

Mate & Plate™ Yeast Two-Hybrid cDNA Libraries

Ready-made or make your own; by far the easiest screening method for yeast two-hybrid interactions

- No library-scale plasmid amplification or transformation required for premade Mate & Plate Libraries
- Select from a wide variety of cDNA libraries: human, mouse, normalized & universal
- Make your own pretransformed library, simply and efficiently, using SMART™ technology
- Perfect for use with Matchmaker™ Gold Yeast Two-Hybrid Systems

Clontech's Matchmaker Systems, including our latest version, the **Matchmaker Gold Yeast Two-Hybrid System** (see pages 1–3), are highly optimized tools for screening cDNA libraries in yeast to identify novel DNA-binding proteins or protein-protein interactions (PPIs). Traditional construction of a GAL4-AD fusion protein library for yeast two-hybrid (Y2H) screening requires a significant amount of effort, including: cDNA synthesis, cloning the cDNA library in a yeast expression vector, library-scale amplification in *E. coli*, plasmid purification, and library-scale transformation into yeast—all this before screening can even begin.

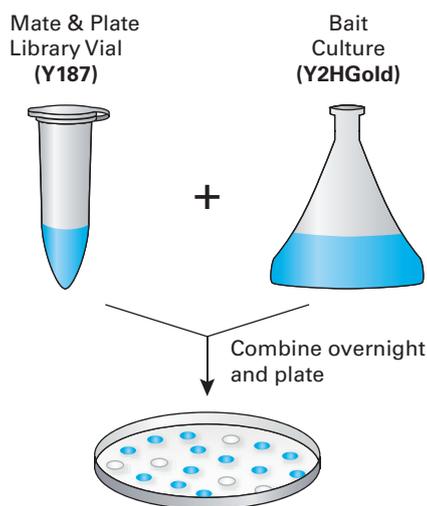


Figure 1. The Mate & Plate Protocol.

To screen a Mate & Plate Library, an aliquot of the library in the *MAT α* Y187 strain is simply mixed with a bait-expressing, *MAT α* reporter strain culture (Y2HGold or AH109). The two strains are co-cultured overnight and then plated on selective agar medium.

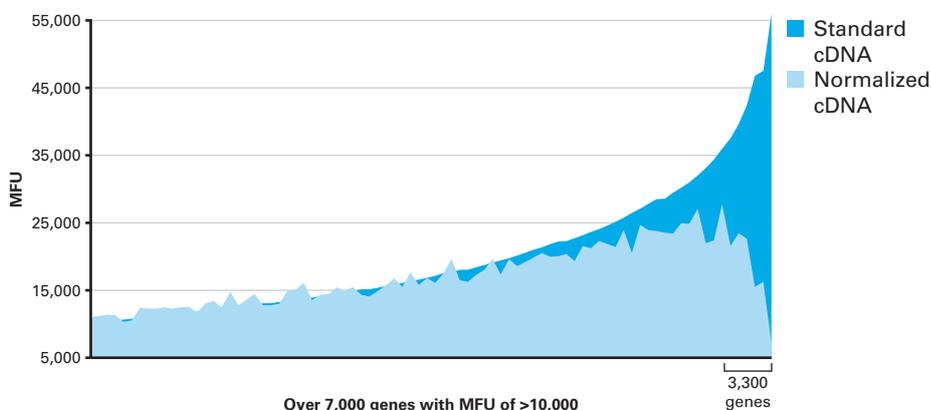


Figure 2. Normalization reduces the abundance of cDNAs derived from highly expressed genes. Universal cDNA, synthesized using RNA from mixed human tissues, was analyzed before and after normalization on a NimbleGen® *Homo sapiens* microarray (Cat. No. A4542-00-01). Data are shown for the 7,000+ genes which exhibited greater than 10,000 Mean Fluorescence Units (MFU). The signal intensities for approximately 3,300 of the most highly represented genes were significantly reduced following normalization, reflecting a preferential reduction of these abundant cDNAs.

Simple screening with Mate & Plate Libraries

Fortunately, Clontech has an extensive variety of pretransformed cDNA libraries in yeast for which all the exhaustive steps of library construction and transformation have already been performed and verified. **Mate & Plate Libraries** are cDNA libraries of GAL4-AD prey fusion proteins that are ready for immediate screening in Y2H systems. These libraries have been transformed into the *MAT α* haploid yeast strain, **Y187**, which can be easily mated to a haploid *MAT α* reporter strain, such as **AH109** or **Y2HGold**.

Our "Mate & Plate" protocol makes library screening a very simple task accomplished by combining the Mate & Plate library culture with a culture of your bait-expressing reporter strain (Figure 1). Co-culturing the two strains overnight produces an array of diploid yeast clones, each coexpressing your bait with a different library prey protein. The clone pool can then be plated on selective media to screen for individual clones that express the appropriate reporter genes and markers, indicating the presence of interacting hybrid protein pairs.

Normalized Libraries Produce Fewer False Positives

Clontech also offers a selection of *normalized* Mate & Plate libraries, which further simplify the search for novel protein-protein interactions. Duplex-specific nuclease (DSN) normalization selectively removes abundant, and therefore redundant, cDNAs from the total pool and enriches the library for rare and less abundant sequences (1, 2; Figure 2). This process eliminates a major source of potential false positives. Library complexity effectively increases, which reduces the number of independent clones that must be screened in order to detect genuine positive interactions, and lowers the frequency of false positives that emerge from primary, low stringency screens.

By using a normalized Mate & Plate library together with the stringent screening methodology provided by the Matchmaker Gold System, your primary screens will greatly favor the identification of genuine positives, produce few false positives, and yield virtually no background colonies.

Mate & Plate™ Yeast Two-Hybrid cDNA Libraries...continued

Balanced Gene Representation

To illustrate how DSN normalization results in more balanced gene representation, cDNA samples taken before and after normalization were compared on a NimbleGen microarray containing 47,633 human genes (Figure 2). In this analysis, cDNA species from the most highly expressed genes were preferentially eliminated, while less abundant cDNAs remained largely unaffected. Consequently, the representation of low-copy-number sequences increased within the total cDNA pool. Figure 3 provides specific examples of how the cDNA levels of two highly-expressed housekeeping genes, β -actin and GAPDH, were effectively reduced by normalization.

Universal Libraries for Universal Gene Coverage

Our universal libraries provide the broadest and most complete coverage of genes that are expressed in almost any tissue. To create these all-purpose, normalized libraries, we combined RNAs from a diverse collection of either mouse or human whole tissues specifically chosen to represent an expansive range of expressed genes (3). These same RNA pools are used for our **qPCR Human Reference cDNA** and **Total RNA**, as well as for our **Human and Mouse Universal Reference Total RNAs** (3). Following cDNA synthesis and

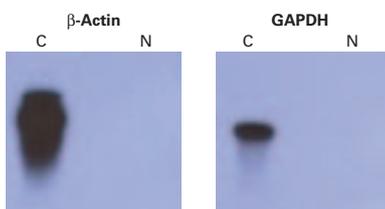


Figure 3. DSN-Normalization removes highly abundant cDNAs. Normalized (Lanes N) and non-normalized (Lanes C) human HeLa S3 cDNAs were compared using virtual Northern blot analysis and ^{32}P -labeled probes. The levels of these two highly abundant cDNAs were sharply reduced following normalization.

amplification using SMART™ technology, we normalize each cDNA pool before constructing and transforming the library into yeast. Combining “across-the-board” gene representation with the enrichment of low-copy-number cDNA, our universal normalized libraries offer the greatest capacity for identifying genuine binding partners of your protein of interest.

Efficient Two-Hybrid Screening

To illustrate the improved Y2H screening qualities of a normalized library, we searched for binding partners of a murine p53-bait, using our **Mate & Plate Library - HeLa S3 (Normalized)**. A medium stringency screen of only 279,000 clones yielded 62 colonies that could possibly contain p53-binding proteins (1). In contrast, we recommend screening 1–2 million clones for a standard library. Of eight colonies that were selected for further analysis, four contained three different well-known binding partners of p53: PCNA, PRMT3, and PTEN. Thus, it is possible to screen a significantly smaller number of clones in a normalized library and still generate valuable data.

Make Your Own “Mate & Plate” Library

For researchers wishing to construct and transform their own cDNA library, we offer the convenient and straightforward **Make Your Own “Mate & Plate” Library System**. This system combines SMART technology with highly efficient homologous recombination in yeast, allowing you to efficiently synthesize cDNA and then clone it into the **pGADT7-Rec AD Cloning Vector** directly in yeast. Your library is constructed and transformed into the Y187 yeast strain in a single step. The result is a custom-made library ready to be used in our easy Mate & Plate protocol.

Mate & Plate Libraries, coupled with the stringent screening of the Matchmaker Gold, provide the most complete and advanced

| Product | Size | Cat. No. |
|---|----------|----------|
| Mate & Plate Library - Universal Human (Normalized) | 2 x 1 ml | 630481 |
| | 5 x 1 ml | 630480 |
| Mate & Plate Library - Universal Mouse (Normalized) | 2 x 1 ml | 630482 |
| | 5 x 1 ml | 630483 |
| Mate & Plate Library - HeLa S3 (Normalized) | 5 x 1 ml | 630479 |
| Mate & Plate Library - Human Bone Marrow | 5 x 1 ml | 630477 |
| Mate & Plate Library - Human Fetal Brain | 5 x 1 ml | 630469 |
| Mate & Plate Library - Human Heart | 5 x 1 ml | 630471 |
| Mate & Plate Library - Human Liver | 5 x 1 ml | 630468 |
| Mate & Plate Library - Human Skeletal Muscle | 5 x 1 ml | 630473 |
| Mate & Plate Library - Human Testis | 5 x 1 ml | 630470 |
| Mate & Plate Library - Human Ovary | 5 x 1 ml | 630474 |
| Mate & Plate Library - Mouse Embryo 11-day | 5 x 1 ml | 630478 |
| Mate & Plate Library - Mouse Embryo 17-day | 5 x 1 ml | 630476 |
| Systems | | |
| Make Your Own “Mate & Plate” Library System | 5 rxns | 630490 |
| Matchmaker Gold Yeast Two-Hybrid System | each | 630489 |

Notice to Purchaser

Please see the Matchmaker™ Two-Hybrid System and Reverse Two-Hybrid System Technology licensing statements on page 40.

system for discovering new protein relationships. These tools afford you the greatest opportunities for Y2H screening success—and require the least amount of time and effort.

References

1. Pretransformed Normalized Matchmaker™ Library (January 2007) *Clontechiques* **XXII**(1):21–23.
2. Pretransformed Normalized Matchmaker™ Libraries (January 2008) *Clontechiques* **XXIII**(1):14–16.
3. High-Performance Reference RNA and cDNA (July 2008) *Clontechiques* **XXIII**(2):18–20.