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.

Sample Preparation and Prescreening
A high throughput prescreening based on gold nanoparticles (d-AuNPs).
We distinguished between on-target DNA probe for EPO gene (red) with off-target DNA probe (purple).

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.

Fusion Protein dxCas9-Tn5
Targeted library preparation relies on our innovative fusion protein consisting of a dxCas9 and a Tn5 transposase for sequencing.

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.

Achievements
Co-organized the first EurAsian Meetup in China with BSCG-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).

Adopted a method to detect gene expression in blood with sensitivity for sample preparation.
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.
Fig. 3. EPO gene doping in the human body.
Fig. 4. Targeted integration of DNA fragments (purple) into EPO human DNA.
Fig. 5. Targeted adapter integration on nanopore sequencing. The fusion protein dxCas9-Tn5 is guided to a specific DNA target and adds sequencing adapters required for nanopore sequencing.
Fig. 6. The sequencing adapters added by our fusion protein result in nanopore sequencing.

Fig. 8. MinION device.

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

Fig. 10. Alignment of the DNA sequences read with reference to EPO CDS in planned target. Fusion protein detected on array representing binding of barcoding position (bar arrow) and the addition of sequencing adapters (blue arrow).

Fig. 11. EPO Concentration (mU/mL)

Fig. 12. Plasma

Fig. 13. Blood serum

Fig. 14. Red Blood Cell Population (# of cells x10^12)

Fig. 15. Ratio wavelengths: 520/620 nm

Fig. 16. Matrix plot of potential performance enhancements in EPO gene doping.

Fig. 17. Effect of different concentration of NaCl (15, 30, 250, 300, 350, 38)

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