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 radiolabeled cDNA of high specific activity was recovered with $3–8 \times 10^9$ specific dpm per µg 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 hundredfold when calculated per µg 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.

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

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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-

Table 1. Recipes for Synthetic Complete and Semi-Selective Media

	Concentration (mg/L) in:					
Component	**Complete**	**SS-his**	**SS-leu**	**SS-lys**	**SS-trp**	**SS-ura**
Histidine	20	1	20	20	20	20
Leucine	100	100	40	100	100	100
Lysine	50	50	50	10	50	50
Tryptophan	50	50	50	50	5.5	50
Uracil	20	20	20	20	20	2

Synthetic complete medium is a modification of that described by Sherman (10). In addition to the listed ingredients, each growth medium also contains 1.7 g/L yeast nitrogen base without amino acids and $(NH_4)_2SO_4$, 5.0 g/L $(NH_4)_2SO_4$, 10 mg/L adenine sulfate, 50 mg/L arginine, 80 mg/L aspartic acid, 20 mg/L methionine, 50 mg/L phenylalanine, 100 mg/L threonine, 50 mg/L tyrosine, 50 mg/L isoleucine, 140 mg/L valine and 22 g/L agar. This version of synthetic complete medium does not contain glutamic acid or serine, as the cognate markers are rarely mutated in laboratory strains.

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™-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$^+$ 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 *ura3* strains results in slower accumulation of the red pigment that produces the characteristic red colonies of *ade1* and *ade2* 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$^+$ and Ura$^-$ 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$^+$ strain produced normal-sized, red colonies at all uracil concentrations, and the Ura$^-$ strain failed to grow in the absence of uracil. At very low uracil concentrations (0.2–0.4 mg/L), the Ura$^-$ strain produced very small colonies, and colony size increased with increasing uracil. Interestingly, at an intermediate uracil concentration (2 mg/L), the Ura$^-$ strain produced white colonies that were only slightly smaller than those of a Ura$^+$ strain, but these were white (Figure 1B), similar to the results obtained with LB-ura plates. Above this concentration, Ura$^-$ and Ura$^+$ 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 (*LEU2, HIS3, LYS2* and *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

Name	Relevant Genotype (all strains are *ade2-101*)	Reference
DY3025	*his3-200, LYS2, trp1-D1, leu2-D1, URA3*	13
DY3026	*his3-200, LYS2, trp1-D1, leu2-D1, ura3-X764*	13
DY3029	*his3-200, lys2-801, trp1-D1, leu2-D1, ura3-X764*	13
DY3052[a]	*his3-200, LYS2, TRP1, leu2-D1, ura3-X764*	This study
JW3082	*his3-200, LYS2, trp1-D1, ura3-X764:LEU2:ura3R-HO432*	3
DY3411[b]	*his3-200, lys2-801, trp1-D1, LEU2, ura3-D*	This study
DY3415[c]	*HIS3, lys2-801, trp1-D1, LEU2, ura3-D*	This study

[a]Chromosomal locus is *trp1-D1* complemented with a *TRP1/ARS1/CEN4* plasmid.
[b]Chromosomal *URA3* completely deleted by replacement with pUC19:*LEU2*.
[c]Derivative of DY3411 with *his3-200* complemented with a *HIS3/ARS1/CEN4* plasmid.

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

	***URA3 ade2* (DY3025)**		***ura3 ade2* (DY3026)**	
Uracil (g/L)[a]	**Growth**	**Color**	**Growth[b]**	**Color**
0	+++	Red	-	-
0.2	+++	Red	+	White
0.4	+++	Red	+	White
2[c]	+++	Red	++	White
4	+++	Red	++	Red
5	+++	Red	++	Red
10	+++	Red	+++	Red
15	+++	Red	+++	Red
20	+++	Red	+++	Red

[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.

mutagenic. These media provide an alternative way to identify *ura3* and *lys2* mutants when they are expected at relatively high frequencies ($>5 \times 10^{-5}$), 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

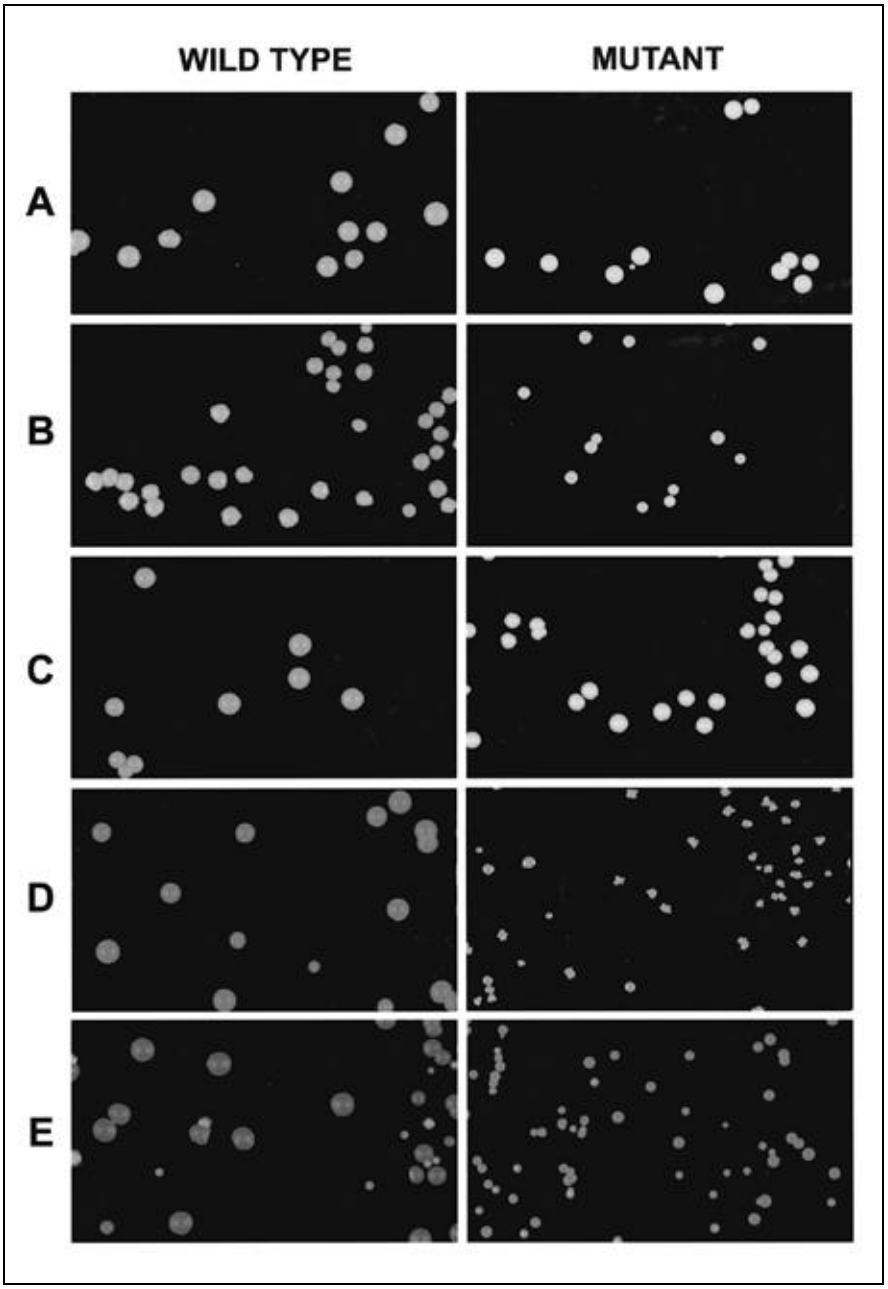

Figure 1. Color phenotypes of auxotrophic and prototrophic strains on semi-selective media. (A) *LYS2/lys2* (strains DY3026/DY3029). (B) *URA3/ura3* (strains DY3025/DY3026). (C) *LEU2/leu2* (strains JW3082/DY3026). (D) *HIS3/his3* (strains DY3415/DY3411). (E) *TRP1/trp1* (strains DY3052/DY3026). In DY3052 (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 petites at very high frequencies, but these can be easily distinguished from auxotrophs by their growth properties on selective media, and because petites will not grow on media containing glycerol as the sole carbon source. Another potential problem is that cells might switch from a red to white phenotype through loss of other genes in the adenine biosynthetic pathway, such as *ADE3*. These false positives also can be identified by use of appropriate selective media.

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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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Western blot analysis is a widely used method for detection of specific proteins in a variety of systems. The proteins are separated by gel electrophoresis and transferred to a suitable support membrane. The membrane is then probed with specific antibodies, which are in turn detected through reaction with secondary antibodies usually conjugated to horseradish peroxidase (HRP). A colorimetric reaction between the peroxidase and a chromogenic substrate results in a blue color at the location of the specific protein band. The introduction of biotinylated secondary antibodies, streptavidin directly conjugated to HRP (streptavidin-HRP) (6) and enhanced chemiluminescence (12) has resulted in a large increase in the sensitivity of detection. Streptavidin has four biotin binding sites per molecule. The streptavidin-biotin interaction, with a K_d 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 molecules. This allows, in theory, four streptavidin-HRP molecules to bind to the antibody, and sensitivity is increased fourfold compared to a secondary antibody 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 permanent autoradiographic record of the results is created. Thus, quantification