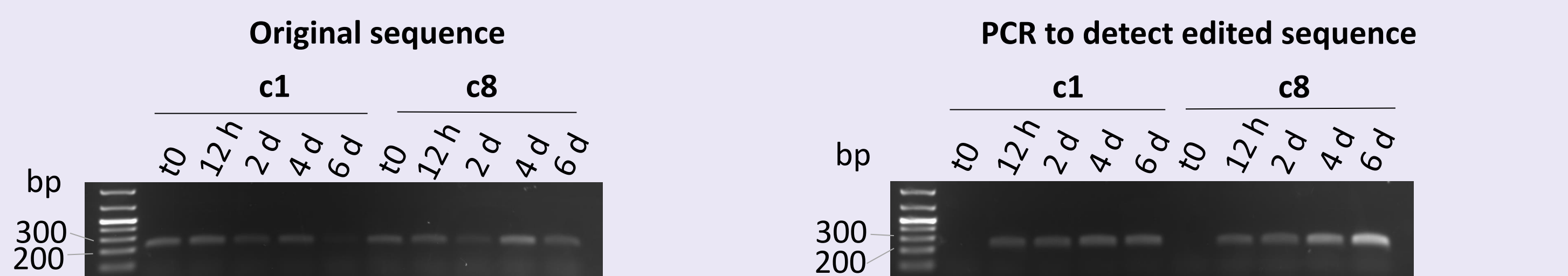
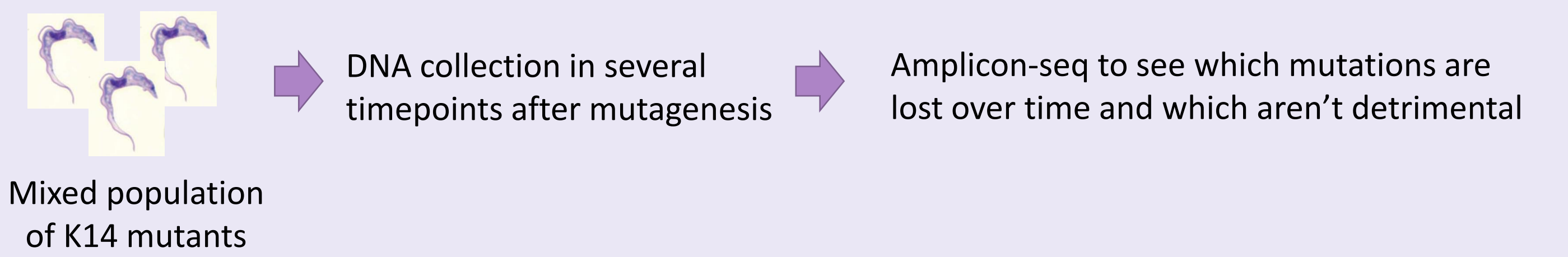
- Hist^{H4}one* strain makes it possible to efficiently edit individual residues on H4 histone gene to investigate their function



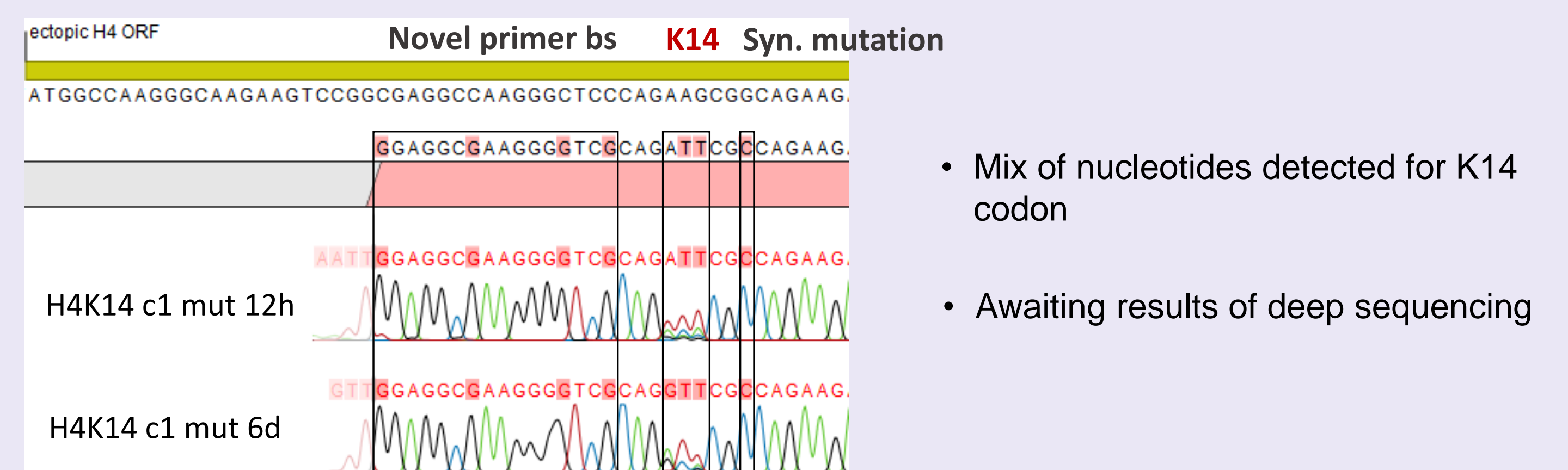
- Original sgRNA was replaced with sgRNA targeting H4K14 codon in *Hist^{H4}one* strain, then Cas9 was induced and cells were transfected with a mix of repair templates, with different codons for position 14



- Fitness of individual H4K14 mutants will be evaluated by analysing which mutants are present in different timepoints after transfection



Sanger sequencing indicates H4K14 editing was successful



Summary

- A single H4 gene supports *T. brucei* growth
- H4 site saturation mutagenesis is now possible
- N-terminal tail truncations may also be possible
- Proposed assays include:
 - fitness-profiling
 - cell cycle - flow cytometry
 - gene expression - RNA-seq