raPOOLs

Robust & Targeted RNA Capture

www.sitoolsbiotech.com
info@sitools.de

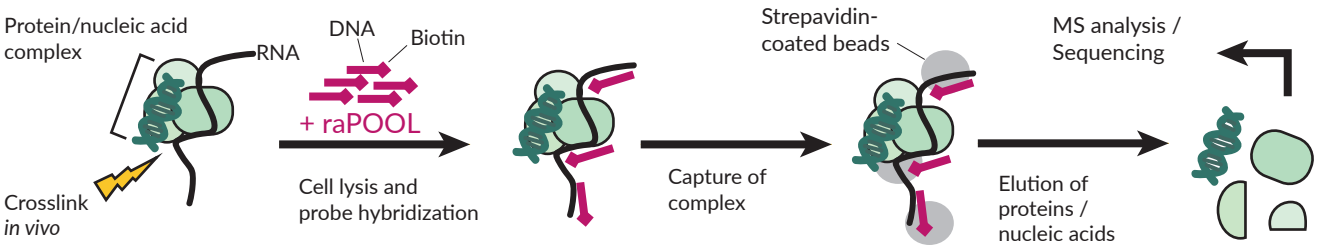
Principle

RNA antisense pools (raPOOLs) enable robust, specific isolation of targeted RNA from cell lysates. They are highly complex, optimally designed sets of **biotinylated DNA probes**, each consisting of 30 3'-biotinylated sequences. Their high-complexity pooling provides maximum coverage of the target RNA for strong enrichment efficiency. The workflow is compatible with cross-linked samples, allowing isolation as well as identification and characterization of nucleic acids and proteins interacting with the target RNA.

raPOOL Benefits

- ✓ highly efficient and specific targeting
- ✓ customizable for every RNA
- ✓ maximizes target RNA enrichment
- ✓ HPLC-purified - low risk of contaminants
- ✓ reproducible and consistent level of RNA enrichment

raPOOL Workflow



Applications

RNA affinity purification

- long non-coding RNAs
- premature/messenger RNAs

RNA interactome

- RNA binding proteins (RBPs)
- interacting RNA and DNA

Available Formats

Kit (incl. probes, beads, and buffers)

6 rx	12 rx	24 rx

Probes alone with nuclease-free water

2 nmol	5 nmol	10 nmol

Use Case 1: 1Enrichment of human MALAT1

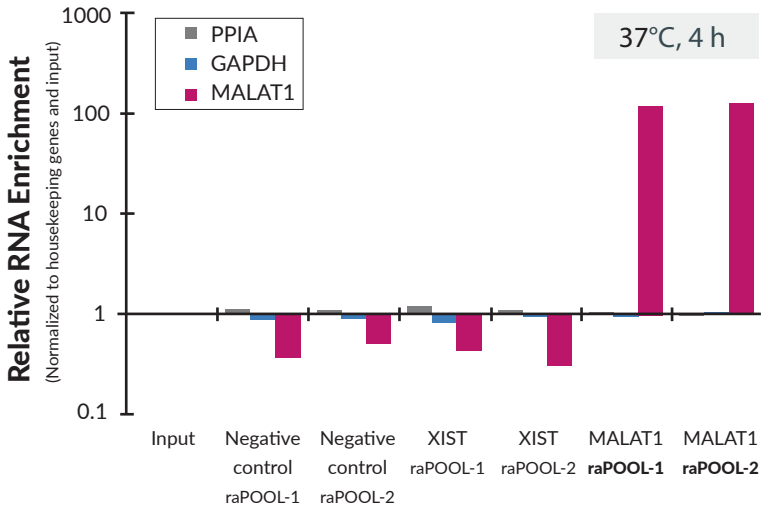


Figure 1: Real-time qPCR quantification of MALAT1 (targeted RNA), PPIA (control) and GAPDH (control) RNA in Hela S3 cell lines after pull down with MALAT1, XIST and negatice control raPOOLs.

HeLa S3 (human) cell lysates were incubated with different raPOOLs against:

- MALAT1 (targeted RNA)
- XIST (control)
- E. coli LacZ (neg. control)

Both raPOOLs against MALAT1 resulted in a **100-fold enrichment of target RNA**, showing that raPOOL capture leads to reproducible pulldown of target RNA.

Use Case 2: lncRNA interactome

A siRNA screen targeting lncRNAs deregulated in cancer, found *LINC00152,* a lncRNA with upregulated expression levels in different cancer types, to affect cell morphology and cell division.

Pulldown of *LINC00152* in Hela cells and identification of its associated proteins by mass spectrometry, revealed that *LINC00152* interacts with a network of proteins associated with the M phase of the cell cycle. This suggests that this lncRNA is crucial for cell cycle progression through mitosis and thus, could act as a non-codig oncogene.

Nötzold et all, Scientific Reports, 2017

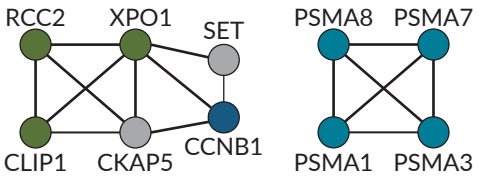


Figure 2: Interaction networks between identified proteins associated with M phase as revealed by the String database. Proteins had a function in microtubule cytoskeleton organization (green nodes) and ubiquitin-protein ligase activity (cyan nodes), with cyclin B (CCNB1) being involved in both (blue).

Use Case 3: Ribosome Heterogeneity

A new highly-divergent type (F-type) of rRNA was identified, that is expressed during the early stages of parasite development in malaria mosquitos.

A custom raPOOL probe set was produced to specifically pull-down down F-type rRNA from *P. falciparum* cell lysates.

Initial validation via qPCR showed a > 30x enrichment of F1 rRNA after pull-down, confirming raPOOL efficiency.

Sebastian Baumgarten, Parasite RNA Biology lab, Institut Pasteur, Paris, France

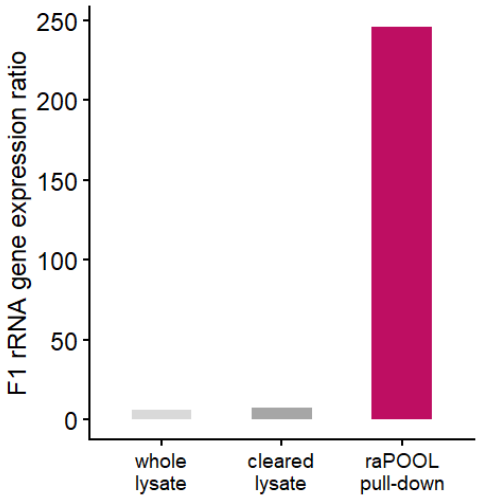


Figure 3: Expression of F-type 28S rRNA (F1) in cell lysates before and after raPOOL enrichment. Reference gene for normalization: seryl-tRNA synthetase.