Mammalian STARR-seq Screening protocol

Recommended:
For BAC screens : T-225 flasks (Thermo Scientific; Cat No. 159934)
For genome-wide screens: Square plates (Thermo Scientific; Cat No. 166508)

Required:
C16 inhibitor targeting PKR (Sigma-aldrich; Cat No. I9785-5MG)
BX-795 inhibitor targeting TBK1/IKK (Sigma-aldrich; Cat No. SML0694-5MG)

1x PBS (Autoclaved), 1x Trypsin (Gibco; Cat No. 25300 054)

DMEM (Gibco; cat. no. 52100-047)
+ 10% heat-inactivated FBS (Sigma; Cat No. F7524)
+ 2 mM L-glutamine (Sigma; Cat No. G7513)

*Electroporation device: **MaxCyte** STX scalable transfection system*
Electroporation buffer (MaxCyte; Cat No. EPB1)
*Clinical Processing Assembly, **OC-100** 100-µL (MaxCyte; Cat No. GOC1)*
*Clinical Processing Assembly, **OC-400** 400-µL (MaxCyte; Cat No. GOC4)*

Focused (BAC) screen:
Grow cells and **split them 24 h before** transfection to reach **8x 10^7 cells** on electroporation day; (e.g. HeLa S3, 2 square plates, 4x10^7 cells/square plate)

Genome-wide screen:
Grow cells and **split them 24 h before** transfection to reach **8x 10^8 cells** on electroporation day; (e.g. HeLa S3 , 25 square plates , 4x10^7 cells/square plate)

Prepare before transfection:
1x / 4x T225 flasks at 37°C, 2 / 7 square plates at 37°C, and 1mM stocks of the C16 and BX-795 inhibitors at -20°C

Electroporation of mammalian cells using the MaxCyte STX transfection system

- Remove medium from cells completely
- Wash cells carefully with 10 ml PBS, remove PBS completely
- Add 1x Trypsin to the cells to cover the plate/flask completely (Square plate: 8ml Trypsin)
- Incubate cells on 37°C / 4-5 min (or until cells completely detach)
- Add 12 ml medium to the cells, resuspend by pipetting up and down thoroughly
- Collect cells in a T-225 flask
- Count cells and use 8x 10^7 / 8x 10^8 cells for the transfection (it is possible that the amount of medium & cells for a gw-screen will not fit in 1x T225 flask, use 2x T225 flasks and measure each flask separate
- Spin the cells down (in 50 ml Falcon tubes) at 125xg for 5 min
- Remove the medium, resuspend pellets in 5 ml Electroporation buffer and pool all

pellets in a 50 ml falcon tube (do not exceed ~40 ml if you have many pellets, reuse cell suspension of one pellet to resuspend the next if necessary)

- Spin the cells down 125xg, 5 min
- Remove medium and add 500 µl / 5 ml Electroporation buffer to the cells (after resuspension of the cell pellet the volume is around 650 µl/ 7 ml)
- Resuspend the cells and add appropriate amount of library to the cells (library concentration should be > 1.0 µg/ µl)
    - *Use 80 µg library per 4x 10^7 cells in 400 µl Electroporation buffer per OC-400 cuvette*
    - *for a focused screen 8x10^7 cells are needed, (2x OC-400 cuvettes*
    - *for a genome-widew-screen 8x10^8 cells are needed, (20x OC-400 cuvettes)*
- Measure volume and fill resuspension up to 800 µl/ 8 ml total with Electroporation buffer
- Add 400 µl cell suspension/ DNA mix per OC-400 cuvette and electroporate using the cee-type specific pre-set protocols or optimization protocol (if no pre-set protocol is available for your cell line use the best fitting optimization protocol for your cell line) MaxCyte STX scalable transfection system
- Put 2x / 10x EPOs into 1x pre-warmed T225 flask, **without adding medium**
- Put cells into the incubator, 37°C for 30min
- Repeat with the additional 10 EPOs ands put them T225 flasks → 37°C for 30min
- After 30 min add 100ml/ 350ml medium **without antibiotics** on the cells
- Start counting the **6 hours incubation time** after medium has been added to the cells (count 6 h until cells lysis during total RNA extraction; see below)
- For cells with Interferon (INF) response add 100 µl or 350 µl of a **1 mM** stock of the C16 and BX-795 inhibitors to the cells (final concentration 1 µM/ inhibitor).
- Don't pipette cells up and down BUT close flasks and shake them mix with inhibitors
- Take out 2x / 7x 50ml and put cells into the pre-warmed 2 / 7 square plates or flasks

Harvesting of mammalian cells 6 hours post electroporation

Note:

Harvesting of adherent cells, especially for a genome-wide screen can take up to 1.5h → start therefore early to stay in the 6h time window, ideally work together with a partner

- Harvest the medium of the cells (20% of cells are still not fully attached to the plates), add 10 ml PBS, harvest PBS, add 8 ml of 1x Trypsin, put cells in incubator 37°C wait 4-5 min, then add 12 ml of medium (to save some medium: Re-use the pre-harvested medium to inactivate the trypsin), resuspend thoroughly and harvest
- Spin the cells down (in 50ml Falcon tubes) at 125xg for 5 min
- Remove medium
- Wash cells once with 10 ml/ 40 ml 1x PBS
- Spin the cells down (in 50 ml Falcon tubes) at 125xg for 5 min
- Remove 1x PBS but leave a rest of the 1x PBS (0.5 ml/ 1 ml) to cover the cells in the 50 ml falcon
- Resuspend the cells in the 1x PBS by softly flicking on the side of the 50 ml falcon

Total RNA Purification (RNeasy Maxi Kit, Qiagen; Cat No. 75162)

Prepare before RNA purification:
Work for this step in in a RNAse free fume-hood to avoid β-Mercaptoethanol exposure;
Tissue Ruptor (Qiagen; Cat No. 9001271), RNase Zap (Ambion; Cat No. AM9780)
→ clean all equipment with RNAse Zap

prepare
15 ml RLT (in the RNeasy Maxi Kit) buffer
15 ml 70% ethanol per cell pellet (8x 10^7- 5x 10^8)

- add **150 µl β-Mercaptoethanol (β-ME)** to **15 ml RLT** buffer
- add 4 volumes of 100% **ethanol** to RPE buffer
- prepare **70% ethanol** (dilute with **DEPC-treated sterile MonoQ water**)

Try to keep everything RNase free

- add **15 ml RLT** (+**β-ME**) buffer **while** vortexing to the resuspended pellet
- disrupt and homogenize the cells with TissueRuptor for **4.5 min** per cell pellet
- add **15 ml 70% ethanol** to the lysate, shake/mix vigorously (20'')
- transfer to RNeasy Maxi column (max. volume 15ml) centrifuge for **5 min** at >3200xg, RT, discard the flow-through
- repeat with the remaining 15ml lysate/EtOH mix
- add **15 ml RW1 buffer**, centrifuge for **5 min** at > 3200xg, 25°C, discard the flow-through
- add **10 ml RPE buffer**, centrifuge for **2 min** at > 3200xg, 25°C, discard the flow-through
- add **10 ml RPE buffer**, centrifuge for **10 min** (to dry the membrane) at > 3200xg, 25°C, discard the flow-through
- take new tubes and elute **3x** in RNase free H_2O: 1st in **1.2 ml,** 2nd in **1 ml**, 3rd in **0.5 ml** -> incubate for 2 min and centrifuge for 5 min

If concentration is expected to be low e.g for focused screens -> elute in 0.5 ml steps only
If concentration after 3rd elution > 300 ng -> elute further in 0.5 ml steps

- if using more than one column per sample purification (typically for genome-wide screens), pool same elution fractions to obtain pools of the 1st, 2nd and 3rd elution steps
  -> measure RNA concentration of **pooled elution fractions**
  -> mix 1st, 2nd and 3rd elution fractions to obtain a pool with ~750 ng/µl (*that's the max. concentration you can use in the next step*)
- Samples can be stored at -80°C
  - safe 10 µl for gel analysis to determine integrity of RNA (*check for degradation*)

Oligo-dT mRNA Isolation (Dynabeads Oligo(dT)$_{25}$, Invitrogen; Cat No. 61005)

Prepare before starting

Binding buffer
Washing buffer
Storage buffer

warm all buffers up to room temperature (RT) (buffer composition are on page 12)

you need *(referring to starting volume of beads)*:
Binding Buffer: 2.5x **Washing Buffer: 2x** **Storage Buffer: 1x**

Prepare RNA for binding to Dynabeads
*The maximum concentration of RNA is **750 ng/µl***

For 15 ml tubes (polystyrene tubes Cat No. 05-527-90)

- heat total RNA for **12 min at 65°C**, place **on ice** immediately for **5 min**, wait for **1 min**
  at room temperature

For 1.5 ml DNA LoBind Tubes (Eppendorf; Cat No. 0030108051)

- heat total RNA for **7 min at 65°C** and incubate **for 3 min on ice; 1 min, room temperature**

Oligo-dT selection of mRNA with Dynabeads

*Use **2x vol.** of Oligo(dT)$_{25}$ beads for **1x vol.** of total **RNA** solution*
Do not vortex as long as RNA is bound to beads!
Oligo dT$_{25}$ beads are re-usable (see page 12 for recovery protocol). Use beads max. 8 times

- **resuspend** the Dynabeads Oligo(dT)$_{25}$ beads thoroughly by vortexing to obtain a uniform brown suspension, distribute beads to appropriate tube & place the tube on a **magnet**
- remove **Storage Buffer** completely from beads
- wash beads **2x** with **2x Binding Buffer** (same volume like beads starting volume) -> after adding buffer -> vortex and incubate beads on a magnetic separator for **1.5 min** (until solution is clear)
- resuspend the beads in **½ volume** *(referring to starting volume of beads)* of **2x Binding Buffer**
- add **1 vol. total RNA** solution – **to 1 vol. resuspended beads > mix gently** (pipet up and down)
- incubate on a rolling shaker for **10 min at RT**
- place the tube on the **magnet for 2 min** and completely **remove supernatant**
- **carefully** wash the beads **2x** with **Washing Buffer B** (use same volume as beads starting volume) -> after adding buffer mix gently by inverting the tube (**no vortex**) and incubate for **1.5 min** on the magnetic separator

- before elution spin tube very shortly and remove the remaining buffer on the magnet
- elute by adding **50 µl** + **40 µl** 10 mM **Tris-HCl** per **1 ml beads** (*starting volume*); vortex
  *-> place beads on **80°C** heat block **for 3 min, 750rpm**; transfer tubes **immediately** to the magnet and incubate for **1 min** -> transfer supernatant to a new RNase-free tube*
- pool all 1st and 2nd elutions from same sample
- measure RNA concentration of final pool
- add **Storage Buffer** (same volume as starting volume of beads) to used beads -> store beads at **4°C**
- **safe 10 µl of pooled elution fractions for gel analysis**
  - based on determined RNA concentration load appropriate amounts on gel (do not load more than 1 µg of RNA per lane)

Turbo DNaseI Digest

Turbo DNase I (Ambion; Cat No. AM2238) is sensitive to mechanical stress! Do not vortex.
Process the entire mRNA, scale up number of reactions accordingly.
For one reaction:

88 µl mRNA (max. 200ng/µl)
10 µl Turbo DNase Buffer
2.4 µl Turbo DNase I
-> make **Master Mix,** invert **gently,** distribute 100µl MM to PCR-stripes
incubate **at 37°C for 30 min at 37°C; 4°C for ∞**

Clean up with Qiagen RNeasy MinElute clean up kit (Cat No. 28204)

(*to inactivate TurboDNaseI and to concentrate the RNA*)
Prepare 80% EtOH (with DEPC water) and 100% EtOH
DNA LoBind Tubes 2 ml (Eppendorf; Cat No. 0030108078)

- pool **2 DNase rxns** (or fill up with water to **200 µl**) in 2ml LoBind tubes
- add **700 µl RLT buffer,** vortex
- add **500 µl 100% EtOH,** vortex
- transfer to RNeasy MinElute spin column (max. vol. 750µl)
- centrifuge at **12000xg**, **30 sec** in a table top centrifuge and discard the flow-through
- place RNeasy MinElute spin column in a new **2 ml** collection tube (provided in kit)
- add **500 µl RPE buffer**, centrifuge at **12000xg**, **30 sec** and discard the flow-through
- add **500 µl 80% EtOH**, centrifuge at **12000xg**, **2 min** and discard the flow-through
- place RNeasy MinElute spin column in a new **2 ml collection tube** (provided in kit)
- to dry the membrane, centrifuge **5 min at full speed with open lid**
- take elution tube (from kit)-> elute in **20 µl + 10 µl RNase-free H_2O**
  - incubate for 1 min and centrifuge for 3 min at full speed
- pool eluates from all columns
- measure RNA concentration of final pool
- measure volume of final pool

- **safe 5 µl of final pool for gel analysis**
  based on determined RNA concentration load appropriate amounts on gel (do not load more than 1 µg of RNA per lane)
  run agarose gel of samples from: total RNA (before and after freezing),
  at 160V for 15 min mRNA (after Dynabeads purification)
  mRNA (after Turbo DNase digest & clean up

Reverse Transcription (SuperScriptIII; Invitrogen; Cat No. 18080093)

*Use **max. 4-5 µg RNA** per RT rxn*
*Perform **1 negative control (RTminus/ RT-)** (add RNase free H_2O, instead of SSIII)*

If RNA concentration is low (5-10 µg RNA in total) -> do at least 5 rxn!

X µl RNA (*max. conc. 4-5ug*)
1 µl dNTP
1 µl GSP (2 µM) (1:50; gene specific primer;
CTCATCAATGTATCTTATCATGTCTG)
fill up to **13 µl with RNase-free H_2O**

do Master Mix, distribute 65µl MM to PCR-stripes (5 reactions/tube)
-> **65°C for 5 min**, put **on ice** for **1 min**

**RNaseOUT and SSIII are unstable enzymes -> put immediately back to freezer after usage*

RT rxn	**RTminus rxn**
4 µl 5x First-Strand buffer	**4 µl 5x First-Strand buffer**
1 µl DTT (0.1 M)	**1 µl DTT (0.1 M)**
1 µl RNaseOUT*	**1 µl RNaseOUT***
1 µl SSIII*	**1 µl RNase free H_2O**
7 µl/ rxn	**7 µl /rxn**

-> add **35µl** of this enzyme mix to the previous **65 µl rxn** (5 rxns/tube: final vol.: 100µl)
-> incubate at **50°C for 1h, 70°C for 15 min , 4°C for ∞**

optional: samples can be stores at -20°C

RNaseA treatment

- add **1 µl RNaseA (10mg/ml; Fermentas; Cat No. EN0531) per 100 µl reaction (= 5 rxns)**
- add **0.2 µl RNaseA** per **RT- rxn** (= **20 µl)**
  ➔ incubate for **1 h at 37°C**

optional: samples can be stores at -20°**C**

cDNA purification with AMPure XP beads (Beckman; Cat No. A63882)

- pool all cDNA samples, mix thoroughly and distribute up to 300 µl (= 15 RT rxn; keep RTminus separate) to LoBind 1.5ml tubes
- Mix/vortex beads thoroughly before use
- add **1.8 vol. beads to 1 vol. cDNA (36 µl for –RT)**, vortex, pipette up and down **20x**
- incubate **15 min** at room temperature
- place onto magnet
- incubate **15 min** on a magnetic stand
- the beads should be collected to the wall of the tube and the solution should be clear
  - remove all liquid
- wash 2x with **1 ml 80% EtOH**, incubation time **2 min** (beads have to be covered)
- dry beads at room temperature for **5-10 min** (keep the tubes with open lids on the magnet)
- elute by adding **20 µl EB per rxn (15 rxn -> elute in 300 µl)**
- take tubes off the magnet
  - *pipet up and down 25x*
  - *place tubes on **37°C** heat block, shake at 300 rpm **for 3 min,***
  - *put tubes immediately on magnetic stand, carefully tilt stand to allow beads to migrate upwards on wall, incubate for **1 min***
  - *transfer supernatant (=sample) to new tube*
  - ***pool all cleaned cDNA samples** (except RTminus)*

Junction PCR (jPCR)

number of jPCR = number of RT reactions
jPCR primers depend on intron used (chimeric etc.)

jPCR mix:

20 µl cDNA (*do 1 jPCR per RT rxn*)
25 µl KAPA 2xHiFi
2.5 µl junction fwd (10 µM) (TCGTGAGGCACTGGGCAG*G*T*G*T*C)
2.5 µl junction rev (10 µM) (CTTATCATGTCTGCTCGA*A*G*C)
fill up with **H_2O** to **50 µl**

* = phosphorothioate bond
-> *protection of3' ends of primers from exonuclease activity of KAPA Polymerase*

PCR cycle:

Denaturation: 98°C- 45 sec

98°C- 15 sec ⎫
Annealing: 65°C- 30 sec ⎬ → 15 cycles (=repeats)
Elongation: 72°C- 70 sec ⎭

Final elongation: 72°C- 60sec

jPCR purification with AMPurexP beads

- pool all reactions (up to 12 jPCR rxn = 600 µl), keep –RT separate
- Measure volume of pooled reactions (always lower than added volumes of individual reactions)
- Mix/vortex beads thoroughly before use
- add **0.8 vol. beads to 1 vol. cDNA (40 µl for –RT)**, vortex, pipette up and down **20x**
- incubate **15 min** at room temperature and another **15 min** on a magnetic separator
- the beads should be collected to the wall of the tube and the solution should be clear
    - remove all liquid
- wash 2x with **1 ml 80% EtOH**, incubation time **2 min** (beads have to be covered)
- dry beads at room temperature for **5-10 min** (keep the tubes with open lids on the magnet)
- take tubes off magnets
- elute in **20 µl H_20 per rxn (25rxn -> elute in 500 µl)**
    - *pipet up and down 25x*
    - *place beads on **37°C** heat block, shake at 300 rpm **for 3 min,***
    - *put beads immediately on magnetic stand, carefully tilt stand to allow beads to migrate upwards on wall, incubate for **1 min***
    - *transfer supernatant (=sample) to new tube*
    - ***pool all cleaned jPCR samples** (except RTminus)*

Sequencing ready PCR

Test PCR:
-> to test, how many cycles are necessary → You need to see a clear band corresponding to the length of the library input (5, 9 cycles)

PCR mix:

for RT: 5 and 9 cycles	for RTminus: 9 cycles
20 µl jPCR (from pool)	***20 µl jPCR (RT- rxn)***
2.5 µl PE1.0 (10 µM)	***2.5 µl PE1.0 (10 µM)***
2.5 µl MP2.0 (10 µM)	***2.5 µl MP2.0 (10 µM)***
***25 µl* KAPA 2x HiFi Mix**	***25 µl* KAPA 2x HiFi Mix**

PCR cycle:

Denaturation: 98°C- 45 sec

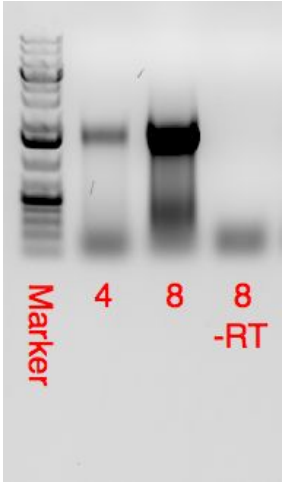
	98°C- 15 sec ⎫	
Annealing:	65°C- 30 sec ⎬ →	4 or 8 repeats (for 5-9 cycles)
Elongation:	72°C- 45 sec ⎭	

Final elongation: 72°C- 60 sec

Gel analysis:

1% agarose gel, 140V, 15 min
load 10 µl of each test PCR + 2 µl of 6x DNA loading dye

Determine how many cycles to use based on band intensity after 5 and 9 cycles.
For the example below we see a faint band at 5 cycles and overamplification after 9 cycles -> we recommend 5 cycles on the jPCR product below on the gel image, perform a minimum of 5 cycles. If 5 cycles already result in an overamplification, reduce the amount of template (divide by 2 per cycle less, for example 5 µl template and 5 cycles if 3 cycles would suffice. Do not reduce number of cycles for the junction PCR to compensate!)



Loaded:
1)Fermentas GeneRuler 1kb plus
2)10 µl of seqready PCR with 4 repeats (5 cycles)
3)10 µl of seqready PCR with 8 repeats (9 cycles)
4) 10 µl of seqready PCR with 8 repeats (9 cycles) of the –RT

Sequencing ready PCR:

PCR mix:

20 µl jPCR (purified)
2.5 µl PE1.0 (10 µM)
2.5 µl TruSeq IDX (10 µM) (*appropriate barcodes, see sequences on next page)*
25 µl KAPA 2x HiFi Mix

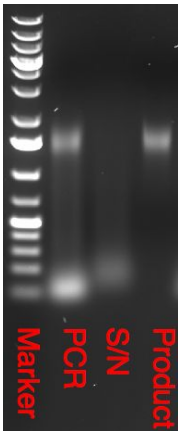
PCR program and number of cycles: *see Test PCR*
Number of reactions: 2 for focused and 20 for genome-wide screen

PE1.0: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
MP2.0: CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

SeqReady PCR purification with SPRIselect beads (Beckman; Cat No. B23318)

*It is critical to use the **exact beads to PCR ratio** – using less than 0.5 leads to loss of sample.*

- pool **2 rxn** or **10 rxn** **(pipette 45 µl out of each PCR reaction to get the exact volume)**
- Mix/vortex beads thoroughly before use
- add **0.5 vol. beads to 1 vol. DNA**, vortex, pipette up and down **20x**
- incubate **10 min** at room temperature and another **5 min** on magnet in LoBind 1.5ml tube
- the beads should be collected to the wall of the tube and the solution should be clear
    - remove all liquid
- ***keep tubes on magnets all the time (except elution), pipette on opposite tube wall***
- wash 2x with **1 ml 80% EtOH**, incubation time **2 min**
- dry beads at room temperature for **2-5 min** (*keep the tubes with open lids on the magnet*)
- elute by adding **10 µl RNase free H_2O (no EB) per rxn (2 rxn -> 20 µl, 10 rxn -> 100 µl)**
- take off tubes from magnet
    - *pipet up and down 25x*
    - *place tubes to **37°C** on heat block **for 3 min,***
    - *put tubes immediately on magnetic stand, carefully tilt stand to allow beads to migrate upwards on wall, incubate for **1 min***
    - *transfer supernatant (=sample) to new tube*
    - ***pool all cleaned seq ready PCR samples** (except RTminus)*



Loaded:
1) Fermentas GeneRuler 1kb plus
2) 10 µl of the pooled PCRs
3) 10 µl of the supernatant, taken after PCR bound to the beads
4) 2 µl final PCR product

TruSeq IDX 1	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 2	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 3	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 4	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 5	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 6	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 7	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 8	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 9	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 10	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 11	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 12	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 13	CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 14	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 15	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 16	CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 17	CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 18	CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 19	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 20	CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 21	CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 22	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 23	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 24	CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 25	CAAGCAGAAGACGGCATACGAGATATCAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 26	CAAGCAGAAGACGGCATACGAGATGCTCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 27	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 28	CAAGCAGAAGACGGCATACGAGATCTTTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 29	CAAGCAGAAGACGGCATACGAGATTAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 30	CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 31	CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 32	CAAGCAGAAGACGGCATACGAGATTGAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 33	CAAGCAGAAGACGGCATACGAGATCGCCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 34	CAAGCAGAAGACGGCATACGAGATGCCATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 35	CAAGCAGAAGACGGCATACGAGATAAAATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 36	CAAGCAGAAGACGGCATACGAGATTGTTGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 37	CAAGCAGAAGACGGCATACGAGATATTCCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 38	CAAGCAGAAGACGGCATACGAGATAGCTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 39	CAAGCAGAAGACGGCATACGAGATGTATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 40	CAAGCAGAAGACGGCATACGAGATTCTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 41	CAAGCAGAAGACGGCATACGAGATGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 42	CAAGCAGAAGACGGCATACGAGATCGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 43	CAAGCAGAAGACGGCATACGAGATGCTGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 44	CAAGCAGAAGACGGCATACGAGATATTATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 45	CAAGCAGAAGACGGCATACGAGATGAATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 46	CAAGCAGAAGACGGCATACGAGATTCGGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 47	CAAGCAGAAGACGGCATACGAGATCTTCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
TruSeq IDX 48	CAAGCAGAAGACGGCATACGAGATTGCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Dynabeads Oligo(dT)$_{25}$ recovering

warm up buffers to RT before recovery: reconditioning buffer: 3x volume
storage buffer: 4x volume

Always remove previous buffer complete before adding the next buffer -> spin down the beads (with hands) and remove residual buffer!

- put Dynabeads Oligo-dT$_{25}$ on the magnet separator**,** wait for **1.5 min** until solution is clear
- remove supernatant completely
- add one volume **Reconditioning Solution**
- place beads on **65°C** heat block, shake at 300 rpm **for 10 min**
- wash **2x** with **Reconditioning Solution,** incubation time on the magnet: **1.5 min**
- wash **3x** with **Storage Buffer,** incubation time on the magnets: **1.5 min**
- add **Storage Buffer** and transfer to **new tube**
- store the beads **at 4°C**
- **beads can be reused 7 times**

Buffers for Dynabeads Oligo(dT)$_{25}$

DEPC treated water: add 750 µl DEPC to 500 ml MonoQ water, incubate o/n, autoclave

		binding buffer (2,5x per Screen)		**washing buffer** (2x per Screen)		**storage buffer** (5x per Screen)	
	Stock		**500 ml**		**500 ml**		**500 ml**
TrisHCl pH 7.5	1 M	20m M	**10 ml**	10 mM	**5 ml**	250 mM	**125 ml**
LiCl	5 M	1 M	**100 ml**	0.15 M	**15 ml**		
EDTA pH 8	0.5 M	2 mM	**2 ml**	1 mM	**1 ml**	20 mM	**20 ml**
Tween-20						0.1 %	**500 µl**
NaN_3	10%					0.02 %	**1 ml**
DEPC treated MonoQ			**388 ml**		**479 ml**		**353.5 ml**

10mM TrisHCl (provided by kit)

	Stock		**100 ml**
TrisHCl pH 7.5	1 M	10 mM	**1 ml**
DEPC treated MonoQ			**99 ml**

Reconditioning Solution: (3x per Screen)

	Stock		**500 ml**
NaOH	5 M	0.1 M	**10 ml**
DEPC treated MonoQ			**490 ml**