InDelphi and Pythia in Genome Editing

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Introduction to CRISPR Repair Challenges

- CRISPR/Cas9 creates double-strand breaks (DSBs) in DNA.
- Repair pathways Non-Homologous End Joining (NHEJ), Microhomology-Mediated End Joining (MMEJ), Homology-Directed Repair (HDR) lead to varied outcomes, often unpredictable.
- Predictable repair is essential for precise gene editing applications.

Microhomology-Mediated End Joining (MMEJ) (*explain*)

- MMEJ aligns DNA ends at a double-strand break (DSB) using short matching sequences called *microhomologies*.
- This alignment leads to the removal of non-matching sequences between microhomologies (orange), creating a small deletion in the repaired DNA.

Introduction to InDelphi's Prediction Model (*explain*)

- *InDelphi* predicts gene editing outcomes by analyzing DNA sequence contexts around breaks. Here Cas9 introduces DSB...
- Microhomology sequences play a significant role in guiding deletion patterns. (panel a,b)
- Different DNA sequences lead to varied deletion profiles, showing the flexibility of *InDelphi*. (panel c)

Experimental Setup and Junction Analysis (*explain*)

- **Panel a**: Shows the design of the CRISPR/Cas9 strategy for inserting an eGFP gene using repair templates.
- **5' and 3' Junctions** (Panel g): - Demonstrates real outcomes at both junctions, confirming deletions depend on microhomology (mH) usage.
- Each scaffold (1, 2, and 3) represents different configurations and the resulting sequence after repair.

eGFP Expression in Xenopus Embryos

- **Figure b** Visualizes eGFP expression in various stages (neural, tadpole, forelimb).
- eGFP expression indicates successful integration of the repair template.
- Different levels and patterns of expression across developmental stages confirm successful integration into the genome.

eGFP and dsRed2 Expression Validation in F2 Generation

- **Panel j** Shows eGFP expression in F2 tadpole kidney, confirming inheritance of the transgene.
- **Panel k-l** Visualizes dsRed2 expression in muscle tissue of F2 homozygote, showing stable integration and expression in F2 offspring.
- This confirms that the genome edits are heritable, supporting *Pythia*'s efficacy in generating inheritable modifications.

In vivo Genome Editing in Neuronal Cells: Experimental Setup (*explain*)

- **Panel e,f** shows the repair template design targeting the Tubb2a gene, using trimology sequences to predict specific deletions (Δ3, Δ6, Δ9) in Tubb2a.
- **panel a** shows Design strategy using *Pythia* to target the Tubb2a gene with CRISPR/Cas9 and repair templates.
- **panel b**: Injection setup in mouse brain regions (V1 and hippocampus) for precise targeting.

Repair Outcomes and Neuronal Localization

- The figure shows Efficiency of repair across both brain hemispheres, demonstrating reliable on-target editing.
- The high-resolution images also confirm successful localization of edits within cortical and hippocampal neurons.

Deep Learning Prediction Model for Single Nucleotide Edits (*explain*)

- The Pythia model also uses its deep learning model to predict and design single nucleotide edits, here the authors Demonstrate the convertion of eGFP to eBFP.
- Deep learning selects optimal gRNAs and repair template designs based on SNP location. (*explain*)
- the scoring system for repair arms, optimizes the edit based on predicted left and right junction outcomes. (*Explain*)
- So, the Pythia matrix helps us find the optimal length for the repair arms.

Experimental Validation for Single Nucleotide Editing (*explain*)

- This eGFP to eBFP conversion was demonstrated Experimentally.
- The predicted deep learning model for the conversion of GFP to BFG was implemented comparing three different Pythia scores.
- The SNP-based conversion was then confirmed through flow cytometry, aligning with Pythia's high prediction scores.
- Higher Pythia scores are associated with greater SNP conversion efficiency.

Correlation of Pythia Scores with SNP Editing Efficiency (*explain-quick*)

- Scatter plot also confirmed a significant correlation between Pythia scores and successful SNP conversion rates (panel d).
- Which means that higher Pythia scores correlate strongly with higher conversion efficiencies for precise SNP editing (panel e)
- This finding validated Pythia's reliability for predicting SNP editing outcomes.

Clinical Application for SNP Corrections in RPE65 Gene Therapy (*explain*-quick)

- The authors also applied Pythia on to the RPE65 gene for single nucleotide mutation correction.
- The Pythia scores identified optimal repair arm lengths to maximize editing accuracy.
- This demonstrated Pythia's potential for potential for designing precise therapeutic templates for single nucleotide corrections in clinical gene therapy.

Conclusion

- **Precision in CRISPR Editing**
  - InDelphi + Pythia enable controlled, predictable edits
  - Effective near microhomology regions for guided repairs
- **Clinical Suitability**
  - Suitable for single-gene corrections with accessible PAM sites
  - Challenges: Complexity in repetitive regions, off-target risks
- **Key Applications**
  - Therapeutic gene editing (e.g., RPE65-related diseases)
  - High-fidelity gene tagging in research
  - Improved precision in gene drive systems

Thank You!