Materials List for:

Generation of RNA/DNA Hybrids in Genomic DNA by Transformation using RNA-containing Oligonucleotides

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Materials

A. Transformation reagents and media (modified from Storici and Resnick, 2006

1. YPD (Yeast Peptone Dextrose): For 1 L, 10 g yeast extract, 20 g soy peptone, 20 g dextrose. Add 15 g agar to make YPD solid media. Autoclave before use. Store at room temperature.
2. Solution 1: 0.1 M of lithium acetate. Prepare immediately before transformation. Solution 1 is a working solution, thus it is prepared directly from the powder. No stock solution is made. Keep at room temperature. LiAc increases the yeast cell wall permeability to DNA.
3. Solution 2: 0.1 M of lithium acetate and 50 % of polyethylene glycol 4000. Also solution 2 is a working solution and it is made directly from the powder. No stock solution is prepared. Keep at room temperature. PEG deposits oligos onto yeast cells.
4. RNA-containing oligos (Thermo Scientific Dharmacon), 50-80-mers, desalted, deprotected and non-purified. Resuspend to 250 pmoles/µl. Store at -80 °C.
5. DNA-only oligos, 50-80-mers, desalted and non-purified. Resuspend to 50 pmoles/µl. Store at -20 °C.
6. SC-Trp (Synthetic complete media lacking tryptophan) solid media.
7. 0.5 cm diameter glass beads, sterilized by autoclaving.
8. RNase-off: RNase decontamination solution.
9. DNase/RNase-free, sterile centrifuge tubes.
10. DNase/RNase-free, sterile conical tubes.
11. DNase/RNase-free, sterile aerosol pipette tips with ZAP: 1-200 ml, 100-1000 ml.

B. Colony PCR materials.

1. DNA primers, desalted and non-purified. Dissolve in sterile water to 50 pmoles/µl. Store at -20 °C.
2. Taq DNA polymerase, buffer, dNTPs.
3. PCR tubes.

C. PCR purification.

1. PCR purification kit.

D. Gel Electrophoresis.

1. Agarose.
2. 1 x TBE running buffer (45 mM Tris-borate and 1 mM ethylenediamine tetraacetate) diluted from 10x TBE.
3. Prestained molecular weight marker.
4. DNA loading dye.

E. Restriction digestion.

1. Restriction enzymes, 10x buffers, BSA.

F. Alkali treatment for the RNA-containing oligo.

1. 1 M of NaOH solution.
2. 1.2 M of HCl solution.

3. 1 M of Tris-HCl, pH 7.4 solution.