

CGTC-HEK293: Advanced Suspension Cell Line for rAAV Therapy Manufacturing

CATAPULT
Cell and Gene Therapy

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Abstract

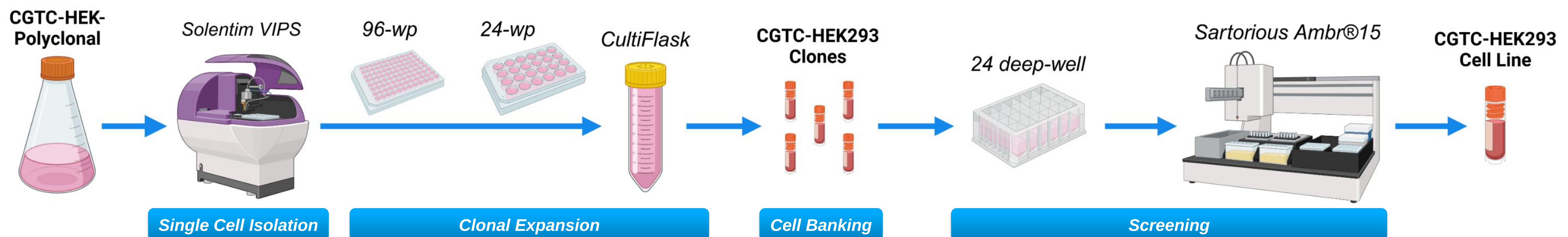
The generation of cost-effective and reliable recombinant adeno-associated viral (rAAV) vectors is crucial for the development and success of rAAV-based advanced therapy medicinal products (ATMP). The Cell and Gene Therapy Catapult (CGTC) has demonstrated the derivation of a HEK293 cell line for rAAV production as well as a tool for cell engineering, to support the development and manufacture of more affordable rAAV therapies. The CGTC-HEK293 cell line was derived from an adherent HEK293 parental line that underwent suspension adaptation, clonal selection, expansion, and screening for rAAV production. After rAAV production platform development, the final clone selected demonstrated enhanced productivity across multiple serotypes to an industry-standard level, making it a potential alternative for rAAV production and further optimization through cell engineering.

Adaptation to Suspension



An adherent HEK293 cell line was thawed in serum-supplemented media to maintain its phenotype. It was then passaged into suspension culture with 2.5% less serum, gradually reducing serum over several passages. The cell line was successfully adapted to serum-free conditions once viability remained above 85%, with cell densities and doubling time matching the adherent control. This process was fully documented for subsequent GMP approval, and a cell bank was generated for the resulting CGTC-HEK-Polyclonal cell line.

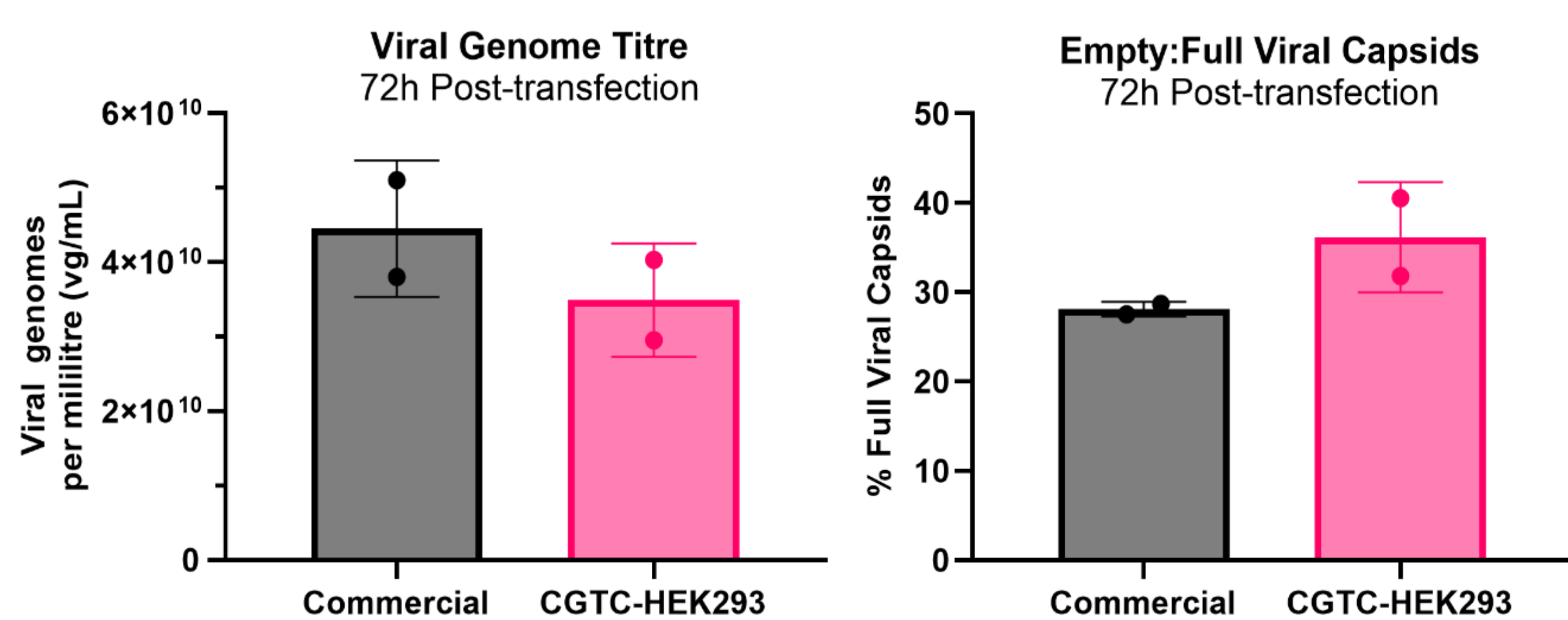
Clonal Selection, Expansion and Screening



The suspension-adapted CGTC-HEK-Polyclonal cell line underwent monoclonal selection using the VIPS™ instrument (Solentim) to isolate and expand 147 clones. 143 clones were expanded to 24-well plates, and 83 were moved to 5-mL cultiflasks. 57 surviving clones were banked, expanded, and screened for growth and viability in 24-deep well plates. Thirteen clones were selected for rAAV2 productivity screening in an Ambr®15 bioreactor (Sartorius) but exhibited lower productivity than expected. Growth, viability, and rAAV production data were used to select the best-performing clone for further development. The clonal isolation, expansion and cell banking was documented for subsequent GMP approval, and a research cell bank was generated for the resulting CGTC-HEK293 cell line.

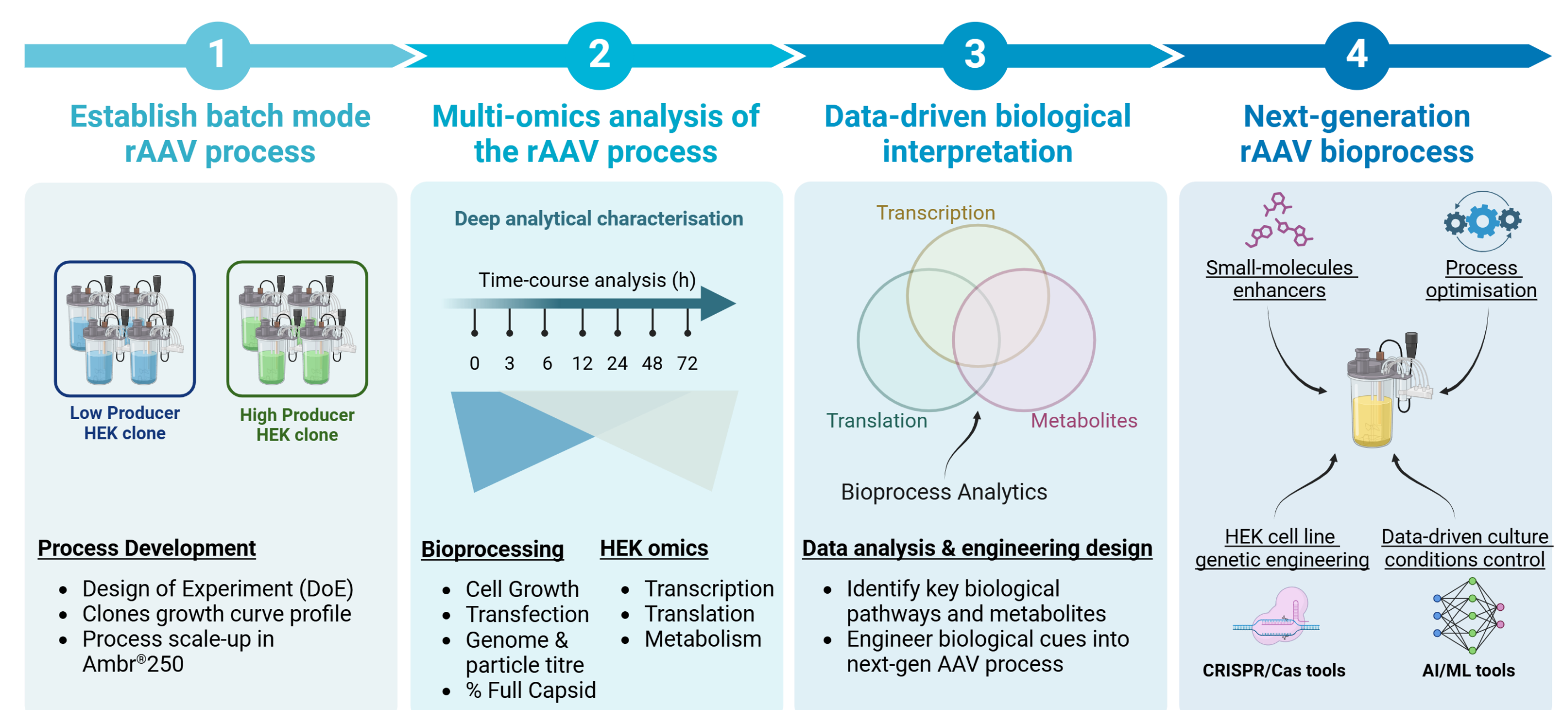
CGTC-HEK293 rAAV Productivity

rAAV8 Production at Ambr®250 scale



A design of experiment (DoE) study optimized transfection parameters for the CGTC-HEK293 cell line in 24-deep well plates, focusing on cell density, transfection reagents, DNA ratio, and total DNA to achieve maximum genomic titre and full rAAV capsids. The optimal conditions were scaled up to Ambr®15 and subsequently to Ambr®250 bioreactors, confirming that the CGTC-HEK293 cell line met expected rAAV productivity for rAAV2 and rAAV8 (data shown). Comparisons showed that the **CGTC-HEK293 cell line exhibited industry-standard productivity for viral genome titre (by qPCR) and a higher percentage of full capsids (by SamuxMP)** in harvest material 72 hours post-transfection.

Cell Engineering for Enhanced Productivity



Multi-omics data has been gathered from both high and low producer clone derivatives of the CGT line, informing cell engineering targets and enhancing the ability to optimize production efficiency. CRISPR-based tools and validation readouts have also been established for precise modifications to the cell line. By integrating these capabilities with the CGTC-HEK293 cell line, higher rAAV productivity can be achieved.

We welcome collaboration and partnerships to leverage our innovative cell line and engineering technologies, aiming to enhance rAAV production and benefit the ATMP industry.

Conclusion

CGTC has developed a HEK293 suspension cell line, **CGTC-HEK293**, and enhanced its rAAV productivity to industry-standards, making this cell line **a potential alternative for rAAV production and further optimisation through cell engineering**, with the aim to improve patient accessibility by supporting the development of cost-effective rAAV therapies.

- Available for in-house evaluation with provision of cell vial, protocol for rAAV production and recommendations for testing/optimisation
- Opportunities to collaborate and access the CGTC-HEK293 cell line



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If you are interested in our CGTC-HEK293 cell line, please, contact our team at info@ct.catapult.org.uk or maria.barreira@ct.catapult.org.uk