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MATERIALS

All DNA oligonucleotides were ordered from Sigma-Aldrich Co. (St. Louis, MO, USA) unless stated otherwise (see sequences below). Primer/probe mixes for qPCR analysis were purchased from Integrated DNA Technologies ((IDT), Coralville, IA, USA) (see sequences below). 20% sodium dodecyl sulfate (SDS) (161-0418) was purchased from Bio-Rad (Hercules, CA, USA). 10X phosphate buffered saline (PBS) (P0191), 5 M Tris pH=8 (T5581), 1 M Tris pH=7 (T1070), 5 M sodium chloride (NaCl) (S0250), and 500 mM tetraethylenediaminetetraacetic acid (EDTA) (E0307-06) were purchased from Teknova (Hollister, CA, USA). 20X saline sodium citrate (SSC) buffer (V4261) and Tween-20 (H15152) were purchased from Promega (Madison, WI, USA). RNase A (12091-039) was purchased from Life Technologies (Carlsbad, CA, USA). 384-well plates for fluorescence measurements were purchased form Sigma-Aldrich Co. (CLS3676). 96-well plates (04729692001) and Master Mix solutions (0470749001) for qPCR were purchased from Roche USA (Nutley, NJ, USA). *Saccharomyces cerevisiae* strain Y1788 was obtained from Professor David Mitchell (University of Texas). Yeast extract peptone dextrose (Y1375, abbreviated YPD), 37% formaldehyde (F38775), and protease inhibitors for fungal growth (P8215) were purchased from Sigma-Aldrich Co. DNA array fabrication materials were purchased as described previously[1] aside from dicyanoimidazole (DCI) activator, which was purchased from Sigma-Aldrich and the Cap B solution which was mixed in house as described below. Tetrahydrofuran (THF), 2,6-lutidine, dimethyl sulfoxide (DMSO), imidazole, and 1-methylimidazole were purchased from Sigma-Aldrich. 2-dimethylaminopyridine (D1680) was purchased from Tokyo Chemical Industry Co. LTD (Tokyo, Japan). The phosphoramidite functionalized polyethyleneglycol 2000 (CLP-2119) was purchased from ChemGenes Corporation (Wilmington, MA.) Labeled DNA oligomers for the surface displacement were purchased from IDT.

METHODS

DNA oligonucleotide hybridization

Sequence information for all oligonucleotides is contained in supplementary tables S2-S6 below. In general, all capture oligonucleotides contained a biotin at the 3' end and all target oligonucleotides contained a flourophore at the 3' end. To prevent photobleaching, all hybridization experiments were performed in the dark. Each hybridization experiment was done using 5 pmol of the capture oligonucleotide and 10 pmol of the target oligonucleotide in 100 µl 1X SSC. Samples were incubated at 37°C for one hour. Hybridized complexes were captured using streptavidin coated magnetic particles (50 µL slurry) in a total of 500 µL 1X SSC buffer for one hour at room temperature. After capture, the beads were washed three times with 1X SSC to remove non-hybridized target oligonucleotide. Release oligonucleotide was added to the beads in a total of 500 µL 1X SSC. Depending upon the molar excess of release oligonucleotide used, 5 nmol (1000X molar excess) to 50 pmol (10X molar excess) was added. The magnetic particles were rotated at room temperature with care to ensure the constant suspension of the particles. Periodically, 15 µL aliquots of the release sample were removed and pipetted into a black, round-bottom 384-well plate after removal of beads. Fluorescent readings were measured using a Perkin Elmer Envision 2100 Multilabel Reader (Waltham, MA, USA). To monitor both FAM and Alexa 488 fluorescence, a 485 nm excitation filter and a 535 nm emission filter were used. To monitor TexasRed fluorescence, a 595 nm excitation filter and 630 nm emission filter were used. Fluorescence values obtained at each release time point were normalized to reflect % release relative to total target captured based on the fluorescence signal remaining in solution after bead capture subtracted from input fluorescence signal prior to hybridization.

Chromatin preparation/hybridization

Yeast cells were grown to saturation in 5ml of YPD overnight at 30°C, shaken at 200 rpm in an Amerex 747 shaker/incubator. The cells were diluted into 1.5 L of YPD and grown to an OD_{600} ~2.0 as measured using an Agilent 8453 UV-Vis spectrophotometer. Formaldehyde was

added to a final concentration just under 3% (122 mL) and incubated for 30 minutes at room temperature, after which unreacted formaldehyde was quenched with 250 mL of 5 M Tris. The cells were collected using an Avanti J-25I centrifuge at 5,000 g for 20 minutes. The cell pellet was washed once with 1X PBS and either used right away or stored at -80°C. Cells from 1.5 L of culture (~2-3 mL cell pellet) were resuspended in 50 mL lysis buffer (20 mM EDTA, 200 mM NaCl, 50 mM Tris pH 7 and protease inhibitors (1/200 from stock)). The cells were lysed at 30kpsi using a Constant Systems TS Series Cell Disruptor. SDS was added to the lysate solution to a final concentration of 1% and the lysate was incubated at 65°C for 5 minutes. The cross-linked chromatin was sonicated in 50 mL volumes using a MisoniX Ultrasonic Processor S4000 at 20 V for a total of 3.5 min with alternating intervals of 4 seconds on and 4 seconds off. The sample was then centrifuged at 8,000 g for 12 minutes to separate the cellular debris from the soluble chromatin. The supernatant was removed from the pellet and diluted 5 fold into lysis buffer to decrease the total SDS concentration to 0.2%. RNase A was added to a final concentration of 60 µg/mL and the solution was shaken at 150 rpm at 37°C for 60 minutes.

For each hybridization experiment, lysate from 250 ml of original cell growth was used. Thus, from a cell lysate preparation from 1.5 L of cell culture, six hybridization experiments were performed in a volume of 50 mLs each. Ten pmol of each capture oligonucleotide for the three genomic loci were added to the cell lysate. The samples were shaken/incubated at 37°C for 3 hours. Streptavidin coated magnetic particles were added (300 µL original slurry) to capture hybridized complexes and the suspension was incubated at room temperature for one hour. The beads were washed four times with wash buffer (50 mM tris pH=8, 200 mM NaCl, 0.2%SDS) and concentrated into 1.7 mL Eppendorf tubes. Release oligonucleotides (10 nmol) were added to 1 mL aliquots of wash buffer and added to the beads. The suspension was rotated at room temperature for 15 minutes. The solution was removed, the beads washed twice with wash buffer and another release oligonucleotide was added to each aliquot of beads. The released material was diluted 10-fold into 1X TE, heated at 95°C for 15 minutes and analyzed using qPCR.

qPCR analysis

DNA from release samples were measured using Taqman assays for each of the three genes studied (25S rDNA, 5S rDNA and X-element). Dilutions of purified yeast genomic DNA were used for a standard curve. Each DNA sample was analyzed in duplicate in a 96-well microtiter plate. Each well contained 5 µL of sample, 10 µL of LightCycler 480 probe master mix, 4.5 µL water and 0.5 µL 40X primer probe mix. After pipetting, each plate was centrifuged for 2 minutes at 2,000 g. The samples were then analyzed using a Roche 480 LightCycler. The qPCR runs included a 5 minute pre-incubation step at 95°C, amplification cycles, and a 2 minute cooling step at 40°C. Each amplification cycle was comprised of a 10 second 95°C incubation with a temperature ramp of 4.4°C/sec, a 30 second incubation at 60°C with a temperature ramp of 2.2°C/sec and a third 1 sec incubation at 72°C with a temperature ramp of 4.4°C/sec. Detection of the FAM flourophore was performed during the 72°C incubation using a 483-533 nm filter set. Analysis of the resultant qPCR curves and calculation of Cp values were performed using the Roche 480 LightCycler software and the 2nd quant/2nd derivative function. Absolute DNA amounts were calculated from the genomic DNA standard curve, and % protein-free DNA values are given by the ratio (protein-free DNA in aqueous phase/input DNA) x 100%).

DNA microarray synthesis

DNA microarrays were fabricated using a custom built maskless array synthesizer (MAS) with minor modifications to previously described chemistry on hydroxyl functionalized glassy carbon surfaces[1, 2]. DCI activator (0.25 M dicyanoimidazole in acetonitrile) was used for the arrays synthesized in the included studies. Exposure solvent consisted of 1% imidazole in DMSO (w/v) and Cap B solution was composed of .5% 2-dimethylaminopyridine, 2% N-methylimidazole and 10% 2,6-lutidine in THF. 5'-nitrophenylpropyloxycarbonyl-dGuanosine (ipac) 3'-β-cyanoethylphosphoramidite and 5'-nitrophenylpropyloxycarbonyl-dAdenosine (tac) 3'- β-cyanoethylphosphoramidite underwent longer coupling steps than previously described (90 seconds) and a polyethyleneglycol 2000 (PEG-2K) polymer functionalized for phosphoramidite

chemistry was employed to act as a spacer at the surface of the array. The PEG-2K spacer underwent two 15 minute coupling steps to attach it to the preceding hydroxyl.

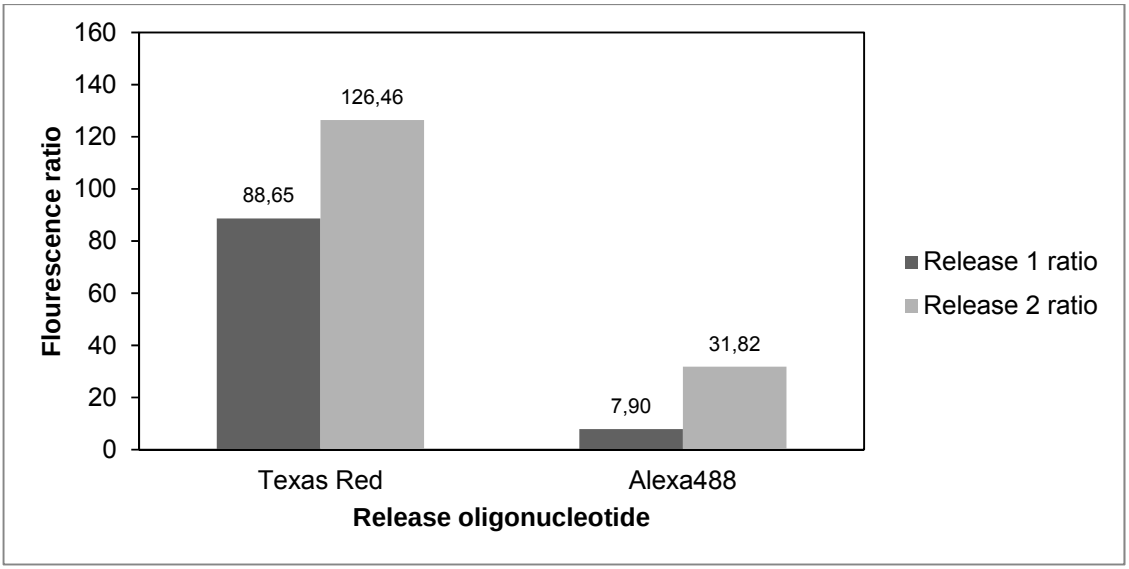
DNA microarray toehold displacements

Displacement reactions on DNA arrays were conducted by hybridizing a labeled oligonucleotide to the entirety of the sequences (38 nt) on the array and imaging followed by the addition of an excess quantity of release oligonucleotide. The initial hybridizations were performed by the addition of a 1 μM solution of the 5'-FAM labeled 30 nt sequence in hybridization buffer consisting of 0.1% Tween-20 and 4X Sodium chloride Sodium Phosphate EDTA (SSPE) solution (150 mM sodium chloride, 10 mM sodium phosphate, and 1 mM EDTA at 1X concentration) to the surface of the DNA array with a Thermo Scientific 125 μL Gene Frame gasket. The array was placed in a humid chamber at 37°C for a minimum of 30 minutes before a 10 minute rinse in 0.5X SSPE at room temperature. Imaging was conducted on a GeneTac UC4x4 scanner using laser/filter settings for FITC and Cy3. The exchange was then performed by the addition of 50 μL of a 200 μM solution of the Cy3-labeled release oligonucleotide in hybridization buffer for 60-80 minutes at room temperature, followed with a 10 minute rinse in 0.5X SSPE prior to reimaging. Arrays were kept wet throughout the entirety of the aforementioned process to prevent non-specific adsorption of duplexed DNA to the carbon surface.

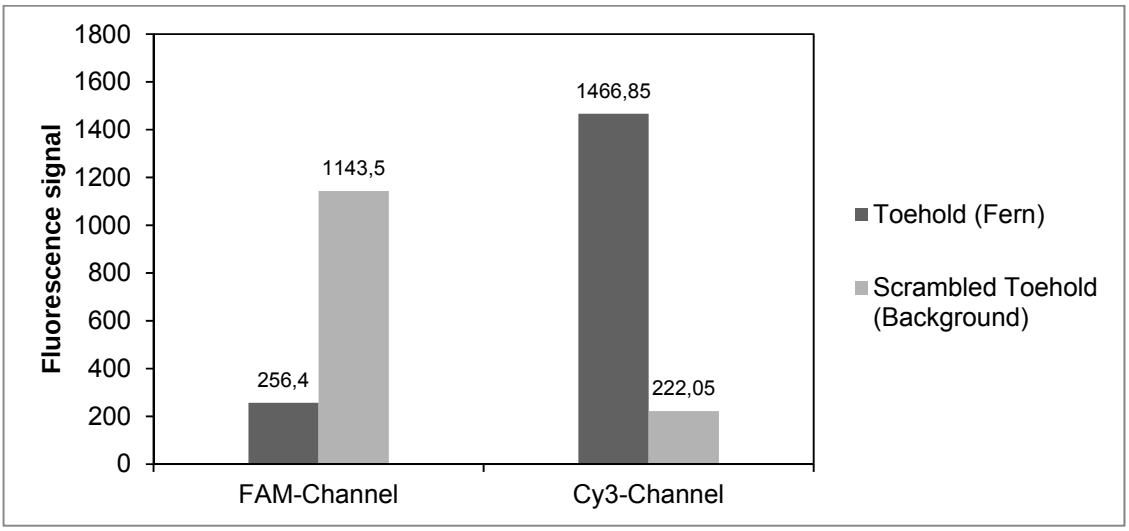
A discussion of DNA array spacer strategies

The interplay between the spacers used in a microarray feature and the feature's density has been known to play a role in DNA binding behavior at a surface[3, 4]. We sought to test whether some common strategies we employ to space a region of DNA from the surface by including polynucleotide regions, polyethylene glycol 2000, or a combination of the two would alter our ability to perform the toehold mediated exchange on an array. An array was fabricated to examine the exchange of a FAM-labeled oligonucleotide (Supplementary Table 2, sequence 3) with a Cy3 labeled release oligonucleotide (Supplementary Table 2, sequence 4) in a similar fashion as

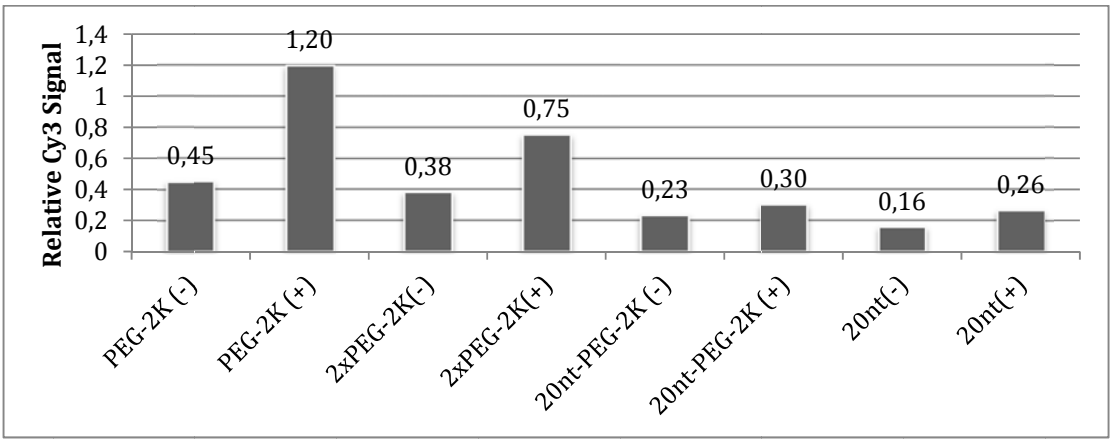
shown in the main text. The array elements were designed to compare the exchange between sequences containing an 8 nt toehold (Supplementary Table 2, sequence 1) to the same sequence without the toehold region. The spacers tested included a single PEG-2K unit, two PEG-2K units, a 20 nt region (Supplementary Table 2, sequence 5), and a 20 nt region followed by a single PEG-2K spacer. Because the toehold and non-toehold sequences are synthesized with different efficiencies, the data was analyzed by examining the Cy3 signal at a feature after the exchange reaction normalized to the FAM signal detected after the first hybridization. This is plotted in Supplementary Figure 3, showing the extent to which the PEG-2K spacer best facilitates the displacement among the strategies we tested.



Supplementary Figure 1. Fluorescence signal ratios between intended target oligonucleotide and alternate target oligonucleotide from sequential release experiments of captured DNA containing different sequences. The Alexa488 and Texas Red fluorescence was measured in each of the four release experiments depicted in Figure 2D. The ratio of the fluorescence signal from the samples using the intended release oligonucleotide to release the specified target oligonucleotide were divided by the fluorescence signal from the samples using the alternative release oligonucleotide. This ratio reflects the specificity of release.



Supplementary Figure 2. Fluorescent signals from the regions of the DNA array depicted in Figure 4. The fluorescent signals from the DNA array from Figure 4 after the displacement reaction are shown. Analysis was performed by using ImageJ to average the intensity of 10 locations inside and outside of the fern region, and subtracting the average value of 20 locations from the background of the fluorescence scan in a region on the array where no DNA was synthesized (not shown in Figure 4)[5].



Supplementary Figure 3. The relative signals of the Cy3-labeled release oligonucleotide detected on a DNA microarray. A 60 minute displacement reaction was carried out on a DNA array as described previously. The Cy3 signal shown was normalized to the strength of the FAM signal present before the displacement reaction to account for differences in the efficiency of the synthesis. "(+)" denotes sequences containing the intended 8 nt toehold, while "(-)" denotes the absence of the toehold.

X-element		
Sample	**X-element/25S rDNA**	**X-element/5S rDNA**
A	281.4	88.7
B	157.4	265.3
C	233.2	36.3
D	33.0	194.0
E	32.8	52.1
F	33.4	34.8
5S rDNA		
Sample	**5S rDNA/X-element**	**5S rDNA/25S rDNA**
A	10.5	57.7
B	8.9	27.1
C	443.4	85.9
D	14.4	11.6
E	36.2	14.9
F	55.0	7776.3
25S rDNA		
Sample	**25S rDNA/X-element**	**25S rDNA/S rDNA**
A	8.5	20.9
B	9.2	39.3
C	11.8	14.3
D	38.3	46.0
E	53.3	39.7
F	41.1	15.7

Supplementary Table 1. qPCR signal ratios between targeted gene release and off-target gene release. For each of the six combinations of captured chromatin target release depicted in Figure 3B/C, the qPCR signal obtained for each of the target genes as well as the two other off-target genes was measured. The ratio of the intended released gene target qPCR signal compared to the two off-target genes is shown.

Region	Surface oligonucleotide (5'-3'-dT-PEG 2K-dT-surface)	Length (nt)
Fern	CTGAACTTAAGCATATCAATAAGCGGAGGA**GTGTATCA**	40
Background	CTGAACTTAAGCATATCAATAAGCGGAGGA**TCGGAATT**	40
5'-Fluorophore	**Hybridization/Release oligonucleotides (5'-3')**	**Length (nt)**
FAM	TCCTCCGCTTATTGATATGCTTAAGTTCAG	30
Cy3	**TGATACAC**TCCTCCGCTTATTGATATGCTTAAGTTCAG	38
---	**Polynucleotide spacer sequence (5'-3')**	**Length (nt)**
---	CTAACAGGTGAGTTCTGATT	20

Supplementary Table 2. Sequences used for toehold displacements on DNA microarrays.

The DNA sequences for the toehold exchange on a DNA microarray are listed with toehold regions in bold. The hybridization and release oligonucleotides were ordered from IDT. The release oligonucleotide contains a 5'-Cy3 attached via 1-[3-(4-monomethoxytrityloxy)propyl]-1'-[3-[(2-cyanoethyl)-(N,N-diisopropyl) phosphoramidityl]propyl]-3,3,3',3'-tetramethylindocarbocyanine chloride and subsequently HPLC purified. The hybridization oligonucleotide contains a 5'-FAM attached via a 6-(3',6'-dipivaloylfluoresceinyl-6-carboxamido)-hexyl-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite.

Toehold length (nt)	Capture oligonucleotide (5'-3'-btn)	Length (nt)
0	AATCTCGAGCCATGATAAACTCTTCAATTT	30
4	TTTTAATCTCGAGCCATGATAAACTCTTCAATTT	34
6	TTTTTTAATCTCGAGCCATGATAAACTCTTCAATTT	36
8	TTTTTTTTAATCTCGAGCCATGATAAACTCTTCAATTT	38
10	TTTTTTTTTTAATCTCGAGCCATGATAAACTCTTCAATTT	40
Toehold length (nt)	**Release oligonucleotide (5'-3')**	**Length (nt)**
0	AAATTGAAGAGTTTATCATGGCTCGAGATT	30
4	AAATTGAAGAGTTTATCATGGCTCGAGATTAAAA	34
6	AAATTGAAGAGTTTATCATGGCTCGAGATTAAAAAA	36
8	AAATTGAAGAGTTTATCATGGCTCGAGATTAAAAAAAA	38
10	AAATTGAAGAGTTTATCATGGCTCGAGATTAAAAAAAAAA	40
---	**Fluorescent target oligonucleotide (5'-3'-FAM)**	**Length (nt)**
---	AAATTGAAGAGTTTATCATGGCTCGAGATT-FAM	30

Supplementary Table 3. Toehold length capture/release/target oligonucleotide sequences.

The DNA sequences for capture/release/target oligonucleotides testing the kinetics of release for different toehold lengths are listed. All capture/release oligonucleotides were ordered from Sigma Aldrich Co.. The capture oligonucleotides were HPLC purified and contain a 3' biotin modification using 1-Dimethoxytrityloxy-3-O-(N-biotinyl-3-aminopropyl)-triethyleneglycolyl- glyceryl-2-O-succinyl-long chain alkylamino-CPG. The target oligonucleotide was ordered from IDT and contains a 3' FAM modification using 1-Dimethoxytrityloxy-3-[O-(N-carboxy-(di-O-pivaloyl-fluorescein)- 3-aminopropyl)]-propyl-2-O-succinoyl-long chain alkylamino-CPG.

Target	Capture oligonucleotide (5'-3'-btn)	Length (nt)
ALEXA488	GTGTATCA CTGAACTTAAGCATATCAATAAGCGGAGGA	38
TexasRed	ACTGCAGT TAGAATATTT TTATGTTTAG GTGATTTTAG	38
Target	**Release oligonucleotide (5'-3')**	**Length (nt)**
ALEXA488	TCCTCCGCTTATTGATATGCTTAAGTTCAGTGATACAC	38
TexasRed	CTAAAATCACCTAAACATAAAAATATTCTAACTGCAGT	38
Target	**Target oligonucleotide (5'-3'-flourophore)**	**Length (nt)**
ALEXA488	TCCTCCGCTTATTGATATGCTTAAGTTCAG-ALEXA488	30
TexasRed	CTAAAATCACCTAAACATAAAAATATTCTA-TexasRed	30

Supplementary Table 4. Sequential release of fluorescent oligonucleotides capture/release/target sequences. The DNA sequences for capture/release/target oligonucleotides testing sequential release of oligonucleotides are listed. All capture/release/target oligonucleotides were ordered from Sigma Aldrich Co.. The capture oligonucleotides were HPLC purified and contain a 3' biotin modification using 1-Dimethoxytrityloxy-3-O-(N-biotinyl-3-aminopropyl)-triethyleneglycolyl- glyceryl-2-O-succinyl-long chain alkylamino-CPG. The target oligonucleotides contain 3' fluorescent modifications: Alexa488 using 5-carboxy-2-(3,6-diamino-4,5-disulfonatoxanthenium-9-yl)benzoate and TexasRed using 1H,5H,11H,15H-xantheno[2,3,4-IJ:5,6,7-I'J']diquinlizin-18-ium, 9-(2,4-disulfophenyl)-2,3,6,7,12,13,16,17-octahydro-succinimidyl ester.

.

Gene	Capture oligonucleotide (5'-3'-btn)	Length (nt)
25S rDNA	GTGTATCA CTGAACTTAAGCATATCAATAAGCGGAGGA	38
5SrDNA	CAACTGTC TTAACGGAAACGCAGGTGATATGAGGGCAG	38
X-element	ACTGCAGTTAGAATATTTTTATGTTTAGGTGATTTTAG	38
Gene	**Release oligonucleotide (5'-3')**	**Length (nt)**
25S rDNA	TCCTCCGCTTATTGATATGCTTAAGTTCAGTGATACAC	38
5SrDNA	CTGCCCTCATATCACCTGCGTTTCCGTTAAGACAGTTG	38
X-element	CTAAAATCACCTAAACATAAAAATATTCTAACTGCAGT	38
Gene	**Target Genomic Sequence**	**Length (nt)**
25S rDNA	TCCTCCGCTTATTGATATGCTTAAGTTCAG	30
5SrDNA	CTGCCCTCATATCACCTGCGTTTCCGTTAA	30
X-element	CTAAAATCACCTAAACATAAAAATATTCTA	30

Supplementary Table 5. Genomic loci capture/release oligonucleotide sequences and native genomic target sequences. The DNA sequences for capture/release oligonucleotides testing sequential release of captured genomic loci are listed. All capture/release/target oligonucleotides were ordered from Sigma Aldrich Co.. The capture oligonucleotides were HPLC purified and contain a 3’ biotin modification using 1-Dimethoxytrityloxy-3-O-(N-biotinyl-3-aminopropyl)-triethyleneglycolyl- glyceryl-2-O-succinyl-long chain alkylamino-CPG.

X-element qPCR assay			
Position	*Sequence (5'-3')*	*Tm*	*Fluorophore/quencher*
Left	GCCGCCGAATGAGATATAG	58.3	---
Right	CGGTTTATACCCTGTGCCAT	59.71	---
Probe	CCCATAAAGCCCACGATTATCCACA	68.47	5'FAM - 3'TAMRA
25S rDNA qPCR assay			
Position	*Sequence (5'-3')*	*Tm*	*Fluorophore/quencher*
Left	TTAGTAACGGCGAGTGAAGC	58.2	---
Right	CAAAGTTGCCCTCTCCAAAT	59.17	---
Probe	TCTGGTACCTTCGGTGCCCGA	69.22	5'FAM - 3'TAMRA
5S rDNA qPCR assay			
Position	*Sequence (5'-3')*	*Tm*	*Fluorophore/quencher*
Left	GTGCATTGTGATGTGGAGAA	58.02	---
Right	CTACCTCTGCATGCCACCTA	58.9	---
Probe	CCGACCAACTTTCATGTTCTGTTTCG	68.55	5'FAM - 3'TAMRA

Supplementary Table 6. qPCR assay sequences. The DNA sequences for the three qPCR assays used in the gene capture experiments are listed. All qPCR assays were ordered from IDT. The assays were designed using the GenScript Real Time Primer Design Tool (https://www.genscript.com/ssl-bin/app/primer). The FAM modification at the probe 5' termini was 6-Carboxyfluorescein, and the TAMRA modification at the 3' termini was 5(6)-Carboxytetramethylrhodamine dT.

SUPPLEMENTARY INFORMATION REFERENCES

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