SUPPLEMENTARY MATERIAL

List of abbreviations and acronyms

AMA	1:1 mixture (v/v) of aqueous ammonium hydroxide and aqueous methylamine used to perform fast oligonucleotide deprotection
CPG	Controlled Pore Glass
DAD	Diode array detector for HPLC
DMT (or tryril)	Dimethoxytrityl group, that is widely used for protection of 5`-hydroxy group in nucleosides.
DMT-ON (or trytil ON) approach	Oligonucleotide purification approach based on hydrophobicity of 5`-dimethoxytrityl (DMT) protecting group of synthetic oligonucleotide
DMT-ON oligonucleotides	Oligonucleotides protected by 5`-dimethoxytrityl (DMT) protecting group
DMT-OFF oligonucleotides	Oligonucleotides without 5`-dimethoxytrityl (DMT) protecting group
dNTP	Deoxynucleotide-5`-triphosphate
EGFP	Enhanced Green Fluorescent Protein
ESI	Electrospray ionization in mass spectrometry
HPLC	High-performance liquid chromatography
LC/MS	Liquid chromatography–mass spectrometry
MALDI	Matrix-assisted laser desorption/ionization in mass-spectrometry
MeCN	Acetonitrile
MS	Mass spectrometric detector for HPLC
ODS	Octadecylsilane
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction

PTFE	Polytetrafluoroethylene
Q-TOF	Quadrupole time-of-flight mass analyser
RP-SPE	Reversed-Phase Solid Phase Extraction
SPE	Solid Phase Extraction
TBC	Thermodynamically Balanced Conventional method for DNA assembly
TBIO	Thermodynamically Balanced Inside-Out method for DNA assembly
TCA	Trichloroacetic acid
TEA	Triethylamine
TEAAc	Triethylamine acetate buffer
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

Table S1 – Oligonucleotides design for EGFP gene synthesis by DNAWorks based on 50-mer oligonucleotides.

№[1]	Sequence (5’ -> 3’) [2]	Length	Tm, °C	Overlap	Coding region
S12	ATGGTGAGCAAAGGCGAAGAA**CTGTTTACCGGCGTGGTG**	39	64.3	18	1-39
S11	**CTGTTTACCGGCGTGGTG**CCGATTCTGGT**GGAGCTTGATGGTGATGTGAA**	50	63.8	21	22-71
S10	**GGAGCTTGATGGTGATGTGAA**TGGCCACAAATT**TAGCGTGAGCGGCGAAG**	50	65.5	17	51-100
S09	**TAGCGTGAGCGGCGAAG**GGGAAGGCGATGCG**ACCTACGGCAAACTGACCC**	50	65.3	19	84-133
S08	**ACCTACGGCAAACTGACCC**TGAAATTCATTT**GCACCACAGGAAAACTGCC**	50	65.2	19	115-164
S07	**GCACCACAGGAAAACTGCC**GGTGCCGTGGCCG**ACCTTGGTGACCACCCTG**	50	64.6	18	146-195
S06	**ACCTTGGTGACCACCCTG**ACCTATGGGGTG**CAGTGCTTTAGCCGGTATCC**	50	63.9	20	178-227
S05	**CAGTGCTTTAGCCGGTATCC**CGA**CCATATGAAACAGCATGATTTTTTCAA**	50	63.2	27	208-257
S04	**CCATATGAAACAGCATGATTTTTTCAA**GAGCG**CGATGCCGGAGGGCTATG**	50	65.7	18	231-280
S03	**CGATGCCGGAGGGCTATG**TGCAGGA**ACGTACCATTTTCTTCAAAGATGAT**	50	62.6	25	263-312
S02	**ACGTACCATTTTCTTCAAAGATGAT**GGGA**ACTATAAAACCCGTGCGGAAG**	50	63.8	21	288-337
S01	**ACTATAAAACCCGTGCGGAAG**TGAAATTTGA**GGGCGATACCCTGGTGAAT**	50	64.9	19	317-366
A01	**CCTTAAAGTCAATGCCTTTCAGTTC**AATACG**ATTCACCAGGGTATCGCCC**	50	64.9	19	348-397
A02	**TTATGGCCCAGAATATTGCCA**TCCT**CCTTAAAGTCAATGCCTTTCAGTTC**	50	64.0	25	373-422
A03	**CATTATGGCTGTTGTAGTTGTATTCC**AGT**TTATGGCCCAGAATATTGCCA**	50	63.0	21	402-451
A04	**TCTGTTTATCCGCCATTATGTACA****CATTATGGCTGTTGTAGTTGTATTCC**	50	63.7	26	426-475
A05	**TTTGAAGTTCACCTTAATGCCG**TTCT**TCTGTTTATCCGCCATTATGTACA**	50	63.4	24	452-501
A06	**CGCTGCCATCTTCGATATTATGA**CGAAT**TTTGAAGTTCACCTTAATGCCG**	50	62.8	22	480-529
A07	**TCTGCTGGTAATGGTCCGC**CAGCTGCA**CGCTGCCATCTTCGATATTATGA**	50	64.1	23	507-556
A08	**AGCAGCACCGGGCCA**TCGCCAATCGGGGTAT**TCTGCTGGTAATGGTCCGC**	50	65.4	19	538-587
A09	**GTGCGCTCTGGGTGCT**CAGATAATGATTATCCGGC**AGCAGCACCGGGCCA**	50	67.3	15	573-622
A10	**ATGTGATCCCGCTTTTCGTTC**GGATCCTTGCTCA**GTGCGCTCTGGGTGCT**	50	65.5	16	607-656
A11	**CTGCCGCGGTCACGAAT**TCCAGCAGGACC**ATGTGATCCCGCTTTTCGTTC**	50	64.6	21	636-685
A12	TTTATACAGTTCATCCATACCCAGTGTAATGC**CTGCCGCGGTCACGAAT**	49	66.1	18	669-717

[1] – S01-S12 – oligonucleotides coding sense strand of EGFP gene;

A01-A12 – oligonucleotides coding antisense strand of EGFP gene.

[2] – Inner overlapping part of each oligonucleotide shown in red;

Outer overlapping part of each oligonucleotide shown in blue.

Table S2 – Oligonucleotides design for EGFP gene synthesis by DNAWorks based on 65-mer oligonucleotides.

№[1]	Sequence (5’ -> 3’) [2]	Length	Tm, °C	Overlap	Coding region
S08	ATGGTGAGCAAAGGCGAAGAACTGTTTACCGGCGTGGTGCC**GATTCTGGTGGAACTGGATGG**	62	63.8	21	1–62
S07	**GATTCTGGTGGAACTGGATGG**CGATGTGAACGGACATAAATTCAGCGTGA**GCGGCGAAGGCGAGG**	65	66.4	15	42–106
S06	**GCGGCGAAGGCGAGG**GGGACGCGACGTATGGTAAACTGACCCTGAA**ATTCATTTGCACCACCGGG**	65	64.4	19	92–156
S05	**ATTCATTTGCACCACCGGG**AAACTGCCGGTGCCGTGGCCGACCCT**GGTGACCACCCTGACCTATG**	65	65.1	20	138–202
S04	**GGTGACCACCCTGACCTATG**GCGTGCAGTGCTTTAGCCGTTATC**CTGACCACATGAAACAGCATG**	65	63.1	21	183–247
S03	**CTGACCACATGAAACAGCATG**ATTTTTTCAAATCTGCGATGCCGGAA**GGCTATGTGCAGGAGCGT**	65	65.5	18	227–291
S02	**GGCTATGTGCAGGAGCGT**ACCATTTTCTTCAAAGATGATGGCAAC**TATAAGACCCGTGCGGAAGT**	65	64.1	20	274–338
S01	**TATAAGACCCGTGCGGAAGT**GAAATTTGAGGGCGATACTCT**GGTGAATCGTATTGAACTGAAAGG**	65	63.3	24	319–383
A01	**GCTTATGGCCCAGAATGTTTC**CATCTTCCTTAAAATCAATT**CCTTTCAGTTCAATACGATTCACC**	65	63.3	24	353–417
A02	**ATCGGCCATGATATACACATTATG**GCTATTGTAATTGTATTCCA**GCTTATGGCCCAGAATGTTTC**	65	63.0	21	398–462
A03	**TGACGAATTTTGAAGTTGACTTTAATG**CCATTTTTCTGTTT**ATCGGCCATGATATACACATTATG**	65	62.1	24	440–504
A04	**AATGATCCGCCAGCTGAAC**GCTCCCATCCTCAATGTTG**TGACGAATTTTGAAGTTGACTTTAATG**	65	62.8	27	479–543
A05	**TCCGGCAGCAGCACAG**GGCCATCCCCAATCGGGGTATTCTGCTGAT**AATGATCCGCCAGCTGAAC**	65	63.8	19	526–590
A06	**TTCATTCGGATCCTTGCTCAG**CGCGGACTGGGTGCTCAGATAATGGTTA**TCCGGCAGCAGCACAG**	65	65.5	16	576–640
A07	**CCTGCCGCGGTCACAA**ATTCAAGCAGGACCATATGATCACGTTT**TTCATTCGGATCCTTGCTCAG**	65	63.5	21	621–685
A08	TTTATACAGTTCATCCATGCCCAAGGTAATG**CCTGCCGCGGTCACAA**	47	65.8	16	671–717

[1] – S01-S08 – oligonucleotides coding sense strand of EGFP gene;

A01-A08 – oligonucleotides coding antisense strand of EGFP gene.

[2] – Inner overlapping part of each oligonucleotide shown in red;

Outer overlapping part of each oligonucleotide shown in blue.

Listing S1 – Python script for calculation of TBIO scheme.

```
import sys
from Bio.Seq import Seq
from Bio.Alphabet import IUPAC
seq01=Seq("acgt", IUPAC.unambiguous_dna)

#arguments for script (input file name, PCR volume, required number of PCR reactions, SPE elution volume)
scheme_file_name = sys.argv[1]
reaction_volume = float(sys.argv[2])
number_of_reactions = float(sys.argv[3])
elution_volume = float(sys.argv[4])

#calculation of oligo extinction coefficients
def e260_func(seq):
   Coefficients = {
      "a":15.4,
      "c":7.4,
      "g":11.5,
      "t":8.7,
      "aa":13.7,
      "ac":10.6,
      "ag":12.5,
      "at":11.4,
      "ca":10.6,
      "cc":7.3,
      "cg":9,
      "ct":7.6,
      "ga":12.6,
      "gc":8.8,
      "gg":10.8,
      "gt":10,
      "ta":11.7,
      "tc":8.1,
      "tg":9.5,
      "tt":8.4
      }
   e260=0
   for i in range(0,len(seq)-1):
      e260 = e260 + 2*Coefficients[seq[i]+seq[i+1]]

   for i in range(0,len(seq)-2):
      e260 = e260 - Coefficients[seq[i+1]]

   return e260

#Input format for TBIO calculation:
#Name   Sequence          Yield, % Target Concentration, nMA260 (1 cm)
oligos_table = [['Name',
            'Sequence',
            'Yield, %',
            'e260, mM-1cm-1',
            'A260, (1cm)',
            'Concentration, nM',
            'Target Concentration, nM',
            'V, ul',
            'Vnorm, ul']]
```

```
volumes = []
total_volume_for_reaction = 0
total_volume_for_mixing = 0
A260_afterSPE_exp = 0

#reading input file
with open(scheme_file_name) as scheme_file:
   lines = scheme_file.readlines()

lines = lines[1:len(lines)]

for line in lines:
   Name = line.split("\t")[0]
   Sequence = line.split("\t")[1]
   Yield = float(line.split("\t")[2])
   e260 = e260_func(line.split("\t")[1].lower())
   A260 = float(line.split("\t")[4])
   Concentration = A260/(e260+(e260/2)*(1-(Yield/100))/(Yield/100))*1000000
   Target_Concentration = float(line.split("\t")[3])
   V = reaction_volume/(Concentration/Target_Concentration)
   Vnorm = reaction_volume/(Concentration/Target_Concentration)

   oligos_table.append([
      Name,                    #0
      Sequence,
      Yield,
      str(round(e260,5)),
      str(A260),
      str(round(Concentration,5)),
      str(Target_Concentration),
      str(round(V,5)),
      str(round(Vnorm,5))
      ])

for oligo in oligos_table:
   if(oligo[7]<>'V, ul'):
      total_volume_for_reaction=total_volume_for_reaction + float(oligo[7])
      volumes.append(oligo[7])

normalization_coefficient_min = round(1.0 / float(min(volumes)),5)
normalization_coefficient = number_of_reactions
normalization_coefficient_relative = round(number_of_reactions / normalization_coefficient_min, 5)

for oligo in oligos_table:
  if(oligo[8]<>'Vnorm, ul'):
     oligo[8] = float(oligo[8])*normalization_coefficient
     total_volume_for_mixing = total_volume_for_mixing + float(oligo[8])

for oligo in oligos_table:
  if(oligo[8]<>'Vnorm, ul'):
     A260_afterSPE_exp = A260_afterSPE_exp +
float(oligo[5])*float(oligo[3])*float(oligo[8])/elution_volume/1000000


result_scheme_file_name = 'result_' + scheme_file_name

#writing TBIO calculation results to file
with open(result_scheme_file_name,'w') as final_scheme_file:
   final_scheme_file.write('TBIO scheme calculated for ' +
                  str(reaction_volume) +
```

```
                    ' ul reaction volume'+' for  ' +
                    str(int(number_of_reactions)) +
                    ' reactions'+'\n')
    for oligo in oligos_table:
        for i in range(0,len(oligo)):
            final_scheme_file.write(str(oligo[i]))
            final_scheme_file.write('\t')
        final_scheme_file.write('\n')
    final_scheme_file.write('\n')
    final_scheme_file.write('Min Normalization coefficient for volumes =' +
                    str(normalization_coefficient_min)+'\n')
    final_scheme_file.write('Relative normalization coefficient for volumes for ' +
                    str(int(number_of_reactions))+' reactions = ' +
                    str(normalization_coefficient_relative) + '\n')
    final_scheme_file.write('Total volume for reaction = ' +
                    str(total_volume_for_reaction) + 'ul' + '\n')
    final_scheme_file.write('Total volume for mixing = ' +
                    str (total_volume_for_mixing) + 'ul' + '\n')
    final_scheme_file.write('A260 (1 cm) after SPE with ' +
                    str(int(elution_volume)) + ' ul elution = ' +
                    str(round(A260_afterSPE_exp,3)) + '\n')


#output to screen
for oligo in oligos_table:
    print(str(oligo)+'\n')

print('\t')
print('Min Normalization coefficient for volumes = ' +
      str(normalization_coefficient_min) + '\n')
print('Relative normalization coefficient for volumes for ' +
      str(int(number_of_reactions)) + ' reactions = ' +
      str(normalization_coefficient_relative) + '\n')
print('Total volume for reaction = ' +
      str(total_volume_for_reaction) + ' ul' + '\n')
print('Total volume for mixing = ' +
      str (total_volume_for_mixing) + ' ul' + '\n')
print('A260 (1 cm) after SPE with 750 ul elution = ' +
      str(round(A260_afterSPE_exp,3)) + '\n')
```

Listing S2 – Input file example for calculation of TBIO scheme for EGFP central fragment.

Name	Sequence	Yield, %	Target conc, nmol/l	Abs260, AU
S06	ACCTTGGTGACCACCCTGACCTATGGGGTGCAGTGCTTTAGCCGGTATCC	72	200	39.33
S05	CAGTGCTTTAGCCGGTATCCCGACCATATGAAACAGCATGATTTTTTCAA	76	120	45.97
S04	CCATATGAAACAGCATGATTTTTTCAAGAGCGCGATGCCGGAGGGCTATG	56	100	37.5
S03	CGATGCCGGAGGGCTATGTGCAGGAACGTACCATTTTCTTCAAAGATGAT	76	80	42.2
S02	ACGTACCATTTTCTTCAAAGATGATGGGAACTATAAAACCCGTGCGGAAG	78	60	40.8
S01	ACTATAAAACCCGTGCGGAAGTGAAATTTGAGGGCGATACCCTGGTGAAT	76	40	44.8
A01	CCTTAAAGTCAATGCCTTTCAGTTCAATACGATTCACCAGGGTATCGCCC	78	40	39.9
A02	TTATGGCCCAGAATATTGCCATCCTCCTTAAAGTCAATGCCTTTCAGTTC	77	60	42.0
A03	CATTATGGCTGTTGTAGTTGTATTCCAGTTTATGGCCCAGAATATTGCCA	75	80	44.0
A04	TCTGTTTATCCGCCATTATGTACACATTATGGCTGTTGTAGTTGTATTCC	74	100	37.4
A05	TTTGAAGTTCACCTTAATGCCGTTCTTCTGTTTATCCGCCATTATGTACA	73	120	42.07
A06	CGCTGCCATCTTCGATATTATGACGAATTTTGAAGTTCACCTTAATGCCG	74	200	29.73

Listing S3 – Example of output file for calculation of TBIO scheme for EGFP central fragment (TBIO scheme was calculated for 50 μl PCR volume, 100 reactions and elution volume was set 300 μl). Yield and A260 columns were deleted as compared original file in order to simplify the output.

TBIO scheme calculated for 50.0 ul reaction volume for 100 reactions

Name	Sequence	e260, mM-1cm-1	Concentration, nM	Target Concentration, nM	V, ul	Vnorm, ul
S06	ACCTTGGTGACCACCCTGACCTATGGGGTGCAGTGCTTTAGCCGGTATCC	460.3	71534.74226	200.0	0.13979	13.979
S05	CAGTGCTTTAGCCGGTATCCCGACCATATGAAACAGCATGATTTTTTCAA	480.8	82573.55166	120.0	0.07266	7.266
S04	CCATATGAAACAGCATGATTTTTTCAAGAGCGCGATGCCGGAGGGCTATG	489.1	55046.16014	100.0	0.09083	9.083
S03	CGATGCCGGAGGGCTATGTGCAGGAACGTACCATTTTCTTCAAAGATGAT	486.2	74959.79956	80.0	0.05336	5.336
S02	ACGTACCATTTTCTTCAAAGATGATGGGAACTATAAAACCCGTGCGGAAG	498.6	71715.40989	60.0	0.04183	4.183
S01	ACTATAAAACCCGTGCGGAAGTGAAATTTGAGGGCGATACCCTGGTGAAT	500.2	77350.87783	40.0	0.02586	2.586
A01	CCTTAAAGTCAATGCCTTTCAGTTCAATACGATTCACCAGGGTATCGCCC	471.2	74211.6709	40.0	0.02695	2.695
A02	TTATGGCCCAGAATATTGCCATCCTCCTTAAAGTCAATGCCTTTCAGTTC	464.5	78670.3399	60.0	0.03813	3.813
A03	CATTATGGCTGTTGTAGTTGTATTCCAGTTTATGGCCCAGAATATTGCCA	478.6	78801.2656	80.0	0.05076	5.076
A04	TCTGTTTATCCGCCATTATGTACACATTATGGCTGTTGTAGTTGTATTCC	466.0	68265.00913	100.0	0.07324	7.324
A05	TTTGAAGTTCACCTTAATGCCGTTCTTCTGTTTATCCGCCATTATGTACA	461.1	76998.8329	120.0	0.07792	7.792
A06	CGCTGCCATCTTCGATATTATGACGAATTTTGAAGTTCACCTTAATGCCG	470.1	53791.92982	200.0	0.1859	18.59

Min Normalization coefficient for volumes =38.66976

Relative normalization coefficient for volumes for 100 reactions = 2.586

Total volume for reaction = 0.87723ul

Total volume for mixing = 87.723ul

A260 (1 cm) after SPE with 300 ul elution = 9.474

Figure S1 – Registered and deconvoluted ESI$^-$ mass spectra of crude and SPE purified oligonucleotide S12. (A – ESI$^-$ mass spectrum of crude oligonucleotide S12, B – deconvoluted ESI$^-$ mass spectrum of crude oligonucleotide S12, C – ESI$^-$ mass spectrum of SPE purified oligonucleotide S12, D – deconvoluted ESI$^-$ mass spectrum of SPE purified oligonucleotide S12).

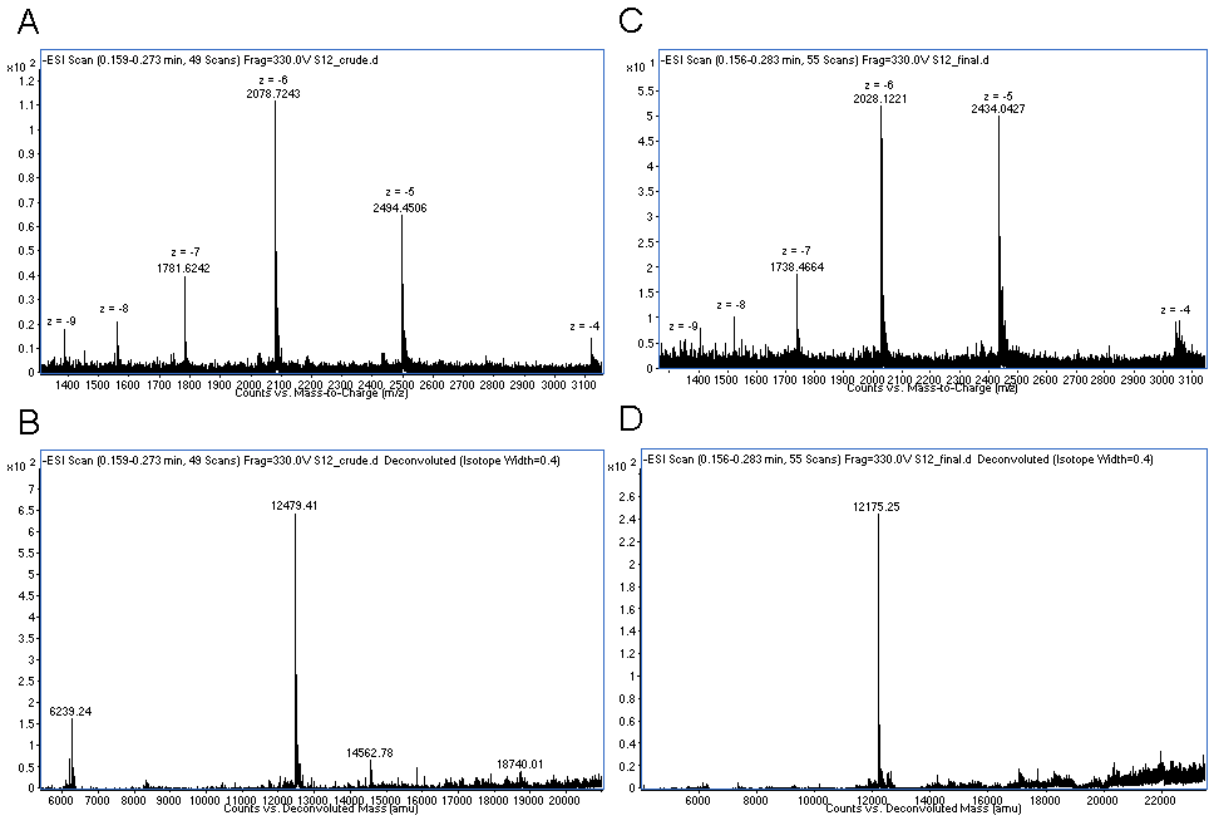

Figure S2 – Registered and deconvoluted ESI$^-$ mass spectra of crude and SPE purified oligonucleotide A06. (A – ESI$^-$ mass spectrum of crude oligonucleotide A06, B – deconvoluted ESI$^-$ mass spectrum of crude oligonucleotide A06, C – ESI$^-$ mass spectrum of SPE purified oligonucleotide A06, D – deconvoluted ESI$^-$ mass spectrum of SPE purified oligonucleotide A06).

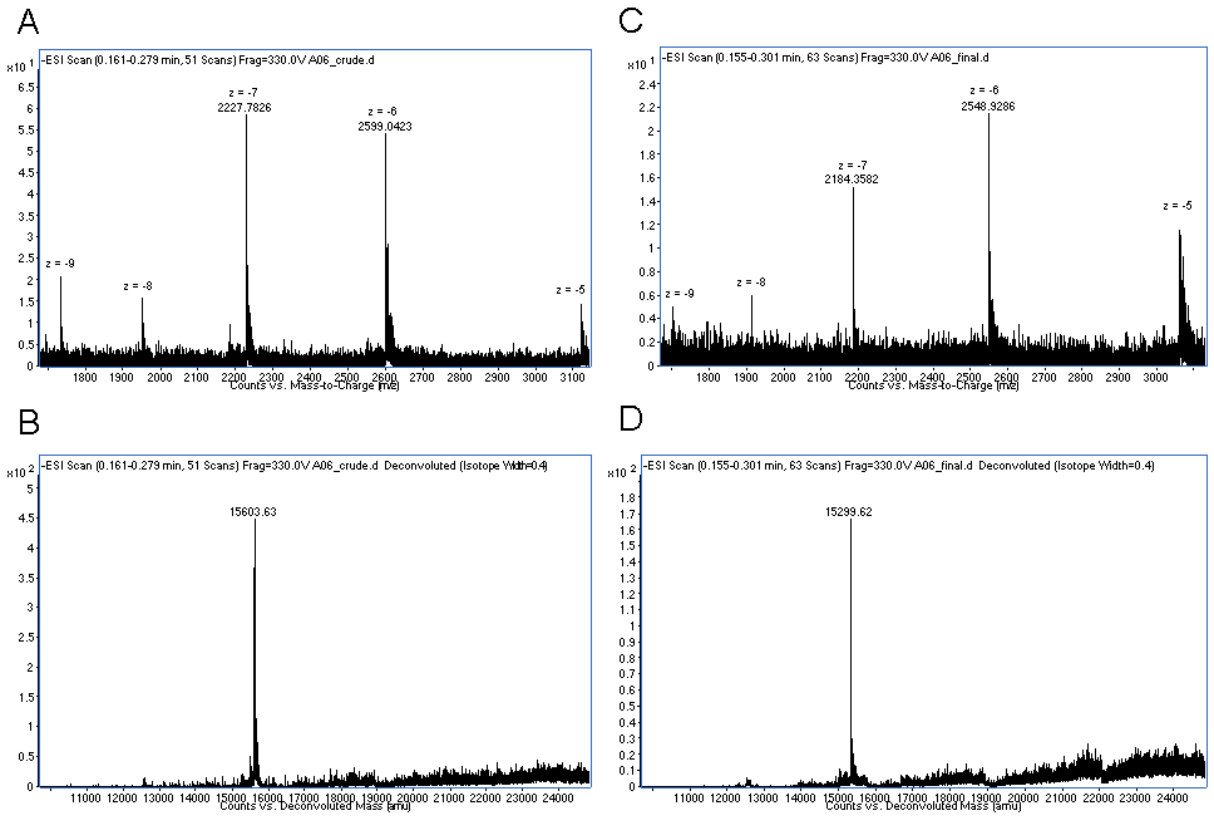

Figure S3 – Polyacrylamide gel electrophoresis (18% gel) of crude, SPE column purified and SPE ZipTip purified oligonucleotides S12 and A06 (1 – crude 5`-DMT-protected oligonucleotide S12 desalted by SPE column; 2 – SPE column purified oligonucleotide S12; 3 – SPE ZipTip purified oligonucleotide S12; 4 – crude 5`-DMT-protected oligonucleotide A06 desalted by SPE column; 5 – SPE column purified oligonucleotide A06; 6 – SPE ZipTip purified oligonucleotide A06). SPE column desalting of crude 5`-DMT-protected oligonucleotides leads to partial purification as follows from PAGE results. SPE ZipTip purified oligonucleotides were more than twice overloaded as compared to crude 5`-DMT-protected oligonucleotides in order to reveal remaining impurities.