Hybrid selection assay

General considerations:

Estimate abundance of your RNA in the tissue/cell of origin. Hybrid selection will work well for RNAs representing >0.1 % of total poly A^+-RNA. If it is rarer, increase concentration of poly A^+-RNA to >500 µg/ml. In order to detect a translation product from a hybrid selected RNA, about 10 ng of this RNA are required. It is useful to choose a tissue in the expression of this RNA is maximal.
As controls, include: plasmid without insert, cDNA coding for a prominent RNA (eg. actin). For in vitro translation, always translate an aliquot of same RNA used in hybrid selection assay.

- Make sure all reagents all of very high purity and free of RNAse.

- Linearize plasmid with a uniquely cutting enzyme. If using lambda DNA or PCR fragments no linearisation is required. About 2-4 µg of specific DNA is sufficient which means in case of lambda you need quite a lot of DNA. Keep in mind that nitrocellulose binds ~80 µg of DNA per cm^2. Include a positive and a negative control template (see above).
- Adjust DNA concentration to 0.5 µg/µl in TE. Denature by heating to 95 °C for 10 min and snap-cool on ice. Add 0.1 vol of 10 M NaOH and incubate at RT for 5 min. Add 0.5 vol of 20 x SSPE and place on ice immediately.

- Spot DNA in 20-30 µl aliquots onto 13 mm nitrocellulose filters sitting on clean 3MM paper. Let filters air-dry in between.

- Wash dried filters 2 x in Falcon tube in 30 ml of 6 x SSPE (can be done in batch if different templates are tested). Place filters on 3 MM paper, and dry. Fix DNA by baking filters at 80°C for 60-90 min. Keep filters separate on 3MM paper while baking.

- Wash filters twice with 20 ml of boiling water for 1 min each in Falcon tube. Remove excess water and place filters individually in 24 well microtiter plate.
- Add 400 µl prehybridization solution per filter. Prehyb. is 65 % formamide, 0.4 M NaCl, 20 mM Pipes-KOH pH 7.4, 0.4 % SDS, 250 µg/ml herring sperm DNA. Before closing lid place clean sheet of Nesco film over wells. Incubate at 42 °C for 30-60 min.

- Remove prehybridization and add 100-200 µl of hybridization solution: as above, plus 200 µg/ml poly A^+-RNA or more (total RNA can be used at 2-5 mg/ml). Before adding hybridization solution heat 10 min at 70°C and snap-cool on ice. Incubate for 3 hrs at 50 °C (stringent condition, lower temperature and/or formamide concentration may be used for cross-reactive conditions. RNA conc. may be adjusted as well).

- Wash filters 10 x with 0.1 x SSPE, 0.1 % SDS at 68 °C, followed by 3 washes in 0.1 x SSPE at 68 °C. Perform washes in batch in Falcon tube using appr. 30 ml of buffer each time. SSPE must be pre-warmed. Washing conditions may be altered, example is for stringent conditions.

- Dry filters individually on 3 MM paper and place in Eppendorf tubes. To each filter add 400 µl of TE and 1 µl of glycogen. Release bound RNA by heating at 95 °C for 90 sec. Snap-cool on ice and transfer spnt. into new tube. Add 0.1 vol of 3 M NaAc pH 5.5 and 2 vol of EtOH. Mix and spin in benchtop for 15 min full speed. Discard spnt and wash pellet once with 400 µl of 70 % EtOH. Discard and air-dry pellet.
- Resuspend RNA in 5-8 µl water and use half for in vitro translation in reticulocyte lysate or for cDNA synthesis.
- Use ultrapure Sigma water.