

Monitoring Protein-RNA Interaction Dynamics in vivo at High Temporal Resolution using χ CRAC

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Materials

| Name | Company | Catalog Number | Comments |
|--|--------------------------|----------------|--|
| 1,4-dithioereitol | Merck | 10708984001 | Buffer component in mammalian cell lysis |
| 1.5 mL tubes | Eppendorf | 0030 120.086 | General reaction tube |
| 2 mL tubes | Eppendorf | 0030 123.344 | For holding columns and collection of waste |
| 32P-yATP | Perkin Elmer | NEG502Z-250 | For radiolabelling the 5' end of the RNA |
| 4-12% Bis-Tris gel | Invitrogen | NP0321BOX | SDS-PAGE gel |
| 4X loading buffer | Novex | NP0008 | Protein loading dye concentrate |
| 50 bp ladder | New England Biolabs | N3236 | Reference ladder for excising region of interest from the amplified cDNA library |
| 50% PEG | NEB | B100045 | For the L5 linker ligation |
| 6% TBE gel | Invitrogen | EC6265BOX | For separation and purification of the cDNA library |
| Acetone | ACROS Organics | 423245000 | Washing of TCA-precipitated proteins |
| anti-FLAG beads | Sigma Aldrich | M8823-1ML | For purification of FLAG-tagged RBPs |
| ATP (100 mM) | Thermo Fisher Scientific | R0441 | For ligation of the L5 linker onto the 5' end of captured RNAs |
| Beta-mercaptoethanol | Sigma Aldrich | M3148-100ML | Buffer component |
| Biomax MS intensifying screen | Sigma Aldrich | Z363162-1EA | For intensifying the autoradiogram signal |
| Chloroform | Thermo Fisher Scientific | 1010219 | For phenol-chloroform extraction following RNA purification |
| cOmplete EDTA-free protease inhibitor cocktail | Roche | 11873580001 | For inhibition of cellular proteases after lysis |
| Complete supplement mixture -TRP | Formedium | DCS0149 | For preparation of synthetic defined medium |

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| Costar Spin-X 0.22 µm filters | Sigma Aldrich | CLS8160 | For isolating the excised cDNAs following gel extraction |
| DNase RQ1 | Promega | M6101 | For DNA digest following cell lysis |
| dNTPs (10 mM) | Sigma Aldrich | 4638956001 | For reverse transcription and PCR |
| Ethanol | Thermo Fisher Scientific | 10041814 | For phenol-chloroform extraction following RNA purification and DNA precipitation |
| Ethylenediaminetetraacetic acid | Invitrogen | AM9261 | For protease K buffer |
| Exonuclease I | New England Biolabs | M0293 | For degradation of primers following PCR |
| Glass microfiber filters | Whatman | 1823-010 | For isolating the excised cDNAs following gel extraction |
| Glucose | Formedium | GLU03 | For preparation of glucose-containing, synthetic defined medium |
| Glycogen (20 mg/mL) | Roche | 10901393001 | Precipitation of proteins, RNA and DNA |
| GST-TEV | Homemade | | Construct and purification protocol is available upon request |
| Guanidium hydrochloroide | Thermo Fisher Scientific | 10071503 | Required for pulldown denaturing conditions and washing buffer |
| IgG beads | GE Healthcare | 17-0969-01 | For purification of protein A-tagged RBPs |
| Imidazole | Sigma Aldrich | I2399-100G | For elution of captured proteins from Nickel beads |
| Isoamyl alcohol | Thermo Fisher Scientific | A393-500 | For phenol-chloroform extraction following RNA purification |
| Luna Universal One-Step RT-qPCR | NEB | E3005S | For qPCR of the cDNA in order to calculate required number of PCR cycles |
| Magnesium chloride | Fluka Analytical | 63020-1L | For PNK buffer |
| Membrane filters | Millipore | AAWP09000 for yeast or HAWP09000 for bacteria | For vacuum filtration of cells |
| Micro bio-spin columns | Biorad | 732-6204 | For collecting eluate after gel extraction |
| Ni-NTA beads | Qiagen | 30210 | For secondary protein capture |
| NP-40 | Sigma Aldrich | I8896-100ML | Buffer component |
| Pfu polymerase | Promega | M7741 | For amplification of the cDNA library |
| Phenol | Sigma Aldrich | P4682-400ML | For phenol-chloroform extraction following RNA purification |
| Pierce spin columns | Thermo Fisher Scientific | 69725 | For on-column enzymatic reactions |
| Protease K | Roche | 3115887001 | For degradation of the RBP following gel extraction |
| Quartz Petri dish | UVO3 | N/A | For cross-linking of adherent cells. Available from https://www.vari-x-link.com for 400 GBP |
| Radiography films | Amersham | 28906843 | For autoradiography visualisation |
| RNAClean XP beads | Beckmann | A63987 | SPRI beads for clean up of RNAs and cDNAs |
| RNase H | New England Biolabs | M0297 | For degradation of RNAs following reverse transcription |
| RNase-It | Agilent | 400720 | For RNA digestion |
| rRNasin | Promega | N2511 | For inhibition of any contaminating RNases during enzymatic reaction |
| Sodium acetate | Sigma Aldrich | S2889-1KG | For phenol-chloroform extraction following RNA purification and DNA precipitation |

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| Sodium chloride | Thermo Fisher Scientific | 7647-14-5 | Buffer component |
| Sodium deoxycholate | Sigma Aldrich | D6750-100G | Buffer component in mammalian cell lysis |
| Sodium dodecylsulfate | Sigma Aldrich | L3771-1KG | For protease K buffer |
| SUPERase-In | Invitrogen | AM2694 | For inhibition of cellular RNases after lysis |
| SuperScript IV | Thermo Fisher Scientific | 18090010 | For reverse transcription |
| T4 PNK | New England Biolabs | M0201 | For radiolabelling the 5' end of the RNA |
| T4 RNA ligase 1 | New England Biolabs | M0204 | For ligation of the L5 adaptor onto the RNA 5' end |
| T4 RNase ligase 2, truncated K222Q | NEB | M0351S | For ligation of the App_PE linker onto the 3' end of captured RNAs |
| TBE buffer (10X) | Invitrogen | 15581-028 | For running TBE gels |
| TEV protease | Homemade | | For eluting captured proteins following FLAG capture |
| Thermosensitive alkaline phosphatase | Promega | M9910 | For 5' and 3' dephosphorylation of RNAs |
| Trichloroacetic acid (100%) | Sigma Aldrich | T0699-100ML | For precipitation of RBP-RNA complexes |
| Tris hydrochloride | Invitrogen | 15504-020 | Buffer component |
| Triton X-100 | Sigma Aldrich | T8787-100ML | Buffer component in mammalian cell lysis |
| Vari Filter | UVO3 | N/A | Device for vacuum harvesting cells. Available from https://www.vari-x-link.com for 100 GBP |
| Vari-X-Linker | UVO3 | N/A | Cross-linker for cross-linking cells. Available from https://www.vari-x-link.com for 16,000 GBP |
| Yeast nitrogen base | Formedium | CYN0410 | For preparation of synthetic defined medium |
| Zirconia beads | Thistle | 11079105Z for yeast or 11079101Z for bacteria | For cell lysis via bead beating |