Supplementary Information

Virtual screening. The coordinates of the RUNX1 Runt domain (1EAN) were obtained from the PDB. The hydrogen atoms were added in InsightII/Builder (Accelrys) program (*Insight 2005 Molecular Modelling Program Package*; Molecular Simulations Inc., San Diego) using the protonation states of protein residues at pH = 7.0. The computer program LUDI/InsightII [1] was applied for virtual screening of CAP (Chemicals Available for Purchase, 78,000 compounds) library to the CBFβ binding interface on the Runt domain structure. Compounds from CAP library were docked and ranked by the scoring function (Energy Estimate 1) implemented in the LUDI program [2]. The values of the most important LUDI parameters used for virtual screening searches were as follows: Min Separation = 3; Link, Lipo and H-Bond Weights were set to 1.0; Aliphatic_Aromatic and Reject Bifurcated parameters were turned off; No_Unpaired_Polar, Electrostatic_Check and Invert parameters were turned on; Es Dist = 2.5Å; Max RMS = 0.8 Å; Number of Rotatable Bonds: two at a time, Radius of Search was 10Å. The 500 best scored hits (compounds with the predicted binding affinity < 300 μM as evaluated by LUDI's empirical scoring function) were subjected to visual inspection of their potential interactions with the Runt domain. Compounds with diverse scaffolds and involved in at least two hydrogen bonds with the Runt domain were selected for experimental evaluation.

Saturation transfer difference NMR

Saturation transfer difference (STD) NMR experiments [3,4] were performed with 30 μM Cerulean-Runt domain or Venus-CBFβ, 800 μM AI-7-54 or AI-8-45, 10% D_2O, and 5% DMSO in 50 mM KP_i, 100 mM KCl, 10 mM K_2SO_4, 2 mM $MgSO_4$, pH 7.5 in a final volume of 200 μL. All STD experiments were performed using a 600 MHz Bruker NMR spectrometer at 25°C with saturation times of 500, 750, 1000, 1500, and 2000 ms. Samples were irradiated at 0.4 ppm (protein) and 30 ppm (off-resonance control) and the difference spectra calculated using MestReNova.

Chemical synthesis.

Commercially obtained reagents were used as received. Progress of reactions was monitored by TLC performed on Analtech 250micron silica gel GF plates visualized with 254 nm UV light and also by mass spectrometry using a Waters single-quadrupole LCMS. All compounds were purified on Biotage Isolera Four Flash Chromatography system, using SNAP cartridges. All final compounds were also purified by HPLC. Melting points were determined on a Mel-Temp manual melting point apparatus with a Fluke 51II thermocouple. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker NMR spectrometer at 600 MHz in $CDCl_3$ and DMSO-d_6, with TMS as internal standard. Chemical shift values are reported in ppm units. Mass spectra were recorded on a Micromass AutoSpec Ultima Magnetic sector mass spectrometer in both positive and negative ESI mode at the University of Michigan Department of Chemistry mass spec facility.

The compounds **4a-4d, 5a-f** and **6a-b** were prepared as described previously [5-7].

5-(thiophen-2-yl)-1,3,4-thiadiazole-2(3H)-thione **(4a**) CAS Registry Number 41526-33-4

5-phenyl-1,3,4-thiadiazole-2(3H)-thione **(4b)** CAS Registry Number 5585-19-3

5-(6-methylpyridin-2-yl)-1,3,4-thiadiazole-2(3H)-thione (**4c**) CAS Registry Number 1093390-88-5

5-(pyrazin-2-yl)-1,3,4-thiadiazole-2(3H)-thione (**4d**) CAS Registry Number 37545-34-9

5-(2-fluorophenyl)-1,3,4-thiadiazole-2(3H)-thione (**5a**) CAS Registry Number 108413-60-1

5-(3-fluorophenyl)-1,3,4-thiadiazole-2(3H)-thione (**5b**) CAS Registry Number 276254-76-3

5-(4-fluorophenyl)-1,3,4-thiadiazole-2(3H)-thione (**5c**) CAS Registry Number 108413-72-5

5-(o-tolyl)-1,3,4-thiadiazole-2(3H)-thione (**5d**) CAS Registry Number 76779-96-9

5-(2-methoxyphenyl)-1,3,4-thiadiazole-2(3H)-thione (**5e**) CAS Registry Number 108413-63-4

5-(4-fluoro-2-methylphenyl)-1,3,4-thiadiazole-2(3H)-thione (**5f**) CAS Registry Number 1093390-85-2

2-(2-fluorophenyl)-5-(methylthio)-1,3,4-thiadiazole (**6a**) CAS Registry Number 276254-77-4

5-(benzofuran-2-yl)-1,3,4-thiadiazole-2(3H)-thione (**6b**) CAS Registry Number 130967-94-1

5-(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)-1,3,4-thiadiazole-2(3H)-thione (6d)

The title compound prepared as described previously[8]**.**

^{1}H-NMR (300 MHz, DMSO): δ 7.66-7.71 (1H, dd, *J*=6.00 Hz, *J*=9.00 Hz), 7.77-7.80 (1H, d, *J*=9.00 Hz), 8.04-8.07 (2H, d, 9.00 Hz); ^{13}C-NMR (600 MHz, DMSO): δ 125.08, 126.50, 127.17, 132.38, 133.32, 134.90, 144.66, 157.08, 164.33, and 189.13

General Synthetic procedure for the Key Intermediates in Scheme 5

The compounds, methyl 5-aryl furan-2-carboxylates in step 1 and 5-arylfuran-2-carbohydrazides in step 2 of **Scheme 5,** were prepared as described previously [9-11].

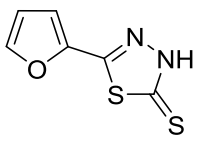
General procedure for the synthesis of Potassium 2-(5-arylfuran-2-carbonyl) hydrazine carbodithioate:

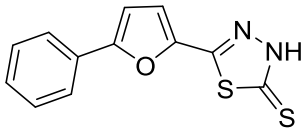
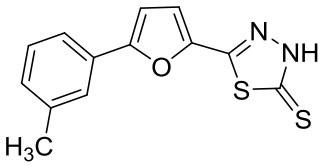
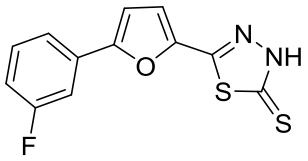
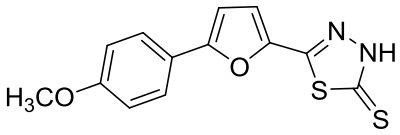
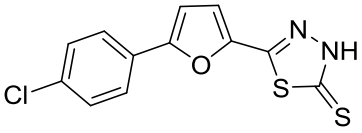
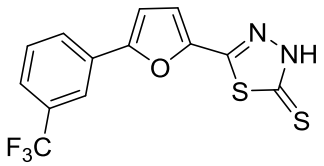
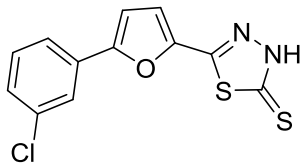
To a solution of potassium hydroxide in ethanol (0.5M) was added 2-carbohydrazide fallowed by carbon disulfide. The resulting solution was allowed to stir overnight at room temperature. The precipitate was diluted with ether, filtered, washed with ether (3X), dried and used in the next step without further purification.

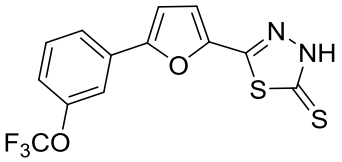
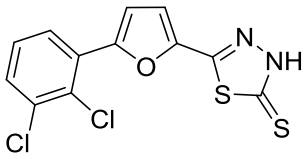
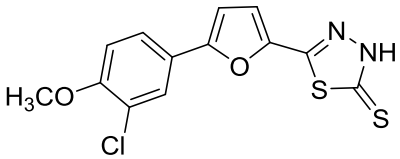
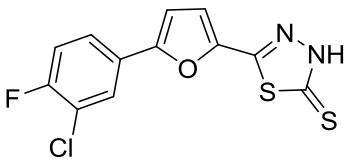
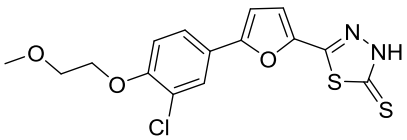
General procedure for the synthesis of 5-(5-(aryl) furan-2-yl)-1, 3, 4-thiadiazole- 2 (3H) - thione

Potassium hydrazine carbodithioate (1 mmol) was added to concentrated sulfuric acid (1mL) at -10^{o}C under argon and stirred at the same temperature for 30 min. The reaction mixture was warmed to 0^{o}C and stirred at that temperature for 2h. The cold reaction mixture was poured over crushed ice, the solid thus separated was filtered, washed with water, and dried. The dried compound was stirred with DL-dithiothreitol (DTT) in ethanol for 3h at room temperature. The resulting solution was diluted with water and solid thus separated was filtered, dried and purified by mass directed HPLC using water/acetonitrile as solvent system on a Waters Sun Fire column.

Compound physical properties and spectral data

Name	Structure	MP (^{o}C)	^{1}H-NMR (δ in ppm)	^{13}C-NMR (δ in ppm)	HRMS
AI-7-54 (3)		214-215	^{1}H-NMR (800 MHz, DMSO): δ6.76-6.77 (1H, dd, *J* = 1.76, 3.52 Hz), 7.20-7.21 (1H, d, *J* = 3.28 Hz), 7.98-7.99 (1H, d, *J* = 1.36 Hz), 14.78 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ112.90, 113.22, 143.57, 146.66, 150.64, 187.33	m/z $[M+H]^{+}$ calcd for $C_6H_4N_2OS_2$; 184.9843; found: 184.9837

AI-8-153 (6c)		237-238	^{1}H-NMR (800 MHz, DMSO): δ7.25-7.26 (1H, d, *J* = 3.60 Hz), 7.33-7.34 (1H, d, *J* = 3.68 Hz), 7.40-7.42 (1H, dd, *J* = 7.36, 7.36 Hz), 7.49-7.51 (2H, dd, *J* = 7.68, 7.68 Hz), 7.81-7.82 (2H, d, *J* = 7.76 Hz), 14.10 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ108.88, 115.29, 124.47, 129.29, 129.33, 129.57, 142.78, 150.37, 156.01, 187.26	m/z [M-H]$^-$ calcd for $C_{12}H_8N_2OS_2$; 259.0005; found: 259.0005
AI-9-24 (7a)		176-178	^{1}H-NMR (800 MHz, DMSO): δ2.38 (3H, s), 7.22-7.23 (2H, m), 7.32-7.33 (1H, d, *J* = 2.48 Hz), 7.37-7.39 (1H, dd, *J* = 7.64, 7.64 Hz), 7.61-7.62 (1H, d, *J* = 7.76 Hz), 7.64 (1H, s), 14.80 (1H, s)	^{13}C-NMR (800 MHz, DMSO): δ21.46, 109.03, 115.46, 121.89, 125.04, 129.23, 129.50, 130.08, 138.90, 142.88, 150.60, 156.23, 187.15	m/z [M-H]$^-$ calcd for $C_{13}H_{10}N_2OS_2$; 273.0162; found: 273.0163
AI-9-13 (7b)		222-225	^{1}H-NMR (600 MHz, DMSO): δ7.23-7.26 (1H, m), 7.34-7.36 (2H, m), 7.53-7.56 (1H, m), 7.65-7.67 (2H, m), 14.81 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ110.43, 111.29, 115.24, 115.96, 120.70, 131.48, 143.27, 150.58, 154.54, 162.34, 163.52, 187.26	m/z [M-H]$^-$ calcd for $C_{12}H_7FN_2OS_2$; 276.9911; found: 276.9913
AI-9-23 (7c)		211-212	^{1}H-NMR (800 MHz, DMSO): δ3.80 (3H, s), 7.04-7.05 (3H, m), 7.25-7.26 (1H, d, *J* = 3.68 Hz), 7.73-7.74 (2H, d, *J* = 8.88 Hz), 14.84 (1H, s)	^{1}C-NMR (800 MHz, DMSO): δ55.77, 107.41, 115.07, 115.66, 122.02, 126.35, 142.09, 150.82, 156.40, 160.26, 187.00	m/z [M-H]$^-$ calcd for $C_{13}H_{10}N_2O_2S_2$; 289.0111; found: 289.0112
AI-8-117 (7d)		>250	^{1}H-NMR (800 MHz, DMSO): δ7.24-7.25 (1H, d, *J* = 3.68 Hz), 7.30-7.31 (1H, d, *J* = 3.28 Hz), 7.54-7.55 (2H, d, *J* = 8.64 Hz), 7.81-7.82 (2H, d, *J* = 8.56 Hz), 14.84 (1H, s)	^{13}C-NMR (800 MHz, DMSO): δ109.76, 115.48, 126.26, 127.97, 129.55, 133.66, 143.02, 150.53, 154.88, 187.16	m/z [M-H]$^-$ calcd for $C_{12}H_7ClN_2OS_2$; 292.9616; found: 292.9615
AI-9-28 (7e)		223-228	^{1}H-NMR (800 MHz, DMSO): δ7.37-7.38 (1H, d, *J* = 3.68 Hz), 7.49-7.50 (1H, d, *J* = 3.68 Hz), 7.73-7.77 (2H, m), 8.12-8.13 (2H, m), 14.86 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ110.80, 115.38, 120.98, 123.50, 125.54, 128.35, 130.27, 130.58, 130.82, 143.68, 150.33, 154.31, 187.37	m/z [M-H]$^-$ calcd for $C_{13}H_7F_3N_2OS_2$; 326.9879; found: 326.9881
AI-8-45 (7f)		220-222	^{1}H-NMR (800 MHz, DMSO): δ7.35-7.36 (1H, d, *J* = 3.68 Hz), 7.38-7.39 (1H, d, *J* = 3.68 Hz), 7.46-7.47 (1H, ddd, *J* = 0.88, 2.00, 7.96 Hz), 7.52-	^{13}C-NMR (600 MHz, DMSO): δ110.30, 115.15, 123.04, 124.03, 128.90, 131.11, 131.53, 134.34, 143.46,	m/z [M+H]$^+$ calcd for $C_{12}H_7ClN_2OS_2$; 294.9767; found: 294.9761

			7.54 (1H, dd, *J* = 7.88, 7.88 Hz), 7.78-7.79 (1H, dt, *J* = 1.24, 7.76 Hz), 7.89-7.90 (1H, dd, *J* = 1.80, 1.80 Hz), 14.80 (1H, s)	150.34, 154.37, 187.32	
AI-9-27 (7g)		226-227	^{1}H-NMR (600 MHz, DMSO): δ7.34-7.35 (1H, d, *J* = 3.66 Hz), 7.39-7.41 (2H, m), 7.62-7.65 (1H, dd, *J* = 8.04, 8.04 Hz), 7.78 (1H, s), 7.84-7.86 (1H, d, *J* = 7.38 Hz), 14.83 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ110.73, 115.27, 117.13, 119.54, 121.38, 123.51, 131.29, 131.64, 143.61, 149.33, 150.37, 154.15, 187.36	m/z $[M-H]^-$ calcd for $C_{13}H_7F_3N_2O_2S_2$; 342.9828; found: 342.9830
AI-8-57 (7h)		202-204	^{1}H-NMR (800 MHz, DMSO): δ7.39-7.40 (1H, d, *J* = 3.86 Hz), 7.42-7.43 (1H, d, *J* = 3.76 Hz), 7.52-7.54 (1H, dd, *J* = 7.96, 7.96 Hz), 7.73-7.74 (1H, dd, *J* = 1.44, 8.00 Hz), 7.87-7.88 (1H, dd, *J* = 1.44, 7.92 Hz), 14.88 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ114.67, 114.76, 127.83, 128.39, 129.17, 129.91, 130.95, 133.67, 143.63, 150.22, 151.53, 187.55	m/z $[M+H]^+$ calcd for $C_{12}H_6Cl_2N_2OS_2$; 327.9299; found: 327.9293
AI-8-103 (7i)		212-214	^{1}H-NMR (600 MHz, DMSO): δ3.91 (3H, s), 7.21-7.22 (1H, d, *J* = 3.66 Hz), 7.28-7.29 (1H, d, *J* = 8.10 Hz), 7.30-7.31 (1H, d, *J* = 3.66 Hz), 7.76-7.77 (1H, dd, *J* = 2.07, 8.61 Hz), 7.89-7.90 (1H, d, *J* = 2.16 Hz), 14.77 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ56.67, 108.46, 113.61, 115.49, 122.10, 123.01, 124.89, 125.87, 142.57, 154.54, 155.31, 187.05	m/z $[M-H]^-$ calcd for $C_{13}H_9ClN_2O_2S_2$; 322.9721; found: 322.9720
AI-8-89 (7j)		>250	^{1}H-NMR (800 MHz, DMSO): δ7.34-7.35 (1H, d, *J* = 3.76 Hz), 7.35-7.36 (1H, d, *J* = 3.68 Hz), 7.55-7.57 (1H, m), 7.82-7.84 (1H, m), 8.06-8.07 (1H, m), 14.84 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ110.08, 115.17, 118.32, 121.08, 125.35, 126.53, 127.39, 143.63, 150.65, 153.44, 156.96, 158.53, 187.13	m/z $[M-H]^-$ calcd for $C_{12}H_6ClFN_2OS_2$; 310.9521; found: 310.9520
AI-9-54 (7k)		175-177	^{1}H-NMR (800 MHz, DMSO): δ3.36 (3H, s), 3.72-3.73 (2H, t), 4.26-4.27 (2H, t), 7.22-7.23 (1H, d, *J* = 3.60 Hz), 7.28-7.29 (1H, d, *J* = 8.80 Hz), 7.31-7.32 (1H, d, *J* = 3.12 Hz), 7.73-7.74 (1H, dd, *J* = 2.20, 8.60 Hz), 7.89-7.90 (1H, d, *J* = 2.24 Hz), 14.80 (1H, s)	^{13}C-NMR (600 MHz, DMSO): δ58.68, 68.88, 70.47, 108.39, 114.47, 115.21, 122.35, 122.88, 124.83, 125.89, 142.71, 150.43, 154.69, 154.83, 187.07	m/z $[M-H]^-$ calcd for $C_{15}H_{13}ClN_2O_3S_2$; 366.9983; found: 366.9986

Mouse and human cell lines

Kasumi-1 (ATCC), Jurkat E6-1 (ATCC), 8946 T-ALL, and 720 T-ALL cell lines were cultured in RPMI 1640 (Cellgro) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C under 5% CO_2. K562 (ATCC) cell line is cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% FBS. 720 T-ALL cells were derived from a *Tcf12*$^{+/-}$ mouse expressing a *Tal1* transgene under the control of the *Lck* promoter [12]. 8946 T-ALL cells are derived from a murine T-ALL induced with a doxycycline-repressible human *c-MYC* transgene [13].

MTT Cell Proliferation Assay

Leukemia cell lines (10^4 cells/200 µl) were plated in a 96-well flat-bottom plate and cultured with Dimethylsulfoxide (DMSO) (vehicle) (Sigma-Aldrich), 1 µM Staurosporine (Sigma-Aldrich), or RDIs (12.5, 25, 50, or 100 µM) for 24, 48, and 72 hours. After treatment, 10 µl of 5 mg/ml MTT solution (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (Invitrogen) was added to each well and MTT assay was performed according to the manufacturer's instructions.

CFU-C Assay

Frozen human AML samples, and mouse leukemic bone marrow cells were thawed and cultured in RPMI 1640 with 10% FBS for two hours. Live cells were washed and recovered, and subsequently plated in Human Methylcellulose Complete Media HSC003 (R&D Systems) in 5% CO_2 at 37°C for 14 days, or in Methocult GF M3434 (Stem Cell Technologies) and incubated in 5% CO_2 at 37°C for 7 days, for human and mouse cells, respectively.

Western blotting

Murine leukemic cells treated with RDIs or DMSO were harvested and lysed with RIPA buffer (25mM Tris•HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). Total protein lysates were resolved on 4-12% SDS-PAGE gels, transferred to a nitrocellulose membrane (GE Life Sciences), and probed with primary antibodies. Proteins of interest were visualized by chemi-luminescence (Pierce). The following antibodies were

used for immunoblotting: caspase-3 (Cell Signaling, #9662), p53 (Santa Cruz, DO-1; Leica, CM5), and actin (Santa Cruz, N21).

Flow cytometry

Mouse and human cells: Cells were stained with fluorochrome-conjugated antibodies for 30 minutes at 4°C and washed with 2% FBS in PBS prior to analysis. Apoptosis analysis (Annexin V-APC; BD Biosciences) was performed according to the manufacturer's recommendations on a LSR II flow cytometer (BD Biosciences). The data were analyzed using FlowJo v.9.8 (TreeStar).

Zebrafish embryos: Transgenic embryos (5 per tube x $\geq$5 replicates) were incubated in 0.5mg/ml Liberase (Roche) solution with gentle agitation at 37°C for 90 minutes, then dissociated, filtered, and washed with PBS. Dead cells were excluded by SYTOX red (5nM, Life Technologies) prior to analysis using a BD FACS Canto II or Beckman Coulter Gallios.

Gene expression analysis (microarray)

RNA for microarray was isolated with the RNeasy Kit (QIAGEN). Total RNA quantity and integrity were verified using Bioanalyzer (Agilent Technologies), and amplified using Ambion WT Expression Kit (Applied Biosystems). Microarray experiments were performed on GeneChip Mouse Gene 2.0 ST Array (Affymetrix) at the University of Pennsylvania Molecular Profiling Facility, according to the manufacturer's instructions. Significantly perturbed KEGG pathways [14] were identified using the functional annotation tool available at DAVID (http://david.abcc.ncifcrf.gov/summary.jsp) [15].

Affymetrix probe intensity (cel) files were analyzed using Partek Genomics Suite (v6.6, Partek, Inc., St. Louis, MO). The data was normalized using Robust Multichip Average Algorithm (RMA), and technical controls were excluded to leave 34,365 transcript IDs available for statistical analysis. A one-way ANOVA followed by 3 pairwise comparisons (t-tests) were performed across the samples, each yielding a p-value for each transcript ID. The p-values were further corrected using the Benjamini-Hochberg procedure for false discovery rate (FDR). Fold-change in expression level for each transcript ID was calculated for the 3 pairwise comparisons.

To identify genes that are differentially expressed following treatment, the data was filtered to retain transcript

IDs that demonstrated a false discovery rate of 5% and have a mean fold change of at least 1.5, up or down in AI-7-54 vs AI-8-45 treated cells. 87 IDs (78 unique genes) met these cutoffs. Hierarchical clustering was performed using Pearson correlation and average linkage. The colors red and blue are used to indicate the log-2 intensity of each gene relative to the mean of AI-7-54 treatment.

Gene expression analysis (quantitative real-time PCR).

RNA for quantitative real-time PCR (qRT-PCR) was isolated with the RNeasy Kit (QIAGEN), and total RNA was reverse-transcribed using cDNA Reverse Transcription Kit (Applied Biosystems). The cDNA produced was used for quantitative real-time PCR using SYBR Green technology (Applied Biosystems).

qPCR primers are listed below:

	Sybr green primer set	
Gene	Forward	Reverse
Dhcr24	CAT CGT CCC ACA AGT ATG	CTC TAC GTC GTC CGT CA
Cdkn1a	TTC CGC ACA GGA GCA AAG T	CGG CGC AAC TGC TCA CT
Deptor	TTG TCG TCT CTG TCA ATG GCC TCA	TTG TCC TTG GGC CTG TCA GAA TCA
Csf1r	ACC AAA TGG CCC AGC CTG TAT TTG	TGC TTG GCA GGT TAG CAT AGT CCT
Cebpa	TGA GAA AAA TGA AGG GTG CAG	CGG GAT CTC AGC TTC CTG T
Hprt	CTC CTC AGA CCG CTT TTT GC	TAA CCT GGT TCA TCA TCG CTA ATC

References

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