A detailed protocol for protein-protein or protein-RNA interaction screening using rec-YnH

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Method Article

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Abstract

Knowing which proteins and RNAs directly interact is essential for understanding cellular mechanisms. Unfortunately, discovering such interactions is costly and often unreliable. To overcome these limitations, we developed rec-YnH, a new yeast two- and three-hybrid-based "screening pipeline":http://www.nature.com/protocolexchange/system/uploads/7059/original/Figure_1.pdf?1533558364 capable of detecting interactions within protein libraries or between protein libraries and RNA fragment pools. rec-YnH combines batch cloning and transformation with intracellular homologous recombination to generate bait-prey fusion libraries. By developing interaction selection in liquid-gels and using an ORF sequence-based readout of interactions via next-generation sequencing, we eliminate laborious plating and barcoding steps required by existing methods. We use rec-Y2H to simultaneously map interactions of protein domains and reveal novel putative interactors of PAR proteins. We further employ rec-Y2H to predict the architecture of published coprecipitated complexes. Finally, we use rec-Y3H to map interactions between multiple RNA-binding proteins and RNAs – the first time interactions between protein and RNA pools are simultaneously detected.

Introduction

Revealing which proteins, protein domains and RNAs undergo direct, physical interactions is an essential prerequisite for understanding the principles by which cellular machines operate. Assays that can detect such interactions are in high demand as they function both to generate system-level views of cellular coordination but also to generate hypothesis for subsequent mechanistic studies. Yeast two and three hybrid \(Y2H, Y3H) screens, allow to detect direct protein-protein and protein-RNA interactions under physiological conditions. The Y2H assay was enhanced significantly in the past. Coupling the readout to next-generation sequencing \(NGS) and multiplexed generation of genetically fused bait-prey libraries resulted in increased assay sensitivity and reproducibility while allowing screening many baits against many preys simultaneously. Improvements of Y3H, however, mostly focused on optimized RNA binding and presentation by linker RNA engineering and selection of stronger MS2-binding proteins. To date, there is no method available allowing the many-by-many direct interaction detection of full-length RBPs and RNA-fragments. With the development of rec-YnH, we aimed at creating "one assay":http://www.nature.com/protocolexchange/system/uploads/7013/original/Detailed_Protocol_rec-YnH.pdf?1533133123 that further advances and integrates past developments in Y2H and Y3H technologies into a single, new assay pipeline allowing screening of protein libraries against protein libraries or RNA fragment libraries. At the same time, we focused on keeping the assay workflow as simple as possible to make it affordable and doable for the standard biomedical research lab. The assay pipeline combines batch-cloning and transformation with intracellular homologous recombination, liquid gel culturing, yeast two or three-hybrid based interaction detection and read out by next-generation-sequencing. This eliminates barcoding, arraying and plating steps needed for available methods. Three compatible vectors allow high-throughput interaction screening within protein libraries and, for the first time, between protein and RNA libraries. We demonstrate that the same assay pipeline can be used for rec-

Y2H and rec-Y3H screening and thus create an assay for both, many-by-many protein-protein or protein-RNA interaction detection.

Reagents

Step 1. Batch Gateway cloning of ORF library into pDEST vectors - pENTR-ORF clones - TE buffer \(10 mM Tris, pH 8.0, 1 mM EDTA) - 10 mM Tris, pH 8.0 - LR clonase II enzyme mix \(ThermoFisher Scientific) - NEB stable cells \(New England Biolabs) - SOC media - 10-cm LB-agar plates - Ampicillin - Spectinomycin - Kanomycin - 1x PBS - QIAprep Spin Miniprep Kit \(Qiagen) Step 2. Competent yeast - Y2HGold \(Clontech) - Loops - Plastic cuvettes - YPDA–agar plates - YPDA liquid media - 50 ml tubes - 250 ml flasks - 500 ml flasks - Sterile H2O - TE buffer \(10x) - 1 M LiAc \(10x) Step 3. Linearization - I-SceI \(\(New England Biolabs) - I-CeuI \(New England Biolabs) - Agarose gel Step 4. Batch yeast transformation - Yeast carrier DNA \(Clontech) - 50% PEG \(Sigma) - 1 M LiAc \(10x) - DMSO \(Sigma) - 0.9% \(w/v) NaCl Step 5. Growth in selection media Yeast dropout agar plates - Square BioAssay dishes, 245×245 mm \(Corning) for screen - 10-cm petri dish for colony counting - Agar \(Conda) - Minimal SD base \(Clontech), - Amino Acid Dropout mixes \(Clontech) - Aureobasidin A \(AbA) \(Clontech) when indicated Yeast dropout liquid-gel media - 5 litre flask - SeaPrep™ Agarose \(Lonza) \(0.5% \(w/v) final concentration) - Minimal SD base \(Clontech), - Amino Acid Dropout mixes \(Clontech) - Aureobasidin A \(Clontech) when indicated Step 6. Colony counting and harvesting yeast - 500 ml centrifuge bottles - 1xPBS - Plastic cuvettes Step 7. Yeast library DNA isolation - Zymoprep™ Yeast Plasmid Miniprep II \(Zymo Research) - DNAse-free water Step 8. Covaris shearing - microTUBE AFA Fiber Pre-Slit Snap-Cap 6x16 mm \(covaris) Step 9. Circularisation - NEBNext® End Repair Module \(New England Biolabs) - MinElute PCR Purification Kit \(Qiagen) - Quick Ligation™ Kit \(New England Biolabs) - DNAse-free water Step 10. R1/R2 PCR - Q5 High-Fidelity 2X Master Mix \(New England Biolabs) - Primer Pr4seq_F_TS_R1 \(5’- ccctacacgacgctcttccgatctcgctgcaggtcgacggatc-3’) \(custom ordered from Integrated DNA Technologies) - Primer Pr4seq_R_TS_R2 \(5’-ttcagacgtgtgctcttccgatctgcagctcgagctcgatggatc-3’) \(custom ordered from Integrated DNA Technologies) - Agencourt AMPure XP beads \(Beckman Coulter) - 10 mM Tris, pH 8.0 Step 11. P5/P7 PCR - Q5 High-Fidelity 2X Master Mix \(New England Biolabs) - NEBNext® Multiplex Oligos for Illumina®, Index Primers Set 1 \(New England Biolabs) - DNAse-free water - Agencourt AMPure XP beads \(Beckman Coulter) - 10 mM Tris, pH 8.0 Step 12. Paired-end sequencing - MiSeq Reagent Kit v2 \(Illumina)

Equipment

o PCR machine o Microcentrifuge o Clinical centrifuge o Ultracentrifuge o Water bath at 30ºC o Water bath at 42ºC o Spectrophotometer o Nanodrop o Shaker at 30ºC o Shaker at 37ºC o Incubator at 30ºC o Incubator at 37ºC o Horizontal Electrophoresis System for DNA o S220 Focused-ultrasonicator with AFA Technology \(Covaris)

Procedure

Please see attached "Detailed Protocol":http://www.nature.com/protocolexchange/system/uploads/7013/original/Detailed_Protocol_rec-YnH.pdf?1533133123 for a full protocol. PARTS OF THE PROTOCOL Please see Figure 1 for a "Workflow Overview":http://www.nature.com/protocolexchange/system/uploads/7059/original/Figure_1.pdf?1533558364 Part I. Bait and Prey library preparation Step 1. Batch Gateway cloning of ORF library into pDEST vectors Part II. Bait and Prey library fusion & Selection in liquid gel Step 2. Competent yeast Step 3. Linearization Step 4. Batch yeast transformation Step 5. Growth in liquid-gel selection media Part III. NGS library preparation Step 6. Harvest yeast Step 7. Yeast library DNA isolation Step 8. Covaris shearing Step 9. Circularisation Step 10. R1/R2 PCR Step 11. P5/P7 PCR Part IV. Paired-end sequencing Step 12. Library quality control and paired-end MiSeq Step 13. Analysis PROTOCOL AT A GLANCE, DAY BY DAY DAY 1 Step 1. Batch Gateway cloning of ORF library into pDEST vectors - Prepare pool of pENTR-ORF clones at 10 nM - Prepare pDEST for Bait and Prey at 10 nM - Set up LR reactions Step 2. Prepare competent yeast - Streak Y2HGold on a YPDA plate DAY 2 Step 1. Batch Gateway cloning of ORF library into pDEST vectors - Continue LR reaction - Transform LR reaction into NEB stable cells - Plate on LB-Agar with corresponding antibiotic DAY 3 Step 1. Batch Gateway cloning of ORF library into pDEST vectors - Colony counting - Harvest colonies from LB-Agar plates - Qiaprep DNA prep of Bait and Prey pDEST-ORF pool Step 2. Competent yeast - Grow 1 colony of Y2HGold during 8 hours - Transfer to 50 ml YPDA media - Grow overnight Step 3. Linearization - Digest Bait and Prey pDEST-ORF pool with I-SceI/I-CeuI - Heat inactivation DAY 4 Step 2. Competent yeast - Transfer yeast to 100 ml YPDA - Grow yeast to OD 0.5 - Wash cells - Resuspend in TE/LiAc Step 4. Batch yeast transformation - Prepare Seaprep liquid-gel selection media - Multiple transformations of yeast with linear Bait and Prey pDEST-ORF pool - Pool together all transformations - Plate dilutions for colony counting Step 5. Growth in selection media - Add transformations to liquid-gel selection media - 1 hour on ice - Grow at 30ºC for 60 hours DAY 7 Step 6. Harvest yeast - Colony counting - Centrifuge liquid-gel selection media with yeast colonies - Wash and resuspend in PBS - OD660 to calculate concentration of cells Step 7. Yeast library DNA isolation - For each selection media, Zymoprep™ Yeast Plasmid Miniprep of recombined Bait and Prey fragments Step 8. Covaris shearing - Shear DNA to 1,500-bp fragments Step 9. Circularisation - End repair - Mini Elute DNA purification - Ligation - Heat inactivation Step 10. R1/R2 PCR - 12 PCR cycles with Q5 polymerase - Purification with AMPure beads Step 11. P5/P7 PCR - 12 PCR cycles with Q5 polymerase and NEBNext® Multiplex Oligos for Illumina - Purification with AMPure beads DAY 8 onwards Step 12. Paired-end sequencing - Library quality control - MiSeq run Step 13. Analysis

Timing

PARTS OF THE PROTOCOL Part I. Bait and Prey library preparation : Day 1-3 Part II. Bait and Prey library fusion & Selection in liquid gel: Day 3-7 Part III. NGS library preparation: Day 7 Part IV. Paired-end sequencing: Day 8-9

Figures

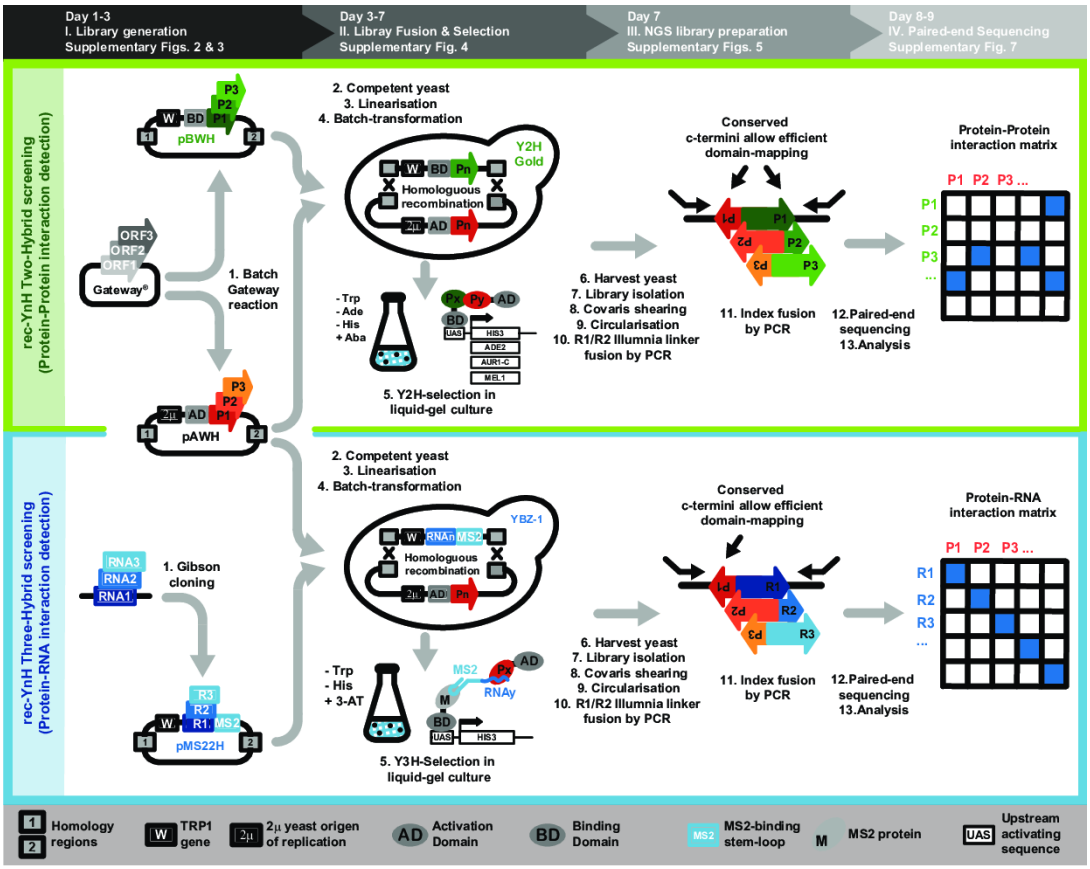

Figure 1. rec-YnH workflow

I: ORF- or RNA-coding fragments are transferred into rec-Y2H or rec-Y3H screening vectors by batch Gateway or Gibson reactions. This step is done only once; the resulting libraries are used for several full-screen replicates.

II: pBWH and pAWH libraries (bait and prey rec-Y2H screening libraries) or pMS22H and pAWH libraries (RNA and prey rec-Y3H screening libraries) are linearized by homing enzyme digests and co-transformed into yeast in batch. The transformed yeast pool is split in a recombination-selection (RS) medium (-Trp, omitted in the figure for simplicity) and a recombination-interaction-selection (RIS) medium (-Trp and different selection markers available) rec-YnH screening is done in liquid-gel cultures. Only correctly fused vectors contain a yeast origin of replication and a TRP1 marker, and are thus able to grow in RS or RIS media.

III: Cells are harvested by centrifugation, the fused plasmid libraries are isolated, and the DNA is fragmented by Covaris and re-circularised by intramolecular ligation. In a first PCR reaction, circular fragments containing the 3' ends of both a bait and a prey are specifically amplified from the pool of fragments, adding R1/R2 Illumina adaptors. A second PCR step then adds a multiplexing index and P5/P7 Illumina attachment sequences, thereby creating a library of fused bait- and prey-coding sequences with conserved C-termini (see Supplementary Fig. 5 for details).

IV: Paired-end sequencing is used to readout library complexity and clone representation (RS condition) and bait-prey interactions (RIS condition). An analysis pipeline normalises the obtained reads, corrects for sequencing depth artefacts and overrepresented clones.

Figure 1

Figure rec-YnH workflow

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement0.pdf