Advanced Detection of Performance Enhancement (ADOPE):

Detecting gene doping with innovative targeted next generation sequencing

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On your marks, get set, DETECT!

Major concerns have risen around the misuse of **gene editing** techniques, particularly for human enhancement. An example of this misuse is **gene doping**, which is a big threat for fair sports. We developed a method to detect gene doping by **targeted sequencing** with our novel fusion protein: **dxCas9-Tn5**. We used EPO as a model gene.

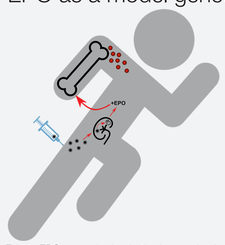

Fig. 1. EPO gene doping in the human body.

Fusion Protein dxCas9-Tn5

Targeted library preparation relies on our innovative **fusion protein** consisting of a **dxCas9** [3] and a **Tn5** transposase[4] for sequencing.

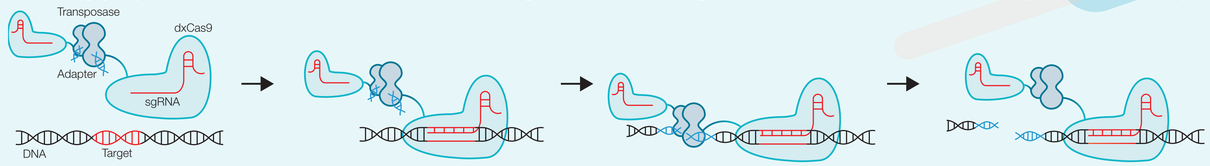

Fig. 5. Targeted adapter integration for nanopore sequencing. The fusion protein dxCas9-Tn5 is guided to a specific DNA target and adds sequencing adapters required for nanopore sequencing.

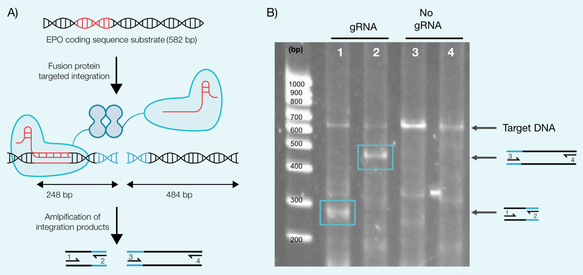

Fig. 6. Targeted integration of DNA adapters (50 bp) in intronless human EPO.

We tested the fusion functionality *in vitro* and showed **targeted adapter integration**.

To cover all variations for the EPO gene, we modeled the extent of codon variation in a heat map and generated the corresponding **sgRNAs** targeting exon-exon junctions.[5]

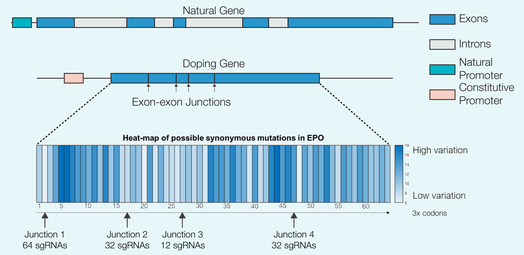

Fig. 7. sgRNA model to find all possible PAM sequences and the minimum number of sgRNAs needed at each exon-exon junction in the human EPO gene.

Integrated Human Practices

Prof. Hidde Haisma - Gene and cell doping expert group WADA
EPO as model gene

Dr. Olivier de Hon - Dutch Doping Authority
High throughput prescreening

Prof. Paul Dimeo - Expert in drug use in sport and antidoping policy
Anticipate athlete behavior

Hackathon Cyber Security Week
Expanding database
Expert discussion, Stirling, Scotland
Future of gene doping

Sample Preparation and Prescreening

A predictive EPO gene doping model to determine the **detection time window** and **sensitivity** for sample preparation.[1]

We extracted artificial EPO gene doping DNA at concentrations predicted by our model. Samples proceed to prescreening.

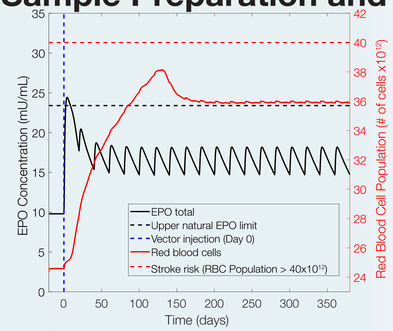

Fig. 2. Effect of EPO gene doping using microdosing every twenty days.

A **high throughput** prescreening based on **gold nanoparticles** (d-AuNPs)[2].

We distinguished between on-target DNA probe for EPO gene (red) with off-target DNA probe (purple).

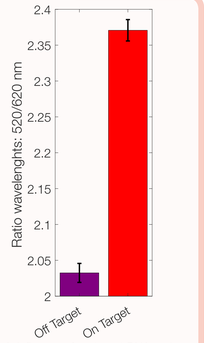

Fig. 4. Influence of on-target DNA probe on d-AuNPs stabilization at 250 mM NaCl.

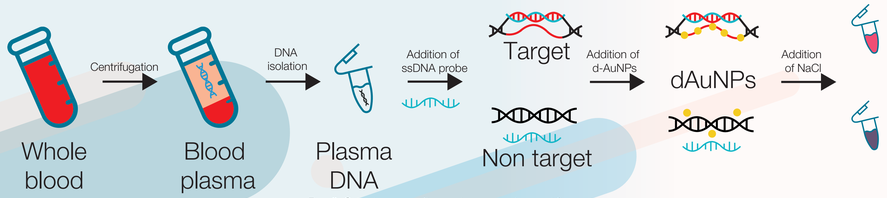

Fig. 3. Sample preparation and prescreening workflow.

Targeted Sequencing

MinION sequencing with a library prepared sample treated with our fusion protein. We obtained **89 unique alignments** to target EPO DNA and 0 alignments of non target DNA.

Fig. 8. MinION device.

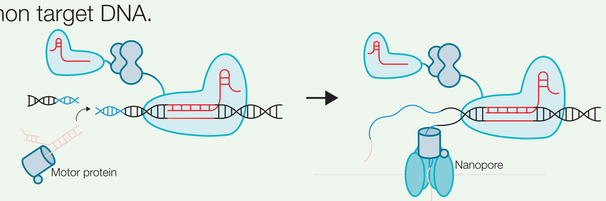

Fig. 9. The sequencing adapters added by our fusion protein result in nanopore sequencing.

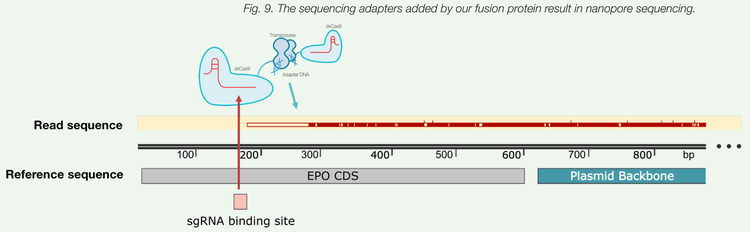

Fig. 10. Alignment of the DNA sequence read with reference EPO CDS in plasmid DNA. Fusion protein depicted with arrows representing binding of dxCas9 position (red arrow) and the addition of sequencing adapters (blue arrow).

Achievements

- Co-organized the first EurAsian Meetup in China with BGIC-Global.
- Modeled the time dependent concentration of EPO gene doping.
- Optimized a protocol for DNA extraction from blood serum to detect gene doping.
- Integrated a prescreening method based on gold nanoparticle stabilization.
- Designed, constructed, expressed and purified a novel dxCas9-Tn5 fusion protein.
- Developed software to generate barcodes and sgRNAs for gene doping detection with targeted sequencing.
- Demonstrated targeted integration by dxCas9-Tn5 for library preparation and targeted nanopore sequencing.
- Developed a software tool for sequencing data analysis.
- Constructed 13 biobricks, including dxCas9-Tn5, dxCas9 and Tn5 (BBa_K2643000-BBa_K2643012).

[1] Ni W., et al. Gene Therapy, 2011, 18, 709–718.
[2] Beatsen-Young A.M., et al. Biosensors and Bioelectronics, 2018, 29-36.
[3] Hu, J.H., et al Nature, 2018, 556(7699),57.
[4] Picelli, S., et al Genome research, 2014,177881.
[5] Beiter T., et al. Gene Therapy. 2011, 18: 225–231.

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