

Supplementary Information

An 8-bit monochrome palette of fluorescent nucleic acid sequences for DNA-based painting

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Experimental Procedures

Sequence Design

The design of DNA oligonucleotides is based on a permutation scheme allowing for the parallel synthesis of all possible combinations of 5 consecutive nucleotides immediately adjacent to a terminal fluorescent dye. The total amount of combinations is 4^5 , or 1024 unique sequences. Downstream of the permutation sequence (P_1 to P_5), an adjustment section of 15 nucleotides is added, separated from the permutation section with a T_{15} spacer. The adjustment section can be represented in the form N_1 to N_5 , where each N_x is a trinucleotide in the form “ACGT - P_x ”. Therefore, if P_1 is T, N_1 is ACG. This adjustment section serves as a way to guarantee the same amount of A, C, G and T nucleotides in each oligonucleotide, regardless of the nature of the permutation. This is important because of the slightly lower coupling efficiency of G phosphoramidites, which would result in a lower amount of G-rich sequences and a deceptively lower fluorescence. By keeping a constant nucleotide content, the synthesis efficiency is expected to be largely homogenous across all 1024 oligonucleotides. A final T_5 linker is added to further distance the oligonucleotide sequence from the microarray surface. Conceptually, the oligonucleotide design can be represented as follows:



Microarray Synthesis

The synthesis of oligonucleotide by Maskless Array Synthesis (MAS) follows previously published procedures that have seen several rounds of technical and experimental improvements over time¹⁻⁷. The synthesis occurs on glass microscope slides (Schott Nexterion D) which have been silanized with hydroxybutyramide prior to synthesis. Synthesis follows standard phosphoramidite chemistry with 3'-protected 5' nucleoside phosphoramidites and is therefore of 5'→3' directionality (so-called “reverse mode”). The sole difference with conventional solid-phase synthesis is the use of a photosensitive protecting group (benzyl-NPPOC, bzNPPOC) instead of the acid-sensitive DMTr group⁸. Photodeprotection is accomplished by exposing the BzNPPOC to 365 nm UV light while the microarray slide is immersed in 1% solution of imidazole in DMSO. UV light, generated by a UV-LED (Nichia), is reflected by a Digital Micromirror Device (DMD, Texas Instruments) as a pattern corresponding to “ON” and “OFF” mirrors, where light reflected from “ON” mirrors will be imaged onto the array surface, triggering photodeprotection on the corresponding area. “OFF” mirrors reflect UV light away from the surface, where the corresponding area will remain unexposed. Only exposed areas and the correspondingly 5'-OH photodeprotected oligonucleotides will be able to couple with the next incoming phosphoramidite (3'-BzNPPOC, Orgentis), while unexposed areas will remain unaffected by the coupling event. Phosphoramidites are prepared as 30 mM solutions in dry ACN and coupled for 60 s in 0.25 M dicyanoimidazole in ACN. After coupling, unreacted 5'-OH are capped by performing a second coupling event with a DMTr-dT phosphoramidite which essentially counts as capping since DMTr group are not removed in MAS. The final coupling is the labelling with Cy3 phosphoramidite

(LinkTech), which is performed with a 50 mM solution of Cy3 in ACN and with 2 x 5 min coupling time. Photolysis is carried out by exposing the selected area to a radiant exposure of 6 J/cm² (~1 min at 100 mW/cm² irradiance) which removes 99% of BzNPPOC groups. The delivery of reagents and solvents to the microarray surface encased in a custom-made reaction chamber is done with an automated DNA synthesizer (Expedite 8909, PerSeptive Biosystems).

Data Extraction and Analysis

After synthesis, the microarrays are immediately washed in ACN for 2 h at r.t. to remove unbound Cy3, dried in a microarray centrifuge then scanned on a microarray scanner at 5 or 2.5 μm resolution (GenePix 4100A or 4400A) using a 532 nm laser excitation wavelength. After the initial scanning, the arrays are transferred in a 1:1 solution of ethylenediamine in ethanol in a glass jar overnight at r.t. to remove phosphate and base protecting groups. The arrays are then washed in 2 × 20 ml H₂O, dried, and scanned again. Scan images in .tif format are analysed with NimbleScan (NimbleGen) and the extracted data processed with Excel. The reported fluorescence intensity data are an average across all replicates, are corrected for background fluorescence and are the result of at least three independent measurements. Background fluorescence corresponds to the fluorescence intensity of a non-labelled version of each sequence and each exposure parameter.

UV exposure gradient and scale calibration

The gradient of UV illumination performed immediately prior to dye coupling ranged from 0 to 6 J/cm² of total irradiance, where ~4J/cm² is expected to remove ~95% of 3'-BzNPPOC protecting groups. UV illumination was carried out in incremental steps of 0.4 J from 0 to 4 J/cm², along with two additional exposure steps at 5 and 6 J/cm², where the photolysis rate has reached a plateau. Smaller increments are difficult to reliably measure because of incompressible time for electronic communication between DMD and shutter. With a total of 13 exposure increments for each of the 1024 sequence permutations, a total of 13312 elements was generated. The synthesis of the exposure gradient for the 3' terminally-labeled sequence permutation library was otherwise carried out as explained in the previous section. After synthesis and deprotection, the overall range of fluorescence was ranked by intensity, there again adopting a sigmoidal shape whose darkest point corresponds to background fluorescence, i.e. sequences exposed for 0 J/cm² (no labeling). The linear section of the curve was selected to construct a linear gradient of fluorescence brightness and signal intensity was divided into 256 increments, or bins. To account for potential errors in identifying a single suitable sequence/exposure tandem for each bin, we instead brought together all elements within ±10% of each incremental fluorescence and randomly chose 5 elements to be representative of each of the 256 bins.

Green scale DNA printing

Input grayscale images in 8-bit format, from public domain or from personal records, were first downscaled to a maximum of 1024 × 768 resolution. The intensity value for each pixel as well as its Cartesian coordinate was fed into a python script that randomly allocates a sequence and its exposure

rate from the bin of corresponding signal intensity. The result grid input was then transformed into a series of digital masks and synthesis instructions using MatLab (MathWorks) and a script designed in-house.

Additional Data

Supplementary File S1. Python script for the automated attribution of a sequence/exposure pair to any given grayscale color intensity in 8-bit monochrome graphical input.

```
-----  
from PIL import Image  
import numpy as np  
import random  
  
# TAKES SINGLE LINED TXT FILE OF SEQUENCES WITH NAMES (RANKED FROM LOWEST TO  
# HIGHEST) AND MAKES AN ARRAY OUT OF IT  
RealSeqRange = np.loadtxt("SelectedSeqGradRange.txt", dtype=str)  
RealSeqRange = RealSeqRange.reshape((len(RealSeqRange), 1), order='F')  
# print(RealSeqRange)  
  
RealSeqIndex = np.loadtxt("SelectedSeqGradIndex.txt", dtype=str)  
RealSeqIndex = RealSeqIndex.reshape((len(RealSeqIndex), 1), order='F')  
# print(RealSeqIndex)  
  
RealSeqCount = np.loadtxt("SelectedSeqGradCount.txt", dtype=str)  
RealSeqCount = RealSeqCount.reshape((len(RealSeqCount), 1), order='F')  
# print(RealSeqCount)  
SequenceListFinal = [None] * (256*5) # from 256  
# print(len(RealSeqCount))  
# print(len(RealSeqRange))  
random.seed(1)  
countA = 0  
countB = 0  
CountC = 0
```

```

for i in range(256):
    countB = countB + int(str(RealSeqCount[i])[2:-2])
    # print(countA, countB)
    y1 = random.choice(range(countA, countB))
    y2 = random.choice(range(countA, countB))
    y3 = random.choice(range(countA, countB))
    y4 = random.choice(range(countA, countB))
    y5 = random.choice(range(countA, countB))
    countA = countB + 1
    # print(countA)
    SequenceListFinal[CountC] = str(RealSeqRange[y1])[2:-3]
    CountC = CountC + 1
    SequenceListFinal[CountC] = str(RealSeqRange[y2])[2:-3]
    CountC = CountC + 1
    SequenceListFinal[CountC] = str(RealSeqRange[y3])[2:-3]
    CountC = CountC + 1
    SequenceListFinal[CountC] = str(RealSeqRange[y4])[2:-3]
    CountC = CountC + 1
    SequenceListFinal[CountC] = str(RealSeqRange[y5])[2:-3]
    CountC = CountC + 1

with open('BinnedSeq8bit.txt', 'w') as file_handle:
    for list_item in SequenceListFinal:
        file_handle.write('%s\n' % list_item)
# OPENS INPUT BMP FILE AS ARRAY
Img = Image.open('ExperimentTest3.bmp') # from ExperimentTest3
Img = np.array(Img)

# CONTROL
# print(Img)

# MODIFIES INPUT ARRAY IN A SINGLE LINE FILE GOING FROM 0-768 (for 1024 times)
grid = (Img.reshape((786432, 1), order='F'))
# print(grid)

# SAVES THE GRID FILE AS TXT AND OPENS IT AS A LIST
np.savetxt("single_line.txt", grid, delimiter="", fmt='%5.0f')

Sequence = np.array(grid)
Sequence = Sequence.astype('str')

```

```

SequenceList = Sequence.tolist()

# OLD PART THAT DEFINES BACKGROUND IN COLOURED BMP INPUT
# for i in range(len(SequenceList)):
#   if SequenceList[i]=='7':
#     SequenceList[i] = 's,Background'

# TAKES SINGLE LINED TXT FILE OF SEQUENCES WITH NAMES (RANKED FROM LOWEST TO
# HIGHEST) AND MAKES AN ARRAY OUT OF IT
RealSeq = np.loadtxt("BinnedSeq8bit.txt", dtype=str)
RealSeq = RealSeq.reshape((len(RealSeq), 1), order='F')
# print(RealSeq)

# SEED IS NOT NEEDED BUT STAYS FOR TESTING JUST IN CASE
random.seed(1)

# LOOPS OVER ARRAY TO MATCH GRAYSCALE PIXEL VALUE-LOCATION TO ALLOCATED
# SEQUENCE (per SEQ MAP)
# AND EXPORT A FILE READY FOR INPUT IN MATLAB PROGRAM
SequenceListFinal = SequenceList
x = (RealSeq.tolist())

# len(SequenceList)
j = 0
for i in SequenceList:
    y = random.choice(range((int(str(i)[2:-2])*5), ((int(str(i)[2:-2])*5)+5)))
    SequenceListFinal[j] = str(x[y])[3:-2]
    j = j + 1

with open('SeqForMatlab8bit.txt', 'w') as file_handle:
    for list_item in SequenceListFinal:
        file_handle.write('%s\n' % list_item)

# CORRECTES FOR EXPOSURE PRIOR TO CY3 COUPLING

ExposureSeq1 = np.loadtxt("SeqForMatlab8bit.txt", dtype=str)
ExposureSeq1 = ExposureSeq1.reshape((len(ExposureSeq1), 1), order='F')
ExposureSeq1 = ExposureSeq1.tolist()

ExposureSeq2 = np.loadtxt("SeqForMatlab8bit.txt", dtype=str)

```

```
ExposureSeq2 = ExposureSeq2.reshape((len(ExposureSeq2), 1), order='F')
ExposureSeq2 = ExposureSeq2.tolist()
```

```
for i in range(len(ExposureSeq1)):
    ExposureSeq1[i] = str(ExposureSeq1[i][-5:-2])
    ExposureSeq2[i] = str(ExposureSeq2[i][37:-2])
    if str(ExposureSeq1[i]) == str(0.4):
        ExposureSeq2[i] = str("9" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "0.8":
        ExposureSeq2[i] = str("99" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "1.2":
        ExposureSeq2[i] = str("999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "1.6":
        ExposureSeq2[i] = str("9999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "2.0":
        ExposureSeq2[i] = str("99999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "2.4":
        ExposureSeq2[i] = str("999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "2.8":
        ExposureSeq2[i] = str("9999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "3.2":
        ExposureSeq2[i] = str("99999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "3.6":
        ExposureSeq2[i] = str("999999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "4.0":
        ExposureSeq2[i] = str("9999999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "5.0":
        ExposureSeq2[i] = str("99999999999" + str(ExposureSeq2[i]))
    if str(ExposureSeq1[i]) == "6.0":
        ExposureSeq2[i] = str("999999999999" + str(ExposureSeq2[i]))
```

```
with open('SeqForMatlabExposure8bit.txt', 'w') as file_handle:
```

```
    for list_item in ExposureSeq2:
        file_handle.write("%s\n" % list_item)
```

Table S1. Fluorescence rankings of 3'-Cy3 labeled ssDNA of all possible pentamers 5' to the dye. Fluorescence is normalized to that of the brightest sequence combination.

Sequence:	Norm. fluor.	SEM									
GCGCG	1.000	0.035	AGAGG	0.594	0.040	AGCCC	0.554	0.034	GGAGT	0.529	0.043
GCGCT	0.964	0.064	AGAGT	0.593	0.044	GGGAG	0.553	0.041	AGATC	0.529	0.026
GCGCC	0.944	0.034	TGGTT	0.593	0.047	GGCAA	0.552	0.040	AAGTT	0.527	0.030
GCGCA	0.876	0.027	GGGGA	0.591	0.028	AGACG	0.552	0.035	AGACC	0.527	0.028
GGCGC	0.793	0.047	AGAAT	0.589	0.024	AGCCT	0.552	0.031	GCGAG	0.526	0.059
AGCGC	0.792	0.034	AGGCA	0.588	0.029	GGCCA	0.551	0.059	AAGCG	0.525	0.028
AGGGG	0.757	0.038	TGGGA	0.587	0.027	CGCGG	0.551	0.064	GGCAC	0.525	0.031
AGGGT	0.725	0.054	TGGGT	0.586	0.044	GGGAT	0.550	0.037	AGTCG	0.525	0.036
AGGGC	0.715	0.052	GGGTG	0.586	0.041	GGGTT	0.549	0.029	GGAGA	0.525	0.055
AGGCC	0.714	0.045	TGGAA	0.585	0.052	GGGTA	0.548	0.037	AGTTT	0.524	0.030
TGCGC	0.697	0.048	GGCAG	0.585	0.076	AGCTG	0.548	0.034	AAGCT	0.524	0.043
AGGCG	0.689	0.033	AGAAG	0.582	0.029	AGTTG	0.548	0.046	AGTAA	0.524	0.030
AGGAG	0.669	0.059	AGCGA	0.580	0.052	AAGGC	0.548	0.058	CGCGA	0.523	0.038
CGCGC	0.660	0.052	AGTAT	0.579	0.047	AGCCG	0.547	0.034	AAAGT	0.522	0.041
ACGCG	0.657	0.043	AGTAC	0.579	0.038	GGTGA	0.546	0.076	GCGGG	0.521	0.062
GGGCC	0.655	0.076	GGGCA	0.579	0.050	TGGTG	0.544	0.031	GGTAC	0.521	0.075
GGGGC	0.650	0.045	GGCGA	0.577	0.042	AGAAC	0.543	0.026	GCGGA	0.521	0.049
AGGCT	0.648	0.024	GGGAC	0.574	0.026	TGGAT	0.543	0.033	AGCAG	0.520	0.025
AGGGA	0.642	0.037	AGAAA	0.568	0.030	GGCGT	0.543	0.032	TGCGA	0.520	0.023
AGGTT	0.638	0.054	TGGCG	0.566	0.036	GGCCT	0.539	0.037	AAAAT	0.520	0.028
GGGGG	0.635	0.030	TGGCA	0.565	0.045	TGAGT	0.539	0.029	AGCAA	0.519	0.070
AGGAA	0.635	0.045	GGCCG	0.564	0.072	TGAGG	0.537	0.048	AGAGA	0.519	0.029
TGGGG	0.626	0.053	AGTGT	0.564	0.039	AGTGA	0.536	0.037	AGTAG	0.518	0.062
TGGCC	0.624	0.064	GGGTC	0.563	0.043	TGGAC	0.534	0.037	GGAAT	0.518	0.045
AGCGG	0.618	0.069	GGGCG	0.563	0.045	GGAGG	0.534	0.029	ATGGG	0.517	0.030
AGGAT	0.617	0.032	GGCCC	0.562	0.072	GGAGC	0.534	0.034	AGCTA	0.517	0.044
AGCGT	0.616	0.044	TGGTA	0.561	0.028	AAAGG	0.534	0.035	TGCGT	0.516	0.061
TGGGC	0.616	0.068	AGTCT	0.560	0.037	AGCTT	0.533	0.067	CGGGT	0.516	0.027
GGGGT	0.613	0.042	TGGAG	0.560	0.050	AGATG	0.533	0.039	GGATA	0.516	0.030
GCGGC	0.611	0.053	AAGGA	0.559	0.021	AAGAT	0.533	0.052	GCGGT	0.516	0.065
AGGTG	0.606	0.037	GGGCT	0.558	0.044	CGGGG	0.532	0.033	GGACG	0.516	0.039
AGGTC	0.605	0.047	GGGAA	0.558	0.029	AGATT	0.531	0.036	AGCCA	0.515	0.031
GGCGG	0.604	0.054	TGCGG	0.558	0.057	AGTGC	0.531	0.037	TGAAA	0.514	0.049
AGGAC	0.600	0.034	TGGTC	0.557	0.035	AGACT	0.531	0.036	GGCAT	0.514	0.040
AGGTA	0.599	0.041	AAGGT	0.557	0.029	AGTTC	0.530	0.070	AAGTC	0.514	0.030
AAGGG	0.596	0.068	AGTGG	0.555	0.040	AGAGC	0.530	0.038	TGGCT	0.513	0.034
			AGTTA	0.555	0.030	TGAAG	0.529	0.056	AAATG	0.513	0.028

AAGAG	0.513	0.026
GCGAA	0.513	0.033
GGAAG	0.512	0.026
AAGTG	0.511	0.032
GGTGT	0.511	0.029
GGACC	0.511	0.057
GGACA	0.511	0.053
CGGCC	0.511	0.041
TGTGG	0.511	0.037
GGATG	0.510	0.100
GGTAA	0.510	0.048
TGACG	0.510	0.055
GAGCG	0.510	0.085
AAGTA	0.510	0.053
GGCTC	0.508	0.037
GAGGA	0.507	0.034
GAGCA	0.507	0.078
AAATA	0.507	0.069
GGATC	0.507	0.036
TGTAA	0.507	0.031
GGCTG	0.506	0.027
TAGGG	0.506	0.024
CGGCG	0.506	0.029
GCCGC	0.506	0.046
ACGGG	0.505	0.024
GGCTA	0.504	0.027
AACGG	0.504	0.041
TGAGA	0.503	0.029
GGAAA	0.502	0.032
GCGAC	0.502	0.043
GAGCC	0.501	0.072
TGATT	0.500	0.030
AGTCC	0.499	0.086
GCGTG	0.499	0.028
AAACA	0.499	0.064
AAACG	0.497	0.030
TGTGT	0.497	0.026
TAGAA	0.496	0.081
GAGGT	0.494	0.035
GCGAT	0.494	0.055

TGTTG	0.494	0.027
ATGAA	0.493	0.074
AATGA	0.493	0.037
ACGGC	0.493	0.034
TGAAT	0.493	0.031
GAGAG	0.492	0.024
ATGGT	0.492	0.033
CGGAA	0.492	0.039
GCAGC	0.492	0.024
ATGAT	0.491	0.042
AGCAT	0.491	0.031
AAAAG	0.491	0.052
AGCTC	0.490	0.030
AAGCC	0.490	0.031
GCGTA	0.490	0.035
GGATT	0.489	0.055
AGATA	0.489	0.045
GGAAC	0.489	0.029
TGTAT	0.489	0.046
GAGAA	0.489	0.086
TTGGG	0.489	0.031
ATGGC	0.488	0.029
TGACT	0.488	0.050
TGCCG	0.487	0.022
GAGGC	0.487	0.024
AAATT	0.487	0.042
ATGTT	0.487	0.053
CGGGC	0.487	0.024
ATGAG	0.486	0.035
TGTTA	0.486	0.037
GGACT	0.486	0.034
GGCTT	0.485	0.024
TCGCG	0.485	0.025
GTGGC	0.484	0.031
AAAGA	0.484	0.055
TGCCT	0.484	0.027
GGTGC	0.483	0.032
GGTGG	0.483	0.055
ACGTG	0.483	0.028
GCGTC	0.483	0.051

GGTAG	0.482	0.023
TGCAT	0.482	0.038
GTGGA	0.482	0.017
TGATA	0.482	0.041
GGTCA	0.481	0.023
AAGAA	0.481	0.022
ATAGT	0.481	0.032
TGCCC	0.480	0.021
CGCGT	0.480	0.034
ATAGG	0.480	0.045
GAGTC	0.479	0.041
TGCCA	0.479	0.034
TGTAG	0.478	0.040
CGGGA	0.478	0.064
GAGCT	0.478	0.035
GAGGG	0.478	0.026
AATGT	0.478	0.030
TGAAC	0.478	0.039
TTGGT	0.477	0.037
GAGAT	0.477	0.059
TGAGC	0.477	0.058
AAAAA	0.476	0.026
GGTTA	0.476	0.055
ACGCC	0.475	0.024
AAAGC	0.475	0.057
AGTCA	0.475	0.024
GCATG	0.475	0.031
TAGCG	0.474	0.037
TGCAG	0.474	0.028
TGACA	0.474	0.030
TGTGA	0.474	0.033
TGCAC	0.473	0.030
GAGTG	0.472	0.057
ACGGT	0.472	0.032
GTGCA	0.472	0.028
TAGGC	0.472	0.033
AGCAC	0.471	0.023
GGTTG	0.471	0.028
AAGAC	0.471	0.027
TAGTA	0.471	0.030

CGGCT	0.470	0.042
GGTCC	0.470	0.054
GGTTC	0.470	0.050
ATGTA	0.470	0.039
GGTCG	0.470	0.032
AAAAC	0.470	0.030
CGGAC	0.469	0.032
TAGAC	0.469	0.021
GGTAT	0.466	0.023
ATGCG	0.466	0.026
GAGTA	0.466	0.040
TGTGC	0.466	0.037
ATGCA	0.465	0.024
ATGGA	0.465	0.060
CGGAG	0.465	0.033
CGGAT	0.464	0.026
TGTAC	0.464	0.031
TTGGA	0.464	0.080
TAGAT	0.463	0.020
AACGT	0.463	0.026
GCTGC	0.463	0.024
GAAGC	0.463	0.021
AATCG	0.463	0.032
ACGGA	0.463	0.017
TAGGA	0.463	0.028
GTGAC	0.463	0.025
AAACT	0.462	0.051
CGCAG	0.462	0.065
GAGAC	0.462	0.030
TAAAA	0.462	0.077
GAATG	0.462	0.069
ATGAC	0.461	0.022
ACGTA	0.460	0.036
GCGTT	0.460	0.036
TGTTT	0.460	0.028
GATGA	0.460	0.032
AGACA	0.459	0.036
TGATG	0.459	0.032
TGTCC	0.459	0.038
TGACC	0.458	0.021

AATGG	0.458	0.031
TAGGT	0.458	0.036
TGTCG	0.458	0.039
ATAAT	0.458	0.047
ATGCT	0.457	0.043
AAATC	0.457	0.021
CGGTT	0.457	0.023
GTGAA	0.457	0.041
GAAGA	0.456	0.024
ACGTT	0.456	0.039
GTGGT	0.456	0.022
TAGTC	0.455	0.036
GCAGG	0.455	0.019
GTGAT	0.455	0.053
TAAAT	0.454	0.038
AATCT	0.454	0.047
AACTG	0.453	0.040
TAGTT	0.453	0.030
TAAGG	0.453	0.043
GTGTA	0.452	0.034
AAGCA	0.452	0.026
AATAG	0.452	0.031
ACGTC	0.452	0.046
ACGAA	0.451	0.026
TGCTG	0.451	0.032
ATAAA	0.451	0.031
TGATC	0.451	0.029
TGCAA	0.450	0.024
TGTCA	0.450	0.060
GCCGG	0.449	0.043
TGCTA	0.449	0.027
TAAGA	0.449	0.024
ACGAC	0.448	0.027
ACGAT	0.447	0.024
ATTGG	0.446	0.071
AATAA	0.446	0.022
GACGC	0.446	0.029
AATTG	0.446	0.027
TTGCC	0.446	0.059
GAAGG	0.445	0.030

AAACC	0.445	0.050
GTGAG	0.445	0.054
CGGTA	0.445	0.026
ACGCA	0.445	0.028
GGTTT	0.444	0.027
GCTGA	0.444	0.036
CGCTG	0.444	0.032
GATGT	0.443	0.063
GCAGA	0.443	0.033
AACGC	0.443	0.035
TCGGT	0.442	0.053
TCGGG	0.442	0.024
AATAT	0.441	0.069
CGATG	0.441	0.081
GTGCT	0.441	0.029
TAGCT	0.441	0.022
GAAGT	0.440	0.024
CGAGG	0.439	0.028
CGCAT	0.439	0.032
AATTT	0.439	0.024
CGCTA	0.439	0.020
CGGTG	0.439	0.041
TAGAG	0.439	0.023
GTGCG	0.439	0.023
GCCAT	0.439	0.027
ACTGT	0.439	0.080
GTAGA	0.439	0.018
CGGTC	0.439	0.035
GATTG	0.438	0.067
CGCAC	0.438	0.036
GTAGC	0.438	0.023
GCCGA	0.437	0.029
TAAGT	0.436	0.035
CGCAA	0.436	0.057
ATGCC	0.436	0.028
TTGTG	0.435	0.082
TCGGA	0.435	0.028
CGAGA	0.435	0.046
GATGC	0.435	0.030
TAATG	0.435	0.043

ACAGG	0.435	0.053
GTGCC	0.435	0.029
TAGCC	0.435	0.022
TTGAC	0.434	0.015
ATGTG	0.434	0.027
ATAGA	0.434	0.019
CGCCG	0.434	0.033
ACGCT	0.434	0.029
AACAT	0.433	0.059
GATCA	0.433	0.025
TTGAA	0.433	0.024
TTGCA	0.433	0.029
GCAGT	0.433	0.063
CGGCA	0.433	0.049
TGTCT	0.433	0.020
ATTTA	0.432	0.031
TTGTA	0.432	0.035
TGCTT	0.432	0.025
AATCC	0.432	0.031
GATGG	0.431	0.046
ATACG	0.431	0.024
TCGGC	0.431	0.021
ATATA	0.431	0.042
GAAAG	0.431	0.056
GTGTG	0.430	0.030
ATTAA	0.430	0.027
GGTCT	0.430	0.035
GCATA	0.430	0.029
ACGAG	0.430	0.036
ACTAG	0.430	0.022
TAGCA	0.429	0.045
GCCTG	0.429	0.081
TAGTG	0.429	0.021
GAGTT	0.429	0.016
ACAGC	0.429	0.053
GAATA	0.429	0.031
TGTTT	0.429	0.024
ATGTC	0.429	0.072
GTGGG	0.428	0.024
GCCAG	0.428	0.043

AATAC	0.428	0.023
AATGC	0.428	0.024
TCGCC	0.428	0.034
AACCG	0.427	0.041
AACGA	0.427	0.030
GCCTA	0.427	0.035
ATAGC	0.427	0.025
GAATC	0.427	0.031
AATTC	0.427	0.039
TTGGC	0.427	0.024
GCCTC	0.426	0.028
CGAAC	0.426	0.070
GCAAA	0.426	0.028
GCAAT	0.426	0.037
CGAAA	0.426	0.021
GACGG	0.426	0.017
CGAGC	0.425	0.035
GAACA	0.425	0.021
GATAG	0.425	0.049
ACCGG	0.425	0.025
GTGTC	0.425	0.020
CGTAG	0.425	0.030
GACGT	0.425	0.029
GTCSA	0.424	0.058
ATTAG	0.424	0.034
GACAG	0.423	0.036
GCCAC	0.423	0.058
ACCGC	0.423	0.051
GTCSG	0.423	0.043
TTGCG	0.422	0.024
GATAC	0.422	0.027
GAACG	0.422	0.028
GTTGC	0.422	0.051
GCACA	0.422	0.054
AACTT	0.422	0.055
CGAGT	0.422	0.080
GATCG	0.422	0.053
TTGAG	0.422	0.024
ATAAC	0.422	0.042
GATTA	0.421	0.058

GACAA	0.421	0.019
GCCAA	0.420	0.035
TCGAT	0.420	0.035
GACGA	0.420	0.020
ATCGG	0.420	0.028
ATAAG	0.420	0.053
TAAAG	0.419	0.025
GTATG	0.419	0.027
TTCGA	0.419	0.041
AACAA	0.419	0.024
CGCCC	0.419	0.034
CGTGG	0.419	0.027
TTAGT	0.419	0.050
GCTCA	0.418	0.030
GATAT	0.418	0.032
TATTA	0.418	0.024
TATGT	0.418	0.031
TCGAC	0.418	0.022
TAATA	0.418	0.026
CGAAT	0.417	0.029
TTGCT	0.417	0.026
TATGA	0.417	0.028
GCATC	0.416	0.022
AATTA	0.416	0.035
TGCTC	0.416	0.024
ATCAG	0.416	0.029
ATTGT	0.415	0.023
AACAG	0.415	0.022
TCGTG	0.414	0.050
ACTGG	0.414	0.020
GATCC	0.414	0.023
AATCA	0.413	0.021
GACAT	0.413	0.030
TATAA	0.413	0.036
CGCCT	0.412	0.025
TCAGG	0.412	0.040
CGCTC	0.412	0.034
TAATT	0.412	0.019
GAATT	0.411	0.026
TTGTC	0.411	0.033

AACTA	0.411	0.035
GACTA	0.411	0.030
GCCCA	0.411	0.030
AACAC	0.411	0.021
ATTTT	0.411	0.028
TTGAT	0.411	0.032
ATATC	0.411	0.023
TATGG	0.411	0.025
ACAGT	0.411	0.026
TTAGA	0.410	0.018
TCGAG	0.410	0.022
GCTAG	0.410	0.025
TAAAC	0.410	0.061
TTAGG	0.410	0.061
TTCGC	0.409	0.047
ATTAT	0.409	0.035
GTACA	0.409	0.023
ATTGA	0.409	0.016
GTAGT	0.409	0.037
GTTGA	0.408	0.034
ATTCG	0.408	0.030
GATAA	0.408	0.017
GCAAC	0.408	0.039
TACTA	0.408	0.028
CTGGC	0.407	0.042
GTATA	0.407	0.024
ACTAT	0.407	0.053
ACAAA	0.407	0.023
ACTGA	0.407	0.029
CGATA	0.407	0.031
CGATC	0.407	0.040
CGCTT	0.406	0.088
CGCCA	0.406	0.026
TACGG	0.406	0.025
TAACG	0.406	0.027
ATCAA	0.406	0.019
ACAAT	0.405	0.033
TTGTT	0.405	0.024
CGTGA	0.405	0.024
TATAT	0.405	0.037

TACGA	0.405	0.032
TTCGG	0.405	0.023
GCTGG	0.405	0.028
ATATG	0.404	0.027
GCAAG	0.404	0.023
CGACA	0.404	0.024
ATCTT	0.404	0.036
TACCG	0.404	0.029
TTTGA	0.404	0.022
TTAGC	0.404	0.025
CAGGT	0.404	0.046
GACTG	0.404	0.051
TTATT	0.403	0.029
CGTCG	0.403	0.029
ATTTG	0.403	0.022
ACCGT	0.403	0.029
GAAAA	0.403	0.030
ATACA	0.403	0.038
TCGAA	0.402	0.026
CGACG	0.402	0.045
CGTGT	0.402	0.027
TAAGC	0.402	0.026
TTTGG	0.402	0.045
CGTTA	0.402	0.051
TTATC	0.402	0.056
AACCA	0.402	0.022
GCTAT	0.402	0.031
CAGGG	0.401	0.025
GATCT	0.401	0.031
TTCAG	0.401	0.021
TATGC	0.401	0.064
TTAAA	0.401	0.019
ATTTT	0.400	0.037
ATCGA	0.400	0.019
GTTCA	0.400	0.024
ATATT	0.400	0.028
CGTAA	0.400	0.035
CCGCG	0.400	0.041
ATCGT	0.399	0.041
GCACT	0.399	0.022

ATCGC	0.399	0.027
TCATG	0.399	0.025
GCTAA	0.399	0.026
ACATG	0.399	0.019
GTCAC	0.398	0.035
ATTAC	0.398	0.027
GCATT	0.398	0.080
ATTGC	0.398	0.052
GTGTT	0.397	0.019
CGAAG	0.397	0.027
CGTGC	0.397	0.027
GAAAT	0.397	0.021
TATAC	0.397	0.025
ACATT	0.397	0.025
GAAAC	0.396	0.026
TTTGT	0.396	0.060
TAACA	0.396	0.021
ATACT	0.396	0.024
TTACG	0.395	0.019
CGACC	0.395	0.020
AACTC	0.395	0.020
GATTC	0.395	0.039
TACGC	0.395	0.021
CGTAC	0.395	0.026
CAGCG	0.394	0.023
CGTCC	0.394	0.032
ACCGA	0.394	0.023
TAATC	0.394	0.027
TAACC	0.394	0.022
ACAAG	0.394	0.024
GCCCG	0.394	0.045
TACGT	0.394	0.020
ACCAG	0.394	0.024
GATTT	0.394	0.048
TCCGA	0.394	0.083
CCGCA	0.393	0.027
TTTGC	0.393	0.026
TCGTA	0.393	0.020
GCTCC	0.393	0.056
CGACT	0.393	0.047

GTTGG	0.392	0.022
GCTGT	0.392	0.023
TATCA	0.392	0.021
TCGCT	0.392	0.085
TTATA	0.392	0.024
TCGCA	0.391	0.029
TCTGG	0.391	0.031
TCGTC	0.390	0.049
GAACT	0.390	0.042
TACTG	0.390	0.030
GTCAG	0.390	0.032
TTTAA	0.390	0.025
GCCGT	0.389	0.029
ACTTG	0.389	0.044
ACCCG	0.389	0.058
CGTTG	0.389	0.025
TCAGA	0.389	0.039
TATTG	0.389	0.017
CAGGC	0.389	0.034
GACTC	0.389	0.030
AACCT	0.388	0.025
TACAG	0.388	0.025
GCTTA	0.388	0.021
ATCAT	0.388	0.028
GTAGG	0.388	0.026
GTCTG	0.387	0.025
CAGTG	0.387	0.064
CTGGT	0.387	0.040
GCACC	0.386	0.021
ACTCG	0.386	0.032
AACCC	0.386	0.022
TAACT	0.386	0.019
TCCGG	0.386	0.035
CGATT	0.385	0.023
GTACG	0.385	0.023
TTAAC	0.385	0.029
GACAC	0.384	0.019
TTCGT	0.384	0.038
GAACC	0.384	0.029
GACCG	0.384	0.031

GCTTC	0.384	0.019
TTACA	0.384	0.021
GTCCA	0.384	0.020
TTTCG	0.384	0.038
CAGGA	0.383	0.033
ACAGA	0.383	0.029
GTATC	0.383	0.029
TTTAG	0.383	0.028
GCACG	0.383	0.036
ACTTC	0.382	0.041
ACTAA	0.382	0.028
TTAAG	0.382	0.030
TATAG	0.382	0.018
TACAT	0.382	0.025
GCCCT	0.381	0.040
GTCAA	0.381	0.029
TTTTA	0.381	0.020
GTTAA	0.381	0.025
ATCTG	0.381	0.022
TTTAT	0.381	0.021
ATACC	0.380	0.033
TTTAC	0.380	0.031
CAGCA	0.380	0.035
ACACT	0.379	0.034
ATTCA	0.379	0.027
ACTGC	0.378	0.032
TCATA	0.378	0.019
TACTC	0.378	0.019
GTTCC	0.378	0.025
TACTT	0.378	0.021
GACCA	0.378	0.028
CAGCT	0.378	0.038
TTAAT	0.377	0.027
GCCCC	0.377	0.026
GTA CT	0.377	0.019
GACCT	0.377	0.020
CTGGG	0.377	0.024
CGTAT	0.377	0.024
ACTCC	0.377	0.042
CTGGA	0.376	0.019

GCTCT	0.376	0.039
CAGTA	0.376	0.020
TATCG	0.376	0.035
CATGC	0.376	0.053
ACACG	0.375	0.026
GTA AA	0.375	0.035
TTATG	0.375	0.030
ACACC	0.375	0.055
ACCAA	0.375	0.053
ACCAC	0.375	0.043
GCTAC	0.375	0.026
GCTCG	0.375	0.032
ACACA	0.374	0.031
GACCC	0.374	0.025
CAGCC	0.374	0.068
TACAA	0.374	0.021
GTTGT	0.373	0.017
GTCAT	0.373	0.027
ACCTA	0.373	0.038
ACTTT	0.373	0.046
ATTCT	0.373	0.022
CTGAC	0.373	0.086
CTGCA	0.373	0.025
ACATA	0.372	0.024
ACTAC	0.372	0.026
CAGAC	0.372	0.017
GCTTG	0.371	0.032
GTCTA	0.371	0.036
CATGA	0.371	0.027
GTTCT	0.371	0.032
TATCT	0.370	0.029
TTTCA	0.370	0.022
CCGGT	0.369	0.031
TCCGC	0.369	0.030
CAGAT	0.369	0.015
ATTCC	0.369	0.029
TATTC	0.369	0.025
ACAAC	0.368	0.022
CCGGA	0.368	0.064
ATCTA	0.368	0.025

CGTCT	0.368	0.024
TACAC	0.368	0.016
TTTTG	0.368	0.022
TCTGA	0.368	0.029
GTAAG	0.367	0.029
TCTTG	0.367	0.027
CCGGG	0.367	0.032
TTCCG	0.367	0.035
TCTGT	0.367	0.028
ATCCG	0.366	0.039
CTGCG	0.366	0.028
GT CCT	0.366	0.034
CAAGC	0.366	0.022
ATCCA	0.366	0.019
ACCTG	0.366	0.023
GCCTT	0.366	0.035
GTATT	0.366	0.024
CGTTC	0.365	0.022
CGTCA	0.365	0.028
TATTT	0.365	0.017
CCGGC	0.364	0.027
GTCCG	0.364	0.018
GTAAC	0.364	0.026
GTCCC	0.364	0.024
CAATG	0.364	0.026
ACATC	0.364	0.024
ACCAT	0.364	0.020
TACCA	0.364	0.024
TTCTG	0.364	0.030
GTAAT	0.363	0.023
GTTAC	0.363	0.021
CAGAA	0.363	0.018
CTGAT	0.363	0.023
TCAGC	0.363	0.028
TTCAA	0.362	0.026
TATCC	0.361	0.028
TCGTT	0.361	0.019
GTACC	0.361	0.035
TCATT	0.361	0.047
TCCAA	0.360	0.039

TCTGC	0.360	0.015
ATCTC	0.360	0.023
TCAAG	0.360	0.026
GTTAT	0.359	0.021
TFACT	0.359	0.028
TTCCC	0.359	0.025
CTGAG	0.359	0.017
CTAGA	0.359	0.023
CAAGA	0.358	0.022
TTCAT	0.358	0.039
GTCGT	0.358	0.022
GACTT	0.358	0.027
TCAGT	0.357	0.022
TTTTC	0.357	0.035
TTACC	0.357	0.020
CAGAG	0.357	0.018
TACCC	0.356	0.015
ACCTC	0.356	0.055
CTGTG	0.356	0.068
TCCGT	0.356	0.021
GTTAG	0.355	0.024
ATCCT	0.355	0.019
GTTTA	0.355	0.025
TCTAG	0.354	0.017
CTAGC	0.354	0.027
CAGTC	0.354	0.033
CAGTT	0.354	0.040
TTTCT	0.354	0.032
TCAAT	0.353	0.027
GTCTC	0.352	0.033
CAAGG	0.352	0.019
CTGTC	0.352	0.018
ATCAC	0.352	0.025
CTGCT	0.352	0.030
ACCCC	0.352	0.021
TTCAC	0.351	0.022
TTCTT	0.351	0.029
TTCCA	0.351	0.018
CTGTA	0.351	0.046
ACCCA	0.351	0.020

ACTTA	0.351	0.021
TTCTA	0.350	0.024
ACCTT	0.350	0.028
CTAGG	0.350	0.055
ATCCC	0.350	0.019
GTCCG	0.350	0.020
TCACA	0.350	0.033
TCAAA	0.349	0.028
CATGG	0.349	0.037
CTGAA	0.349	0.015
ACTCT	0.348	0.022
TCCTA	0.348	0.020
TTCTT	0.348	0.026
TTTCC	0.347	0.023
TCCTG	0.346	0.024
ACTCA	0.346	0.028
ACCCT	0.346	0.035
TCCAT	0.345	0.023
CTGTT	0.345	0.024
CAAGT	0.345	0.021
CTGCC	0.345	0.018
CATGT	0.344	0.022
TACCT	0.344	0.024
GTTTC	0.343	0.027
GTTCC	0.343	0.021
CACGC	0.342	0.060
TCTTC	0.342	0.027
TCAAC	0.342	0.018
CGTTT	0.341	0.032
TTTTT	0.340	0.015
TCCCA	0.340	0.021
TCCAG	0.340	0.026
TCACG	0.339	0.024
TCTAA	0.338	0.020
CATTG	0.338	0.055
GTCTT	0.338	0.046
CATCG	0.338	0.031
CTCGG	0.337	0.050
TCTCG	0.337	0.023
CCGCT	0.337	0.022

CAAAT	0.336	0.028
CACGG	0.336	0.030
CACTG	0.336	0.022
CTTGA	0.335	0.025
CTAGT	0.335	0.028
CATTA	0.334	0.032
CATAG	0.334	0.024
TCACT	0.334	0.020
GTTTT	0.334	0.032
TTCTC	0.334	0.024
CAAAG	0.333	0.045
CATAT	0.333	0.035
CCGTG	0.332	0.028
TCTCT	0.332	0.024
CCAGA	0.331	0.020
TCTCA	0.331	0.022
TCCTC	0.330	0.032
GTTTG	0.330	0.029
TCTAT	0.330	0.017
TCATC	0.330	0.024
CTCTG	0.330	0.043
CCGCC	0.330	0.022
TCTAC	0.330	0.034
TCCCG	0.329	0.019
CCTGA	0.329	0.040
CATCA	0.328	0.035
CACGA	0.328	0.012
GCTTT	0.327	0.027
CAATA	0.327	0.023
TCCAC	0.326	0.030
TCTTA	0.326	0.021
CACTA	0.325	0.032
TCCTT	0.325	0.036
CCAGC	0.324	0.030
CAAAC	0.323	0.031
CTCGA	0.323	0.024
TCACC	0.322	0.023
CCGTT	0.322	0.059
CTATA	0.322	0.017
CTACA	0.322	0.018

CACAG	0.321	0.044
CTTGC	0.321	0.028
CAAAA	0.321	0.019
CACGT	0.321	0.028
TCTTT	0.321	0.019
CCGAC	0.321	0.042
CCGTA	0.319	0.020
CAACG	0.319	0.017
CAACT	0.318	0.025
CTCGC	0.318	0.024
CCTGG	0.317	0.030
CCAGG	0.317	0.023
CTCAG	0.317	0.028
CATAA	0.317	0.019
CTTAG	0.317	0.020
TCCCT	0.317	0.026
CAATC	0.316	0.032
CCTGC	0.315	0.021
CACTC	0.314	0.022
CAATT	0.314	0.026
CATAC	0.314	0.032
CCGAA	0.312	0.023
TCCCC	0.312	0.016
CCCGG	0.312	0.028
CCGTC	0.312	0.018
CTTCA	0.312	0.027
CACAT	0.312	0.014
TCTCC	0.312	0.026
CCGAG	0.312	0.029
CTATT	0.312	0.018
CACAC	0.311	0.024
CCATG	0.311	0.017
CTAAG	0.311	0.025
CCGAT	0.311	0.015
CTTGG	0.310	0.025
CTTGT	0.310	0.025
CTATG	0.309	0.019
CTATC	0.309	0.018
CCCGC	0.308	0.055
CTTTA	0.307	0.019

CACAA	0.306	0.021
CTAAA	0.306	0.019
CATCC	0.306	0.042
CTTTG	0.306	0.036
CTACT	0.305	0.028
CCCGA	0.304	0.018
CTAAT	0.303	0.015
CAACA	0.302	0.032
CATTC	0.300	0.017
CCAGT	0.300	0.020
CCTCA	0.300	0.044
CACCG	0.299	0.015
CTTAC	0.297	0.021
CTTAA	0.297	0.018
CTTTC	0.297	0.050
CATCT	0.296	0.017
CTCAC	0.296	0.022
CTCGT	0.296	0.036
CCATA	0.295	0.018
CCCTG	0.294	0.019

CCCAA	0.294	0.016
CACCC	0.294	0.044
CCTAG	0.294	0.027
CTCAA	0.293	0.019
CTTAT	0.292	0.018
CATTT	0.292	0.021
CTCTA	0.291	0.020
CAACC	0.291	0.020
CCTAA	0.290	0.018
CCAAA	0.290	0.015
CACTT	0.289	0.016
CCTGT	0.289	0.018
CTACG	0.288	0.026
CCAAG	0.288	0.027
CTTCC	0.288	0.030
CCATT	0.286	0.017
CACCA	0.286	0.018
CTCCG	0.286	0.060
CCTCG	0.286	0.021
CTTCG	0.285	0.024

CCTAT	0.285	0.019
CTCTC	0.284	0.019
CTAAC	0.284	0.012
CCATC	0.284	0.016
CCCAG	0.282	0.022
CTTCT	0.282	0.018
CTACC	0.281	0.020
CCACG	0.280	0.022
CCAAT	0.278	0.021
CCCAT	0.276	0.019
CCTTG	0.275	0.015
CCACA	0.275	0.012
CCACC	0.275	0.026
CCTAC	0.273	0.017
CACCT	0.273	0.016
CCTCC	0.273	0.021
CTCAT	0.273	0.018
CCCGT	0.272	0.016
CTCTT	0.272	0.035
CCCCG	0.272	0.012

CCTTA	0.272	0.020
CTCCA	0.271	0.016
CCCAC	0.271	0.019
CCCCA	0.271	0.027
CCCTC	0.269	0.018
CCTCT	0.268	0.019
CCCTT	0.266	0.016
CTCCT	0.266	0.019
CCACT	0.265	0.016
CCAAC	0.265	0.023
CTCCC	0.260	0.019
CTTTT	0.257	0.015
CCTTT	0.253	0.015
CCCCC	0.251	0.018
CCCTA	0.247	0.016
CCCCT	0.243	0.015
CCTTC	0.229	0.015

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