Exploring QRICH2 interactome to understand its role in sperm flagellar assembly and function

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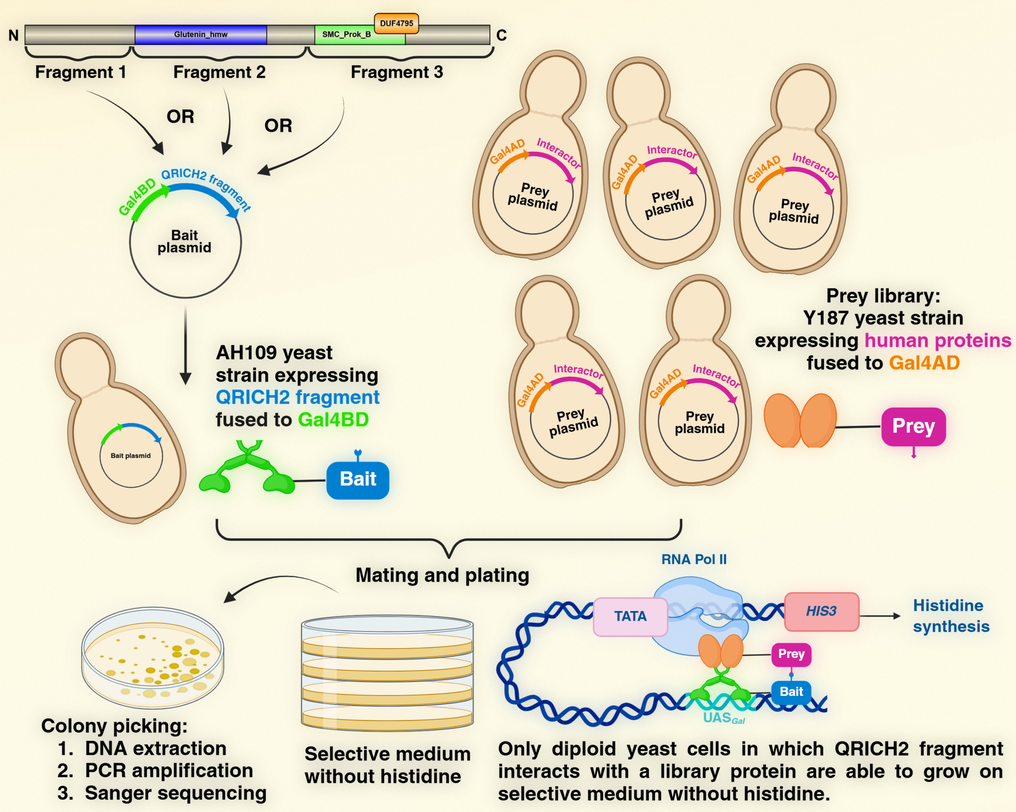
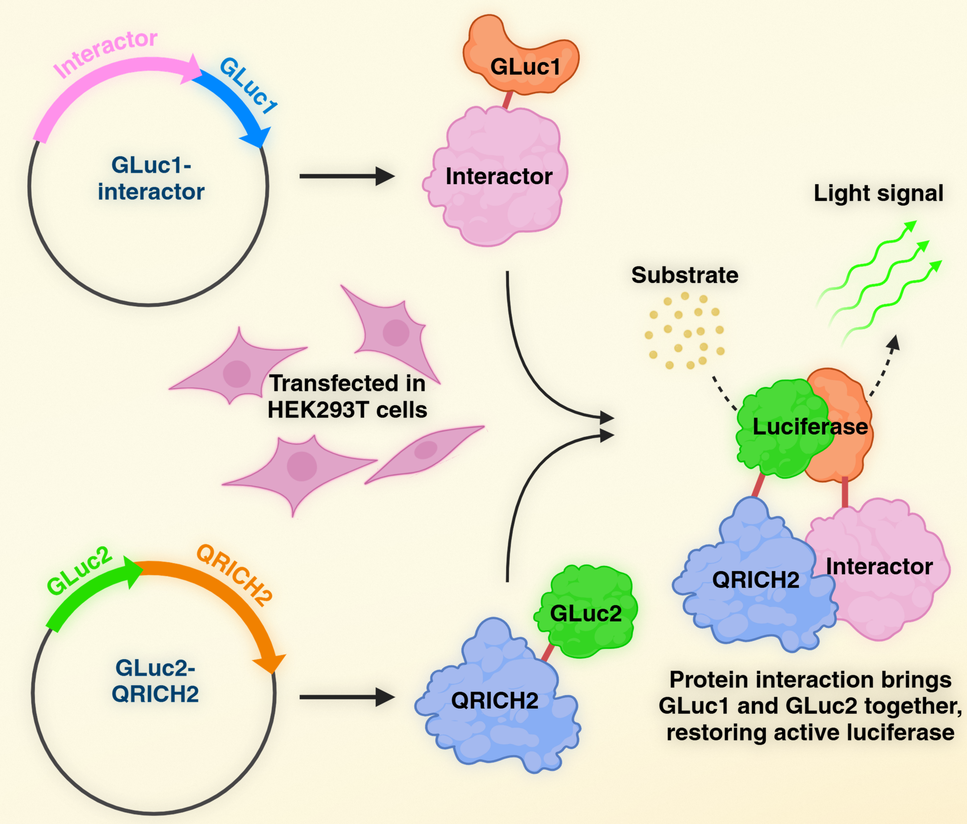
Introduction

Male infertility affects 7% of men worldwide, with a significant proportion linked to **sperm flagellar defects**. Among these, Multiple Morphological Abnormalities of the Flagella (MMAF) represent a severe cause of asthenozoospermia (i.e., reduced sperm motility), characterized by short, coiled, absent, or irregular flagella. Recent genetic studies have identified ***QRICH2*** as a key gene associated with MMAF[1]. Despite this emerging role, the molecular mechanisms underlying QRICH2 function remain poorly understood, and its protein–protein **interaction network** is virtually uncharacterized. To address this gap, we employed two complementary approaches: **Yeast Two-Hybrid** (Y2H) high-throughput screening and ***Gaussia princeps* Complementation Assay** (GPCA) to identify and validate novel QRICH2 interactors.

Screening by Y2H



Validation by GPCA



Results

Due to its large size, QRICH2 was divided into three fragments for Y2H screening. Screening of the C-terminal fragment (Fragment 3) yielded **199 candidate interactors**. Of these, 24 showed testis-enriched expression and 48 were recurrently identified across screening replicates. These high-confidence candidates include flagellar cytoskeletal proteins, gene expression regulators, and ubiquitin-proteasome components. Of the 20 most relevant interactors selected for **GPCA validation**, 12 produced a positive signal. The two remaining fragments have been screened; PCR and sequencing are ongoing.

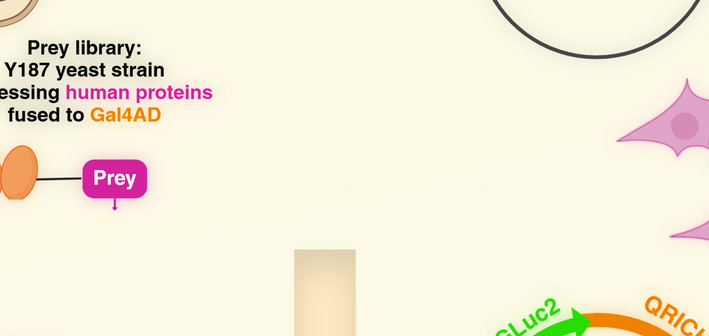


Fig. 1 | Candidate interactors of QRICH2-F3 identified by Y2H screening grouped by functional category. Bold: testis-enriched expression; underlined: identified across multiple replicates.

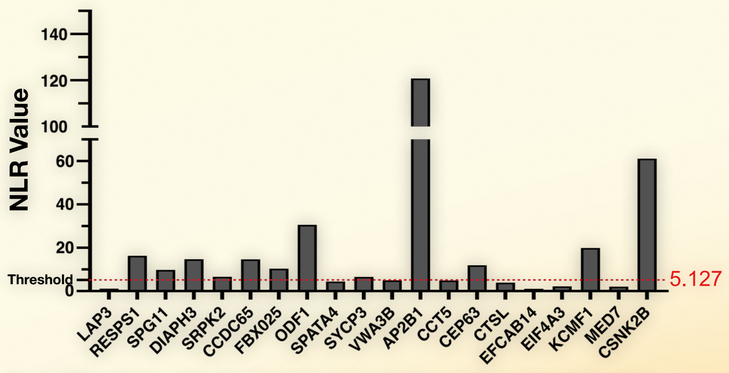


Fig. 2 | GPCA validation of the 20 selected interactors. Red dotted line: background threshold.

Perspectives

Screening of fragments 1 and 2 will, together with the present results, enable broad coverage of QRICH2's interactome across its **full-length sequence**. The interactions validated by GPCA will constitute a prioritized dataset from which the most relevant candidates will be selected for spatiotemporal mapping of QRICH2 complexes throughout spermatogenesis by in situ **Proximity Ligation Assay** (iPLA) on human testis sections.

[1]Shen Y et al. *Nat Commun*. 2019;10:433. doi:10.1038/s41467-018-08182-x

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