products in this RSP technique saves precious biological material. By using a Random Primers DNA Labeling System (Life Technologies) and purifying the resulting labeled cDNA on Sephadex G-50 spin columns, a radio-labeled cDNA of high specific activity was recovered with $3-8 \times 10^9$ specific dpm per $\mu$g DNA as measured by scintillation counting. Hence, the specific activity achieved using RSP with unlabeled cDNA exceeded the one obtained by SR-RT labeling by several hundred-fold when calculated per $\mu$g of poly(A)$^+$ mRNA (Figure 1B). As expected, the hybridization of probes with higher specific activities (made using RSP) on cDNA microarrays (CLONTECH Laboratories, Palo Alto, CA, USA) resulted in the detection of low-abundance mRNAs at a shorter exposure time and with much less background in contrast to the hybridization with SR-RT-labeled cDNAs (Figure 2).

In conclusion, the RSP method described here allows for dramatically increased efficiency of cDNA radiolabeling. Because the use of equal amounts of probe activities as input for the differential hybridization on cDNA microarrays minimizes any bias in the representation of transcripts, it is suitable for the determination of relative expression ratios in differential screening experiments. Using the RSP-labeled probes, the resulting enhanced assay sensitivity at concomitantly low background facilitates the detection of differentially expressed rare transcripts on high-density hybridization targets. Therefore, RSP represents a versatile technique that is effective at low amounts of starting material and allows for use of remaining unlabeled first-strand cDNA in other applications (e.g., subtractive-suppression hybridization). Thus, RSP is an ideal alternative to SR-RT labeling.

REFERENCES


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Nonselective Colony-Color Assays for HIS3, LEU2, LYS2, TRP1 and URA3 in ade2 Yeast Strains Using Media with Limiting Nutrients


Selective growth media are widely used for phenotypic analysis in the yeast Saccharomyces cerevisiae, normally consisting of synthetic complete media lacking one or more amino acids (10). For analysis of recombination or mutagenesis, it is often advantageous to use nonselective assays because selection might prevent certain types of events from being scored. One type of nonselective assay involves the initial growth of colonies on nonselective (rich) medium, such as yeast extract peptone dextrose (YPD) medium, followed by replica-plating to selective medium to distinguish wild-type (WT) and mutant phenotypes (3,15,16). More rapid analysis is made possible if phenotypes can be scored using color markers, as these can be assayed without replica-plating. Colony-color assays have been used in studies of chromosome stability (5–8) with sectoring in colonies reflecting rates of marker loss. In recombination studies, segregation of mismatches in a color marker can yield half-sectored colonies, providing visual evidence of heteroduplex DNA (15).

The color markers used most often are those associated with the adenine biosynthetic pathway, including ADE1 and ADE2. Mutations in either of these genes produce red or pink colonies, while WT yeast colonies are white. We recently described growth medium, called LB-ura, that distinguishes WT URA3 strains from ura3 mutants in ade1 and ade2 backgrounds by colony color (14). On LB-ura plates, ura3 ade1 and ura3 ade2 colonies are white and slightly smaller than URA3 ade1 and URA3 ade2 strains, which appear red. Therefore, this medium allows Ura phenotypes to be distinguished without selection or replica-plating. For certain applications, this medium provides an inexpensive alternative to medium con-
taining 5-fluoro-orotic acid for identification of Ura strains (1). LB-ura medium is a synthetic complete medium lacking uracil (10) plus 3.4 g/L yeast extract, 6.8 g/L Bacto\textregistered-tryptone and 3.4 g/L NaCl. Although it was unclear why LB-ura plates distinguish Ura phenotypes, two clues suggested a possible explanation. First, the growth of Ura strains on LB-ura plates indicated that the LB was contributing uracil. Second, the somewhat slower growth rate of Ura vs. Ura\textsuperscript{+} strains, as evidenced by the smaller Ura colonies, suggested that the uracil concentration was not optimal for growth. We hypothesized that the reduced growth rate of \textit{ura}3 strains results in slower accumulation of the red pigment that produces the characteristic red colonies of \textit{ade}1 and \textit{ade}2 strains. Thus, we reasoned that the different colony colors on LB-ura were solely a consequence of limiting uracil.

To test this idea, we prepared synthetic complete media (Table 1) that either lacked uracil or was supplemented with increasing amounts of uracil. Final uracil concentrations ranged from 0–20 mg/L, the highest concentration being full-strength in synthetic complete medium. Because we had previously observed both colony-color and growth-rate differences on LB-ura medium, all plates in the present study were prepared with equal volumes of medium, so that growth rates would not vary due to differences in the amounts of available nutrients. Isogenic Ura\textsuperscript{+} and Ura\textsuperscript{-} strains (Table 2) were seeded to these plates, and both colony sizes and colors were scored following 2–3 days of growth at 30°C. As expected, the Ura\textsuperscript{+} strain produced normal-sized, red colonies at all uracil concentrations, and the Ura\textsuperscript{-} strain failed to grow in the absence of uracil. At very low uracil concentrations (0.2–0.4 mg/L), the Ura\textsuperscript{-} strain produced very small colonies, and colony size increased with increasing uracil. Interestingly, at an intermediate uracil concentration (2 mg/L), the Ura\textsuperscript{-} strain produced white colonies that were only slightly smaller than those of a Ura\textsuperscript{+} strain, but these were white (Figure 1B), similar to the results obtained with LB-ura plates. Above this concentration, Ura\textsuperscript{-} and Ura\textsuperscript{+} strains could not be distinguished; both produced red colonies. Table 3 summarizes this data, confirming our hypothesis that Ura phenotypes can be distinguished by using medium with limiting concentrations of uracil. We call the medium with 2 mg/L of uracil “semi-selective ura” (SS-ura).

We then reasoned that if colony-color differences on SS-ura plates simply reflect different growth rates due to limiting nutrients, it might be possible to distinguish phenotypes of other yeast markers (\textit{LEU2}, \textit{HIS3}, \textit{LYS2} and \textit{TRP1})...
using analogous semi-selective media. We chose these markers because of the wide availability of strains with mutations in these four genes (plus "URA3") and plasmids with complementing WT copies of these genes (11). The results with isogenic pairs of LEU2/leu2, HIS3/his3 and LYS2/lys2 strains paralleled those described above for "URA3/ura3" strains (Figure 1, A, C and D, respectively); recipes for SS-leu, SS-lys and SS-his media are given in Table 1. However, TRP1 proved more problematic. As with the other markers, both TRP1 ade2 and trp1 ade2 strains produced red colonies on synthetic complete medium (fully supplemented with tryptophan), and the TRP1 strain also produced red colonies without supplemental tryptophan. However, at low tryptophan concentrations (1/9 full-strength), the trp1 ade2 strain produced colonies that were distinctly smaller and not as red as TRP1 ade2 colonies (Figure 1E). At lower tryptophan concentrations, the growth rate was severely reduced, and the colonies were judged to be too small to be useful. Thus, SS-trp media might be used to distinguish TRP1 ade2 and trp1 ade2 strains, but this requires careful inspection of both colony colors and sizes.

The observed colony-color and size differences with limiting concentrations of uracil, histidine, leucine and lysine are consistent with the idea that slower growth rates of auxotrophs result in slower accumulation of red pigment in ade2 mutants. This is similar to the observation that petite ade2 strains, which have defective mitochondrial DNA function (10), also produce small white colonies. This could reflect a growth-rate effect or some other physiological difference between normal and petite cells. Auxotrophs give white colonies on semi-selective media after 2–3 days growth at 30°C; however, after 5 days, colonies develop a faint red color (data not shown), as was found with LB-ura medium (14). Thus, growth on semi-selective media does not completely block accumulation of the red pigment. Although limiting tryptophan also reduced the growth rate of the trp1 ade2 strain, the color difference was less dramatic, suggesting that factors other than growth rate might control or influence colony color. Because LB-ura provided color discrimination for ura3/URA3 strains in both ade2 and ade1 backgrounds (14), it is likely that the semi-selective media will also be useful with ade1 strains. On LB-ura, ade2 and ade1 strains with a variety of combinations of mutant and WT markers at other loci behaved similarly (14), indicating that the status of other markers has little or no effect on color phenotypes.

URA3 and LYS2 are particularly useful markers because forward selection (for URA3 or LYS2) is possible with standard selective media (10), and reverse selection (for ura3 or lys2) is possible using the drugs 5-fluoro-orotic acid and α-aminoadipate, respectively (1,2) These drug-based, negative selection systems are quite useful when ura3 or lys2 mutants are expected at low frequencies, but these systems have two drawbacks in that the drugs are both costly and mutagenic. In contrast, SS-ura and SS-lys media are made with inexpensive reagents, and they are non-

### Table 2. Yeast Strains

<table>
<thead>
<tr>
<th>Name</th>
<th>Relevant Genotype (all strains are ade2-101)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY3025</td>
<td>his3-200, LYS2, trp1-Δ1, leu2-Δ1, URA3</td>
<td>13</td>
</tr>
<tr>
<td>DY3026</td>
<td>his3-200, LYS2, trp1-Δ1, leu2-Δ1, ura3-X764</td>
<td>13</td>
</tr>
<tr>
<td>DY3029</td>
<td>his3-200, lys2-801, trp1-Δ1, leu2-Δ1, ura3-X764</td>
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</tr>
<tr>
<td>DY3052</td>
<td>his3-200, LYS2, TRP1, leu2-Δ1, ura3-X764</td>
<td>This study</td>
</tr>
<tr>
<td>JW3082</td>
<td>his3-200, LYS2, trp1-Δ1, ura3-X764:LEU2:ura3R-HO432</td>
<td>3</td>
</tr>
<tr>
<td>DY3411 b</td>
<td>his3-200, lys2-801, trp1-Δ1, LEU2, ura3-Δ</td>
<td>This study</td>
</tr>
<tr>
<td>DY3415 c</td>
<td>HIS3, lys2-801, trp1-Δ1, LEU2, ura3-Δ</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Chromosomal locus is trp1-Δ1 complemented with a TRP1/ARS1/CEN4 plasmid.
b Chromosomal URA3 completely deleted with replacement with pUC19:LEU2.
c Derivative of DY3411 with his3-200 complemented with a HIS3/ARS1/CEN4 plasmid.

dedefs

### Table 3. Colony Phenotypes of URA3 ade2 and ura3 ade2 Strains with Increasing Uracil Concentrations

<table>
<thead>
<tr>
<th>Uracil (g/L)</th>
<th>URA3 ade2 (DY3025)</th>
<th>ura3 ade2 (DY3026)</th>
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<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>Color</td>
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<tr>
<td>0</td>
<td>+++</td>
<td>Red</td>
</tr>
<tr>
<td>0.2</td>
<td>+++</td>
<td>Red</td>
</tr>
<tr>
<td>0.4</td>
<td>+++</td>
<td>Red</td>
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<tr>
<td>2</td>
<td>+++</td>
<td>Red</td>
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<td>4</td>
<td>+++</td>
<td>Red</td>
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<td>5</td>
<td>+++</td>
<td>Red</td>
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<td>10</td>
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<tr>
<td>15</td>
<td>+++</td>
<td>Red</td>
</tr>
<tr>
<td>20</td>
<td>+++</td>
<td>Red</td>
</tr>
</tbody>
</table>

a Cells were seeded to synthetic complete medium without uracil or supplemented with uracil as indicated.
b Growth rates estimated from colony sizes. Key: **+, normal growth; +, reduced growth; +, very slow growth and -, no growth.
c Uracil concentration in SS-ura medium.

This study
mutagenic. These media provide an alternative way to identify ura3 and lys2 mutants when they are expected at relatively high frequencies (>5 × 10^{-5}), as is typical during the pop-out step of in-out gene replacement (9). Thus, in-out gene replacement can be performed with SS-ura and SS-lys media in place of media with selective drugs. In addition, the other semi-selective media expand the range of markers that can be used in this technique. Because these new media are effectively nonselective, they allow one-step identification of mutants, such as during plasmid curing. These media also allow new markers to be used in

Figure 1. Color phenotypes of auxotrophic and prototrophic strains on semi-selective media. (A) LYS2/lys2 (strains DY3026/DY3029). (B) URAS3/ura3 (strains DY3025/DY3026). (C) LEU2/leu2 (strains JW3082/DY3026). (D) HIS3/his3 (strains DY3415/DY3411). (E) TRP1/trp1 (strains DY3052/DY3026). In DY3052 (Panel E, wild-type), the TRP1 gene is carried on an unstable circular plasmid, and the few small colonies reflect spontaneous loss of this plasmid and reversion to trp1. Also, the color difference between TRP1 and trp1 strains on SS-trp medium is more pronounced than it appears in Panel E.
other techniques and assays, such as chromosome stability assays (5–8), the analysis of telomere silencing or other position effects (4,12) and the detection of heteroduplex DNA (15).

When screening for colonies with a mutant phenotype, there is the potential for false positives. For example, petite prototrophs can yield small, white colonies. Some strains produce peteites at very high frequencies, but these can be easily distinguished from auxotrophs by their growth properties on selective media, and because peteites will not grow on media containing glycerol as the sole carbon source. An -other potential problem is that cells might switch from a red to white phe -notype through loss of other genes in strain. These false positives also can be identified by use of appropriate sele -

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Modification of Enzyme-Conjugated Streptavidin-Biotin Western Blot Technique to Avoid Detection of Endogenous Biotin-Containing Proteins

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Weintraub and D.E. Gottschling. 1998. Genomic protein. The streptavidin-HRP molecule binding sites per molecule. The streptavidin-biotin interaction, with a Kd of 10^-15 mol/L (4), is the strongest noncovalent physicochemical bond known. This strong interaction is advantageous in western blotting, where extensive and stringent washes are needed to obtain clear, unambiguous results reflecting only the specific interaction between primary antibody and target protein. Commercially available systems offer secondary antibodies with Fc portions that are conjugated to four biotin mole -cules. This allows, in theory, four strep -tavidin-HRP molecules to bind to the antibody, and sensitivity is increased fourfold compared to a secondary anti -body conjugated directly to HRP. Sensitivity is increased further by using a phenol-enhanced chemiluminescence technique for the final detection. Chemiluminescence results when HRP catalyzes the oxidation of luminol with consequent light emission, and a per -manent autoradiographic record of the results is created. Thus, quantification