

I RecJ-SSBP experiments, trityl-on

S1 TTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATA
S2 CCATGATGTATACATTGTGTGAGTTATAGTTGTATTCCAA
S3 CACCCTCTCCACTGACAGAAAATTTGTGCCATTAAACATC
S4 ATTTTGAAGTAACTTTGATTCCATTCTTTTGTGTCTG
S5 GTCTGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTA
S6 GAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCC
S7 GGACAGGGCCATCGCCAATTGGAGTATTTTGTGATAATG
S8 CTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAA
S9 CCATGCCATGTGTAATCCCAGCAGCTGTACAAACTCAAG
S10 TGGGATTACACATGGCATGGATGAACTATACAAAATAGGAG

II Failure strand analysis for HPLC, trityl-on / off

40b TTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATT
39b TCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATT
38b CTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATT
35b GTTGAATTAGATGGTGATGTTAATGGGCACAAATT
31b AATTAGATGGTGATGTTAATGGGCACAAATT
20b GATGTTAATGGGCACAAATT

III Secondary structure and temperature studies, trityl-on / off

S11 ACCAGATGGCTGTTCTAGAACCGCTGACGCTGGTTCTCCAGCTGGTTCTGCTTGTGCTGA
S12 GTAAGGCTATCCAATTGCACCCATTGGTTTGTGCTGCTTACAACGCTGACTTCGATGGTG
S13 GTCACTACTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAACGG
S14 TTGGAATACAACATAACTCACACAATGTATACATCATGGCAGACAAAACAAAAGAATGGA

IV PAGE purified samples (IDT), trityl-off

40b TTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATT
Biotin Biotin-TTTTTTTTTTTTTTTTTTTT
Amine Amine-TTTTTTTTTTTTTTTTTTTT
Phosphate Phosphate-TTTTTTTTTTTTTTTTTTTT

Table 1 Sequences for oligonucleotides used for this research (5' — 3')

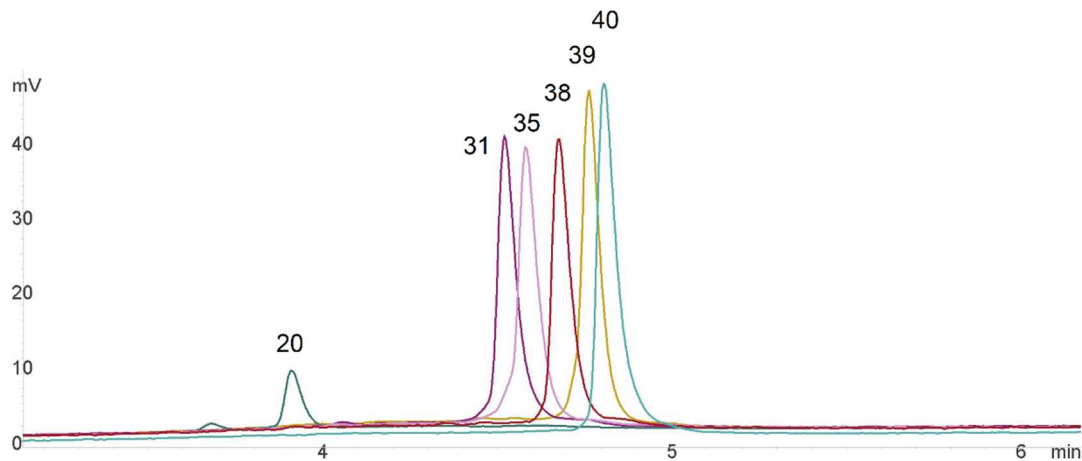


Fig. 1 Control 5' truncated 'failure' strands processed with ion-pairing RP HPLC. This chromatogram shows oligonucleotide strand resolution between 40b - 39b, 39b - 38b, 38b - 35b, 35b - 31b and 31b - 20b, where gradient and method have been optimized for enhanced peak-resolved relative to the full-length 40b strand.

Sample	30m	30m	30m	30m	30m	30m	30m	30m
	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ
	0.5 ug SSBP				0.5 ug SSBP			
	Total digested (pm)	Total digested (pm)	Failures digested (pm)	Failures digested (pm)	Percent failure decrease	Percent failure decrease	Increase purity	Increase purity
S1	20.4	59.2	4	12	15	43	0.8	2.6
S2	11	34.3	10	31	15	47	2.1	7.9
S3	17.4	35.7	13	20	33	52	3.2	5.1
S4	14	30	6	22	20	81	1.3	5.8
S5	18.6	26	17	24	29	40	4.6	6.3
S6	14.1	40	9	13	34	45	2.4	3.2
S7	7.9	30.9	8	30	8	28	0.8	6
S8	8.1	19.6	10	20	27	56	2	4.2
S9	8.3	34	9	29	10	32	1.4	5.7
S10	17.9	54.5	22	44	31	61	4.8	9.7
Average	14	36	11	25	22	49	2	6
Standard deviation	5	12	5	9	10	15	1	2

Table 2 Digestive performance of 30 U RecJ (+ / - 0.5 ug SSBP) on 10 unique 40b samples (crude trityl-on 500 pm) over 30 minutes incubation at 37°C.

Sample	60m	60m	60m	60m	60m	60m	60m	60m
	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ	30 U RecJ
	0.5 ug SSBP		0.5 ug SSBP		0.5 ug SSBP		0.5 ug SSBP	
Total digested (pm)	Total digested (pm)	Failures digested (pm)	Failures digested (pm)	Percent failure decrease	Percent failure decrease	Increase purity	Increase purity	
S1	35	68	15	10	55	36	3.8	1.9
S2	27.4	53.9	25	49	38	73	6	13.3
S3	23.1	52.4	20.8	45.6	30.2	55.3	5.3	8
S4	11.4	39.9	18.4	35.3	29	62.1	3.9	8.5
S5	22.1	59.7	15.6	34.5	28.3	52.1	5	7.5
S6	28.2	49.3	14.1	27.3	29.9	47	4.7	7.3
S7	4.2	48.5	6	47	6	44	0.1	10
S8	22.7	60	13	24	37	66	2.7	5
S9	12.7	38.9	16	38	17	41	3.1	7.8
S10	28.2	57.2	25	40	35	56	5.5	8.7
Average	22	53	17	35	31	53	4	8
Standard deviation	9	9	6	12	13	12	2	3

Table 3 Digestive performance of 30 U RecJ (+ / - 0.5 ug SSBP) on 10 unique 40b samples (crude trityl-on 500 pm) over 60 minutes incubation at 37°C.

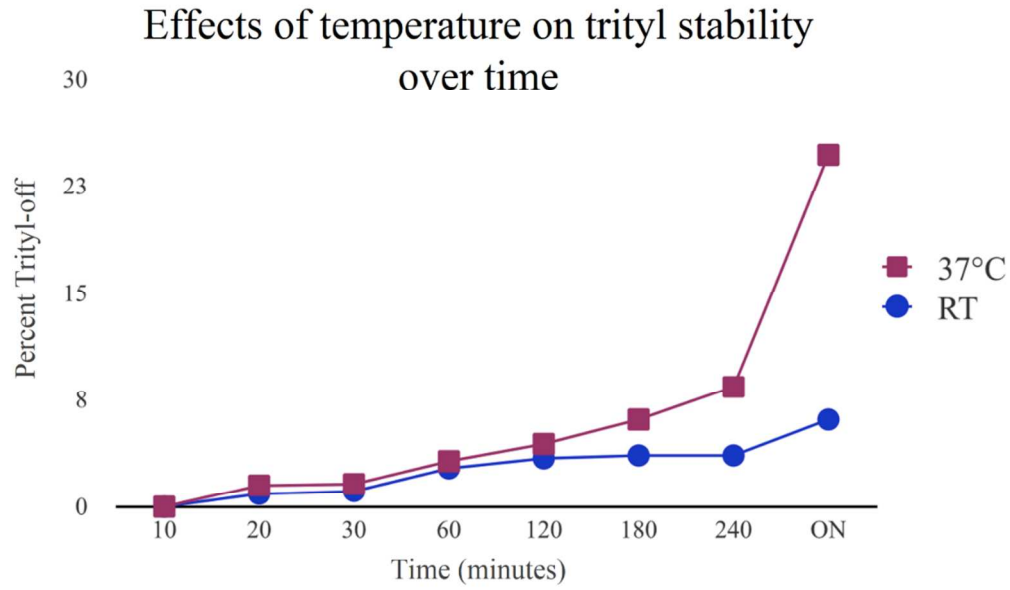


Fig. 2 Effects of time and temperature on the rate of detritylation. Each plot point (time of sample collection) represents an average of three samples (Supplementary Table 1 III) where the percent detritylation was measured (percent peak area from RP HPLC analysis report). (RT, room temperature; ON, overnight; 5 μ M ssDNA in water and 1x Buffer 2 at pH 7).

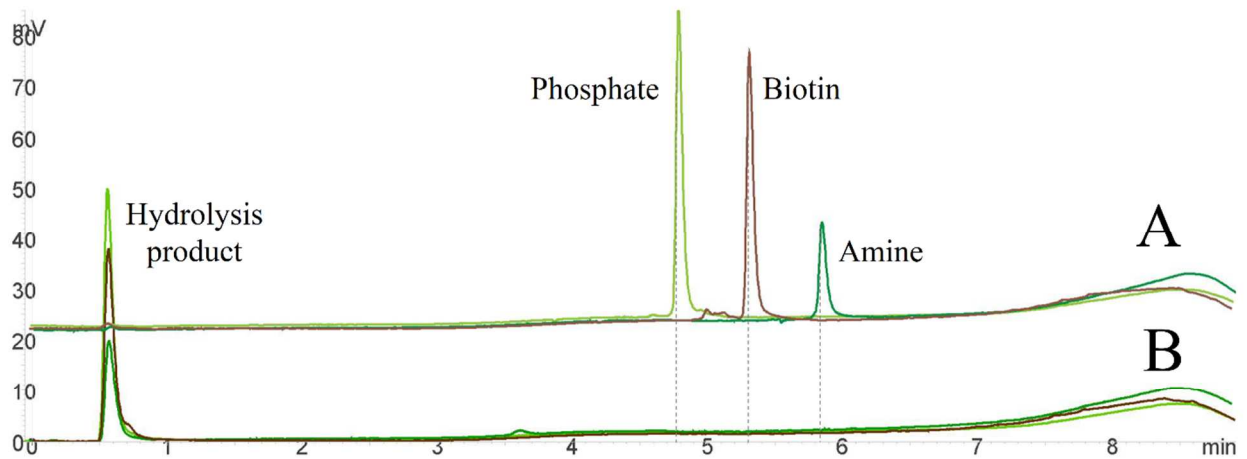


Fig. 3 RecJ-SSBP digestion of oligonucleotides modified at the 5' end with phosphate, biotin and amine (each 20 pm). This figure shows overlapping RP HPLC chromatograms before (A) and after (B) treatment with 30 U RecJ and 0.5 μ g SSBP for 60 min at 37°C.