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High-throughput yeast one-hybrid screens using a cell surface gLUC reporter

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Abstract

Gene-centered yeast one-hybrid (Y1H) screens using arrayed genome-wide transcription factor (TF) clone collections provide a simple and effective strategy to identify TF-promoter interactions using a DNA fragment as bait. In an effort to improve the assay we recently developed a Y1H system that uses a cell surface Gaussia luciferase reporter (gLUC59). Compared to other available methods, this luciferase-based strategy requires a shorter processing time, enhances the throughput and improves result analysis of gene-centered Y1H screens. Here, we described the procedure to perform high-throughput screens using this novel strategy, which involves a protocol for mating two haploid yeast strains carrying an arrayed TF clone collection and a *promoter::gLUC59* reporter, respectively, and a protocol for analyzing gLUC59 activity in the resulting diploid cells.

Keywords

yeast one-hybrid screens; gene-centered Y1H; cell-surface gLUC reporter; transcription factor-promoter interaction.

INTRODUCTION

Identification of transcription factor (TF)-promoter interactions is key to understanding the basic molecular underpinnings of gene regulation. Strategies to identify TF-promoter interactions either focus on a particular TF to search for its interacting promoter regions, or a particular promoter fragment to search for its TF interactors (Reece-Hoyes & Marian Walhout, 2012). The yeast one-hybrid (Y1H) system is among the few strategies widely used for the latter. In this approach, a yeast replicating plasmid encoding a TF fused to a transcription activation domain (AD) (*e.g.* gal4-AD), is introduced into a yeast reporter strain carrying a *promoter::reporter* construct (Reece-Hoyes & Marian Walhout, 2012). An increased reporter activity in the resulting cells then reveals an interaction between the TF-AD protein prey and the promoter bait. Notably, TF preys rely on their intrinsic DNA

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binding activity to bind *cis*-elements within the promoter bait, thus the Y1H system provides a functional approach for identifying TF-promoter interactions.

Before the genomic era, Y1H screens relied on the presence of TF coding sequences in cDNA libraries (Reece-Hoyes & Marian Walhout, 2012). However, TFs are typically expressed at low levels and often restricted to specific tissues, developmental stages or physiological conditions (Czechowski, Bari, Stitt, Scheible, & Udvardi, 2004; Holland, 2002; Vaquerizas, Kummerfeld, Teichmann, & Luscombe, 2009). Thus, cDNA libraries usually contain a partial and biased gene pool, which ultimately limits identifying the complete set of TFs that bind to a promoter region of interest. Recent availability of genome sequences enabled the construction of genome-wide collections of plasmids carrying gene open reading frames (ORFeomes) (Yamada et al., 2003). ORFeome collections of TF-AD encoding plasmids, which include most TFs encoded in a genome (Burdo et al., 2014; Fuxman Bass et al., 2015; Gaudinier et al., 2011; Gubelmann et al., 2013; Hens et al., 2011; King, Foster, Davison, Rawls, & Breton, 2018; Pruneda-Paz et al., 2014; Reboul et al., 2003; Reece-Hoyes, Barutcu, et al., 2011), were instrumental to develop improved Y1H strategies called gene-centered Y1H assays (Deplancke, Dupuy, Vidal, & Walhout, 2004; Deplancke et al., 2006; Pruneda-Paz et al., 2014; Pruneda-Paz, Breton, Para, & Kay, 2009; Reece-Hoyes, Diallo, et al., 2011; Vermeirssen et al., 2007). In the approach that has been most widely used, individual clones in a TF-AD ORFeome collection are organized in an arrayed multi-well format. Keeping this arrangement, each TF-AD clone is introduced into a Y1H reporter strain and the reporter activity is analyzed in the resulting cells (Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011). A major advantage of the strategy is that each TF-promoter combination is evaluated independently, which allows an unbiased survey of all possible interactions. Furthermore, given that the location of each TF-AD plasmid within the array is known and maintained throughout the assay, simply mapping the position of high reporter activities immediately identifies interacting TFs (Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011). Thus, gene-centered Y1H screens provide a rapid and relatively simple assay to comprehensively identify TFs that bind to a single promoter bait.

High-throughput approaches were developed to perform the aforementioned gene-centered Y1H (HT-Y1H) assay using large TF ORFeome collections and to facilitate the simultaneous survey of multiple promoter baits (Gubelmann et al., 2013; Hens, Feuz, & Deplancke, 2012; Kang, Breton, & Pruneda-Paz, 2018; Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011; Vermeirssen et al., 2007). Many of these approaches rely on automation compatible protocols, either in solid or liquid multi-well arrayed formats, to deliver a TF ORFeome clone collection into reporter strains and to analyze the reporter gene activity in the resulting cells (Kang et al., 2018; Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011). In most HT-Y1H protocols, plasmid delivery is achieved by yeast mating, which requires the arrayed ORFeome clone collection pre-transformed into a yeast strain of the opposite mating type as the reporter strain (Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011). On the other hand, reporter genes for auxotrophic selection (*e.g. HIS3*) and/or colorimetric detection (*e.g. lacZ*) were used to identify positive interactions in a high-throughput multi-well format (Pruneda-Paz et al., 2014; Reece-Hoyes, Diallo, et al., 2011). Recently, we implemented a novel cell surface luciferase reporter (gLUC59) that significantly improved the simplicity, reliability and scalability of HT-Y1H screens

compared to our previous method using the *lacZ* reporter gene (Bonaldi, Li, Kang, Breton, & Pruneda-Paz, 2017; Pruneda-Paz et al., 2014). Here, we described the procedure to perform HT-Y1H screens using the gLUC59 reporter, which involves automation compatible protocols to deliver a TF clone collection into yeast reporter strains, and to quantify gLUC59 activity and determine screen results (Fig. 1).

BASIC PROTOCOL 1

Mating TF-library and reporter strains in liquid medium

This protocol describes the delivery of TF-AD coding plasmids (*i.e.* pDEST22-TF) into Y1H reporter strains carrying *promoter::gLUC59* reporter constructs in a 384-well format (Fig. 2A). For that, YU yeast cells (MATα) carrying TF-AD plasmids are mated with YM4271 cells (MATa) carrying the reporter construct. All steps in this protocol are performed in a liquid format and are thus fully compatible with automated liquid handling workstations.

Materials

- YM4271 reporter strains carrying a *promoter::gLUC59* reporter construct (Bonaldi et al., 2017; Breton, Kay, & Pruneda-Paz, 2016).
- Round petri dishes 100 × 15 mm.
- YPD (Yeast Extract-Peptone-Dextrose)-agar medium (see recipe).
- TF-gal4AD clone collection (*i.e.* Arabidopsis pDEST22-TF library (Pruneda-Paz et al., 2014)) transformed into YU yeast cells and arrayed in deep 384-well polypropylene (PP) microplates, v-bottom 240 μL wells (*e.g.* Greiner Masterblock, cat 781271) (5 μL of cells/well) (Kang et al., 2018; Pruneda-Paz et al., 2014). Each microplate must contain 4-6 wells without YU cells (empty well controls) and 4-6 wells containing YU cells transformed with the empty AD vector (*i.e.* pEXP-AD for libraries in pDEST22) (empty vector control) (Pruneda-Paz et al., 2014).

  Note: it is convenient to keep “screen-ready” deep 384-well PP microplates containing the arrayed TF library in YU cells stored as glycerol stocks (5 μL of cells/well) at −80 °C (Kang et al., 2018).
- SD(-Trp) (Synthetic Defined without Tryptophan) minimal medium (see recipe).
- Breathable microplate sealing film (*e.g.* AeraSeal, Excel Scientific, cat B-100).
- Microplate lids (*e.g.* Greiner, cat 656101).
- Yeast growth microplates: deep 384-well polypropylene (PP) microplates, v-bottom, sterile (240 μL/well) (*e.g.* Greiner Masterblock, cat 781271).
- YPD medium (see recipe).
- Yeast mating microplates: 384-well polypropylene (PP) microplates, v-bottom, sterile (*e.g.* Greiner, cat 781281).

- SD(-Trp/-Ura) (Synthetic Defined without Tryptophan and Uracil) minimal medium (see recipe).

Equipment

- Microplate pipetting station with a 384-channel disposable-tip pipette head.
- Incubator and incubator shaker for flasks and microplates (30°C).
- Flexible volume reagent dispenser for 384-well microplates.
- Microplate centrifuge.
- Microplate mixer/shaker (2 mm orbit).

Protocol steps—Step annotations

DAY 1

1. Streak YM4271 reporter strain on YPD-agar medium. Incubate 2 days at 30°C.

DAY 2

2. Dispense 120 µL of SD(-Trp) medium into each well of deep 384-well microplates containing the TF-gal4AD clone collection in YU yeast cells (as described in the materials section (Kang et al., 2018; Pruneda-Paz et al., 2014)). Seal microplates with a breathable sealing film and cover with a plastic microplate lid (to prevent evaporation of well content during incubation). Incubate at 30°C in a microplate shaker (600 rpm) for 36-48 h.

DAY 3

3. Add 50 mL of YPD into a 250 mL flask and inoculate with 2-3 YM4271 reporter strain colonies (use one flask for each reporter strain included in the screen). Incubate overnight at 30°C with agitation (180 rpm).

Note: the number of reporter strains that could be simultaneously screened will depend on the assay setup in each laboratory (e.g. using an automated workstation (Kang et al., 2018) we simultaneously screen up to 16 reporter strains using a ~2000 TF ORFeome).

DAY 4

4. Dispense 40 µL of YPD medium into each well of a new 384-well PP microplate (mating microplate).

5. Mix TF library cell suspension in deep 384-well microplates (prepared in step 2 of this basic protocol) by pipetting up/down (pipetting volume: 50 µL). Transfer 3.5 µL from each well into the corresponding well of the YPD-filled mating microplate (prepared in step 4 of this basic protocol).

Note: when transferring small volumes, it is important to avoid air bubbles at the end of each tip. For that, load 20 µL of the cell suspension, dispense 10 µL back

to the source microplate, then dispense 3.5 μL into the destination microplate, and finally dispense cell suspension leftover back to the source microplate.

6. Add 50 mL of YPD medium to the overnight YM4271 reporter strain culture (started in step 3 of this basic protocol) and aliquot the diluted culture into a new deep 384-well microplate (180 μL/well).

7. Pipette 5-20 μL of the diluted YM4271 reporter strain culture into each well of the mating microplate prepared in step 5 of this basic protocol (containing the TF library).

   Note: a larger volume (20 μL) allows dispensing a drop of the promoter strain culture from the top of the mating microplate so tips do not contact its content, and thus using a single tip load to add the same promoter strain into multiple mating microplates.

8. Mix/shake mating microplate for 10 sec at 1000 rpm, seal microplate with a breathable sealing film, and incubate at 30°C in a humid environment (*i.e.* inside a sealable plastic bag) to prevent evaporation for 12- 24h without shaking.

DAY 5 - part I

9. Centrifuge mating microplate for 3 min at 1000 × *g* (room temperature).

10. Remove supernatant by pipetting (take liquid from 1 mm above the well bottom to avoid disturbing cell pellets).

11. Dispense 40 μL of SD(-Trp/-Ura) medium into each well and vortex for 15 sec at 1000 rpm.

12. Repeat steps 9 and 10 one time.

13. Dispense 65 μL of SD(-Trp/-Ura) medium into each well and mix cell suspension by pipetting up/down.

14. Proceed to step 1 of basic protocol 2.

BASIC PROTOCOL 2

Quantification of gLUC59 activity in liquid cultured yeast cells

This protocol describes the quantification of diploid yeast cell gLUC59 activity in a 384-well format (Fig. 2B). For that, diploid cells obtained after mating are enriched by culturing in auxotrophic selection medium followed by a short incubation in rich growth medium. Cell growth and luminescence (either flash and/or glow emission) are then measured directly in the enriched diploid cell suspension (Bonaldi et al., 2017). Finally, cell growth normalized luminescence is used to determine TF-promoter interactions. All steps in this protocol are performed in a liquid format and are thus fully compatible with automated liquid handling workstations.

Materials

- Diploid YM4271-YU cells carrying *promoter::gLUC59* reporter construct and TF-AD encoding plasmid (*i.e.* from step 13 of basic protocol 1).
- Yeast growth microplates: deep 384-well polypropylene (PP) microplates, v-bottom, sterile (240 µL/well) (*e.g.* Greiner Masterblock, cat 781271).
- SD(-Trp/-Ura) medium (see recipe).
- Breathable microplate sealing film (*e.g.* AeraSeal, Excel Scientific, cat B-100).
- Microplate lids (*e.g.* Greiner, cat 656101).
- YPD medium (see recipe).
- Transparent microplates: 384-well polystyrene (PS) microplates, flat clear bottom (*e.g.* Greiner, cat 781101).
- White microplates: 384-well polystyrene (PS) white microplates, flat bottom (*e.g.* Greiner, cat 781075).
- n-coelenterazine stock solution (see recipe).
- gLUC substrate solution (see recipe).

Equipment

- Microplate pipetting station with a 384-channel disposable-tip pipette head.
- Incubator and incubator shaker for flasks and microplates (30°C).
- Flexible volume reagent dispenser for 384-well microplates.
- Microplate mixer/shaker (2 mm orbit).
- Absorbance and luminescence reader for 384-well microplates (for flash luminescence measurement, reader should be equipped with a reagent injector module).

Protocol steps—Step annotations

DAY 5 - part II (cont. from basic protocol 1)

1) Dispense 45 µL of SD(-Trp/-Ura) medium into each well of a new deep 384-well PP microplate.

2) Mix washed diploid cell suspension in mating microplates (*i.e.* from step 13 of basic protocol 1) by pipetting up/down (pipetting volume: 30 µL). Transfer 2 µL from each well into the corresponding well of the SD(-Trp/-Ura)-filled deep 384-well microplate (prepared in the previous step).

Note: when transferring small volumes it is important to avoid air bubbles at the end of each tip. For that, load 20 µL of the cell suspension, dispense 10 µL back to the source microplate, then dispense 2 µL into the destination microplate, and finally dispense cell suspension leftover back to the source microplate.

3) Seal the deep 384-well PP microplate with a breathable sealing film and cover with a plastic microplate lid (to prevent evaporation of well content during incubation). Incubate at 30°C in a microplate shaker (600 rpm) for 24-36 h.

DAY 7

4) Add 120 µL of YPD into each well of the deep 384-well PP microplate.

5) Seal deep 384-well PP microplate with a breathable sealing film and cover with a plastic microplate lid (to prevent evaporation of well content during incubation). Incubate at 30°C in a microplate shaker (600 rpm) for 5-6 h.

6) Homogenize cell suspension in deep 384-well microplates by pipetting up-down (pipetting volume: 50 µL).

7) Transfer 25 µL of the cell suspension into a transparent 384-well PS microplate, mix/shake for 20 sec at 1000 rpm to even out the liquid surface in each well, and measure OD_{600} in each well using a microplate reader.

Note: to avoid air bubbles at the end of each tip, load 35 µL of the cell suspension, dispense 5 µL back to the source microplate, then dispense 25 µL into the transparent microplate, and finally dispense cell suspension leftover back to the source microplate.

8) Transfer a second 25 µL aliquot of the cell suspension from step 6 into a white 384-well PS microplate, mix/shake for 20 sec at 1000 rpm to even out the liquid surface in each well, and measure luminescence in each well using a microplate reader.

Note: follow the pipetting procedure suggested in the previous step to avoid air bubbles at tip end.

Option 1: Flash luminescence measurement (each well processed independently)

a) Place microplate in a microplate reader equipped with a reagent injection module.

b) Using microplate injector, dispense 25 µL of gLUC substrate solution (see recipe) into a single well (injection speed: 275 µL/sec).

c) Measure well luminescence immediately using a microplate reader (integration time: 1 sec/well).

d) Repeat procedure for all wells in the microplate.

Option 2: Glow luminescence measurement (all wells processed simultaneously)

a) Pipette 25 µL of gLUC substrate into each well of the white 384-well microplate

b) Mix/shake microplate for 20 sec at 1000 rpm.

c) Incubate microplate at room temperature protected from light for 8-10 min.

d) Measure luminescence in each well using a microplate reader (integration time: 1 sec/well).

9) Calculate gLUC enzymatic activity for each well and determine positive interactions:

Note: a separate analysis should be performed for each promoter::gLUC59 construct and each analysis should include all microplates that contain such construct.

a) Calculate the average and standard deviation (SD) of OD_{600} values for all empty wells.

b) Eliminate from further analysis any well with OD_{600} readings below the average value + 3SD for empty wells (calculated in a).

Note: this step will eliminate wells where yeast cells did not grow, which typically result in false positives.

c) Calculate the average and SD of luminescence values for all empty wells.

d) Subtract average OD_{600} and luminescence for empty wells (background values calculated in a and c) from all absorbance and luminescence readings, respectively.

e) Use background corrected readings (obtained in d) to calculate Luminescence/OD_{600} ratio for each well.

f) Calculate the average Luminescence /OD_{600} ratio for empty vector control wells. Establish a cut-off value for positive interactions at 3SD above this average value.

Note: doubling this cut-off value provides a stringent and more reliable criterion to determine positive interactions.

g) Wells with Luminescence /OD_{600} values above the cut-off contain TFs that interact with the promoter bait.

Note: to avoid false positives due to low yeast growth, luminescence values in positive interaction wells should be above the average + 3SD luminescence of empty vector control wells (calculated in c).

REAGENTS AND SOLUTIONS

YPD (Yeast Extract-Peptone-Dextrose) medium

Dissolve 10 g of bacto peptone and 20 g of yeast extract in 800 mL of water. Adjust final volume to 900 mL with water and pH to 5.8 with HCl (for solid medium add 20 g/L of bacto agar). Autoclave. Cool down and add 100 mL of 20 % sterile dextrose solution.

SD (Synthetic Defined) minimal medium

Dissolve 6.7 g of yeast nitrogen base without amino acids (BD Difco, cat DF0919-15-3) and either 0.74 g of dropout mix without tryptophan (SD-Trp) (Clontech, cat 630413) or 0.72 g

of dropout mix without tryptophan and uracil (SD-Trp/-Ura) (Clontech, cat 630427) in 800 mL of water. Adjust final volume to 900 mL with water and pH to 5.8 with NaOH. Autoclave. Cool down and add 100 mL of 20 % sterile dextrose solution.

Coelenterazine stock solution (2.4 mM native coelenterazine)

Dissolve 10 mg of native coelenterazine (Biosynth cat C-7001) in 10 mL of acidified methanol (10 μL of 1N HCl per mL of methanol). Aliquot in amber 1.5 mL tubes and store at −20 °C (use within 2-3 months).

gLUC substrate solution (prepare fresh)

For 100 mL, mix 10 mL of 10x PBS (phosphate buffered saline) pH 7.4, 1 mL of 2.4 mM n-coelenterazine stock solution, 100 μL of 5 M NaCl, and 89.9 mL of water. Incubate for 30 min at room temperature protected from light. Use immediately after.

COMMENTARY

Background Information

Gene-centered Y1H screens provided a remarkably useful strategy to uncover TF-promoter interactions. Despite its unquestionable success we found that using the *lacZ* reporter gene for HT-Y1H screens performed in a liquid format limited the assay throughput (due to procedure steps incompatible with automation) and affected result quality (due to the short linear range of β-galactosidase activity quantification). Using a luciferase (*i.e.* gLUC59) reporter, we recently implemented a novel Y1H system that circumvents these limitations and provides an improved assay (described here) suitable for fully automated gene-centered screens.

Critical Parameters

The following points should be considered when performing these protocols:

- Each deep 384-well microplate containing the TF-gal4AD clone collection in YU yeast cells must include 4-6 wells without YU cells (empty well controls) and 4-6 wells containing YU cells transformed with the empty AD vector (*i.e.* pEXP-AD for libraries in pDEST22) (empty vector control) (Kang et al., 2018; Pruneda-Paz et al., 2014).
- Grow enriched diploid yeast cells in YPD for 5-6 h prior gLUC activity quantification.
- Homogenize cell suspension in absorbance and luminescence microplates before placing into microplate reader.
- Avoid air bubbles after dispensing the cell culture into the absorbance microplates.
- Do not use gLUC substrate solution immediately after preparation (stabilize for 30 min prior usage).

- Determine gLUC activity directly in a cell culture aliquot (washing cells before measurement results in lower luminescence) (Bonaldi et al., 2017).
- Consider that while flash luminescence provides the most sensitive assay, it will require significantly longer microplate reader processing time.
- For glow luminescence quantification, incubate microplates for 8-10 min before measurement to avoid result bias across wells due to luminescence decay (Bonaldi et al., 2017).
- To minimize false positive calls, identify and eliminate from further analysis wells with no yeast cell growth.

Troubleshooting

Understanding Results

To minimize false positive calls, it is recommendable to run replicate screens and to use a stringent positive interaction cut-off threshold. Furthermore, when analyzing high-throughput Y1H screen results it is important to establish systematic steps to confirm that in positive interaction wells: 1) yeast cells are present and 2) luminescence values are statistically different than in empty vector control wells.

Time Considerations

The entire procedure described in these protocols should be performed in 7 consecutive days (Fig. 2).

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Significance Statement

Gene-centered yeast one hybrid (Y1H) screens provide a simple and reliable strategy to uncover transcription factor (TF) - DNA interactions. In this method a genome-wide array of TFs is used to individually investigate all possible TF-DNA interactions to a unique DNA sequence of interest. For that, gene-centered Y1H screens critically rely on high throughput (HT-Y1H) procedures where thousands of such interactions are tested simultaneously. This article presents a streamlined HT-Y1H screen procedure using a recently developed luciferase-based Y1H system that enables full automation and improves result analysis.

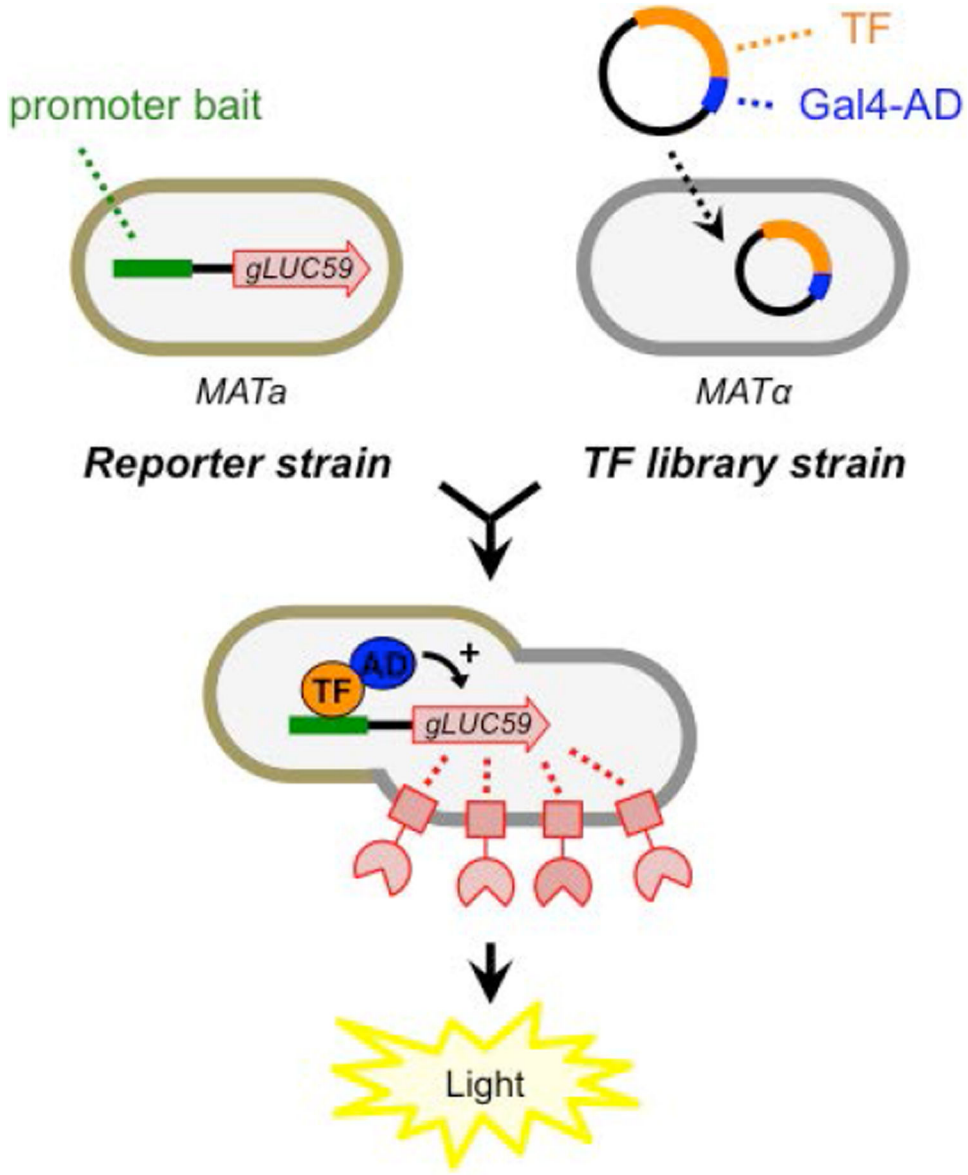

Figure 1. Illustration of the Y1H assay using a cell-surface luciferase reporter (gLUC59).
Main protocols for TF library screening, 1) mating of TF-library and Y1H reporter strains and 2) quantification of gLUC59 activity in liquid cultured yeast cells, are depicted by black vertical arrows.

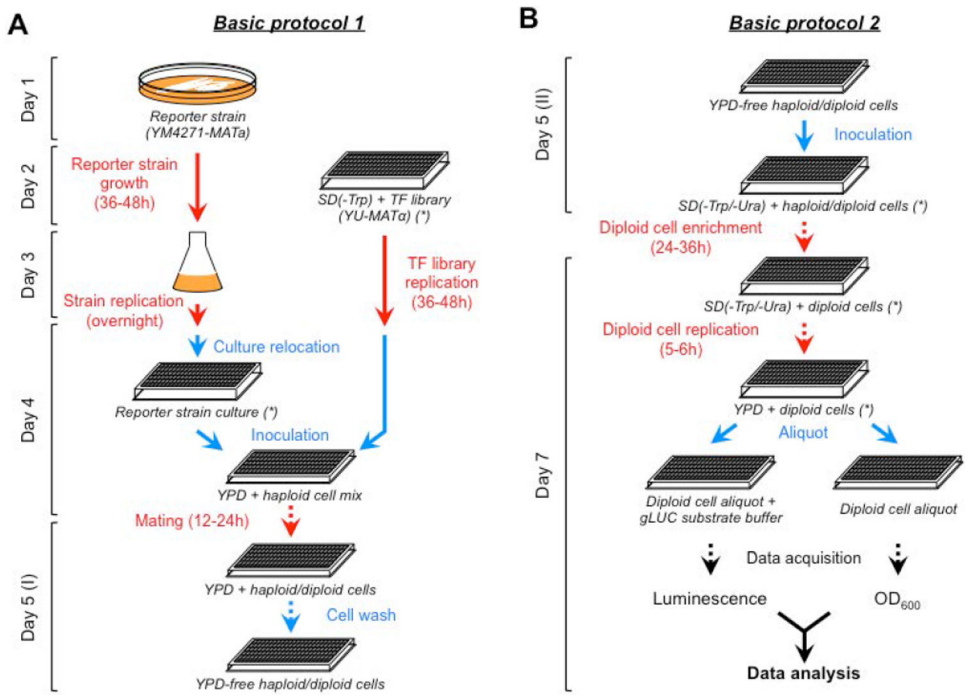

Figure 2. Schematic representation of the two basic protocols described to performing high-throughput gLUC59-based Y1H screens.

A) Basic protocol 1: mating TF-library and Y1H reporter strains in liquid medium. **B)** Basic protocol 2: quantification of gLUC59 activity in liquid cultured yeast cells. All illustrated microplates are 384-well and the text below each one describes its content (a star indicates that a deep-well microplate is used). Arrows indicate protocol incubation steps (red), liquid handling steps (blue) and data processing steps (black). Dotted- or full-lined arrows indicate protocol steps that maintain or introduce a new microplate, respectively. Text next to each arrow provides a brief step description. Procedure timeline is indicated on the left side of each panel.

Table 1.

Potential issues and possible solutions when performing these protocols

Issue	Possible Solutions
Low/no yeast cell growth	Check that the proper haploid or diploid yeast selection medium was used Potential TF toxicity, use TF deletion constructs or inducible yeast expression vectors
No luminescence signal	Incorrect enzyme substrate, only n-coelenterazine should be used
Low/no luminescence signal	Oxidized n-coelenterazine stock solution (dark brown colored), prepare new stock solution
Low luminescence signal	Consider increasing integration time for luminescence measurements Consider measuring flash luminescence emission Low promoter bait activity, consider using shorter or multiplexed promoter baits Consider using haploid YM4271 reporter cells chemically transformed with TF-AD plasmids (Bonaldi et al., 2017)
High basal luminescence signal (*i.e.* in empty vector control wells)	Consider using an alternative promoter bait