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Article

One-Pot production of RNA in high yield and purity through cleaving tandem transcripts

Hannes Feyrer [1], Raluca Munteanu [1], Lorenzo Baronti [1] and Katja Petzold [1,*]

[1] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, 171 77 Sweden;
* Correspondence: katja.petzold@ki.se

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Supplementary information

Construct overview

RNA constructs and ordered DNA sequences. Cleavage guides were ordered at IDT at 100 nmol – 1 µmol scale with standard desalting purification. Chimeric cleavage guides contain mU instead of mT because of cheaper synthesis. All sequences are written in 5′ to 3′ direction.

Nucleotide key:
N: RNA
n: DNA
mN: 2′-OMe nucleotide

pUC19 vector backbone without insert:

gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgag
cgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggata
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Construct 1

Target RNA sequence (20 nt):
AGGGCCACAUCCCACUGCCA

Plasmid insert (27 copies):
taatacgactcactatagggagacactgccaagggccacatcccactgccaagggccacatcccactgccaagggccacatcccactgccaagg
gccacatcccactgccaagggccacatcccactgccaagggccacatcccactgccaagggccacatcccactgccaagggccacatcccactg
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aagggccacatcccactgccaagggccacatcccactgccaagggccacatcccactgccaagggccacatcccactgccaagggccacatccc
actgccaagggccacatcccactgccaaggg

Cleavage guide:
mG mU mG mA c g g t mU mC mC mC

HPLC elution gradient:
18 – 26 %

Construct 2

Target RNA sequence (22 nt):
UGGCAGUGUCUUAGCUGGUUGU

Plasmid insert (26 copies):
taatacgactcactatagggagatggcagtgtcttagctggttgttggcagtgtcttagctggttgttggcagtgtcttagctggttgttggcagtgt
cttagctggttgttggcagtgtcttagctggttgttggcagtgtcttagctggttgttggcagtgtcttagctggttgttggcagtgtcttagctggttg
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Cleavage guide:
mG mC mC mA a c a a mC mC mA mG

HPLC elution gradient:
20 – 30 %

Construct 3

Target RNA sequence (22 nt):
CAAUCAGCAAGUAUACUGCCCU

Plasmid insert (26 copies):
taatacgactcactatagggagacaatcagcaagtatactgccctcaatcagcaagtatactgccctcaatcagcaagtatactgccctcaatcagc
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atactgccctcaatcagcaagtatactgccctc

Cleavage guide:
mA mU mU mG a g g g mC mA mG mU

HPLC elution gradient:
20 – 30 %

Construct 4

Target RNA sequence (22 nt):
CCGGCCGCCUGCGGCACUGCCU

Plasmid insert (26 copies):
taatacgactcactatagggagaccggccgcctgcggcactgcctccggccgcctgcggcactgcctccggccgcctgcggcactgcctccggc
cgcctgcggcactgcctccggccgcctgcggcactgcctccggccgcctgcggcactgcctccggccgcctgcggcactgcctccggccgcctg
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cactgcctccggccgcctgcggcactgcct

Cleavage guide:
mG mG mC mC mG mG A G G C mA mG mU mG mC mC

HPLC elution gradient:
20 – 30 %

Construct 5

Target RNA sequence (22 nt):
AACCAGCUAAGACACUGCCAGG

Plasmid insert (26 copies):
taatacgactcactatagggagaaaccagctaagacactgccaggaaccagctaagacactgccaggaaccagctaagacactgccaggaacc
agctaagacactgccaggaaccagctaagacactgccaggaaccagctaagacactgccaggaaccagctaagacactgccaggaaccagct
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accagctaagacactgccaggaaccagctaagacactgccagg

Cleavage guide:
mG mG mU mU c c t g mG mC mA mG

HPLC elution gradient:
20 – 30 %

Construct 6

Target RNA sequence (75 nt):
CGUCACCCUCCUCAAGUAUACUUCAAAGGACAUUUAACUAAAACCCCUACGCAUUUAU
AUAGAGGAGACAAGUCG

Plasmid insert (5 copies):
taatacgactcactatagggagaacaagtcgcgtcaccctcctcaagtatacttcaaaggacatttaactaaaaccccctacgcatttatatagagga
gacaagtcgcgtcaccctcctcaagtatacttcaaaggacatttaactaaaaccccctacgcatttatatagaggagacaagtcgcgtcaccctcctc
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Cleavage guide:
mG mA mC mG c g a c mU mU mG mU

HPLC elution gradient:
30 – 40 %

Quantification of target RNA using a control standard curve

A control standard curve using 149.8, 74.9, 37.4, 18.7 and 9.3 nmol of construct 2 was created. The area under the curve was plotted against the injected amount of RNA. A linear regression (Figure SI 1) gave the equation

$$AUC_c = 30.8 * n_c$$

This can be used to determine the amount of another RNA construct by determining the area under curve of any RNA x assuming area under the curve is proportional to the injected amount of sample times the molar extinction coefficient according to the Lambert-Beer law.

$$AUC_x = \varepsilon_x * n_x$$

Having a constant flow rate and path length allows to compare between a control sample c (the standard curve) and a target sample t:

$$\frac{AUC_c}{AUC_t} = \frac{\varepsilon_c * n_c}{\varepsilon_t * n_t}$$

Solving this equation for n_t allows us to apply the slope obtained from the standard curve shown in the first equation to determine the amount of substance of a target sample:

$$n_t = \frac{AUC_t * \varepsilon_c * n_c}{\varepsilon_t * AUC_c} = \frac{AUC_t}{30.8} * \frac{\varepsilon_c}{\varepsilon_t}$$

In plain words, we are extracting the n_t from the slope of the standard curve and account for the different ε due to sequence and size differences.

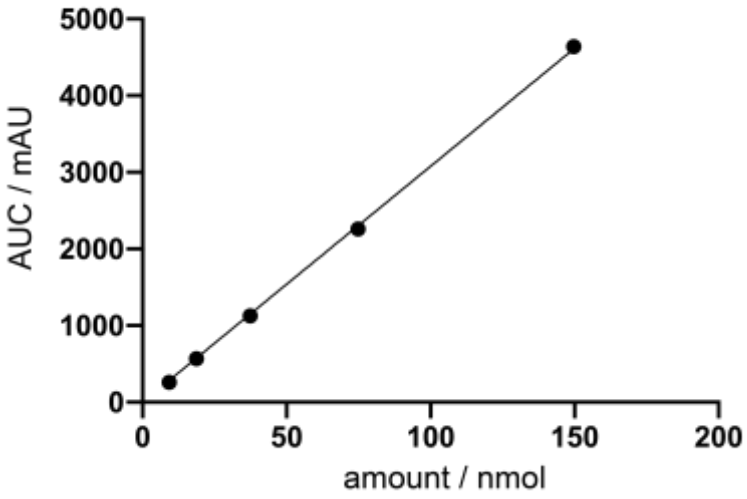

Figure SI 1: Obtained standard curve and linear regression through the data points. The regression was forced through the origin and gave y = 30.8 x.

Table SI 1. Samples of construct 2 for HPLC standard curve and obtained area under the curve (AUC)

m/ mg	n / nmol	AUC / mAU
0.062	9.2	261
0.125	18.7	570
0.25	37.4	1127
0.5	74.9	2260
1	149.7	4641

IVT from single-repeat templates

Templates encoding a single construct repeat are single stranded and only double-stranded in the promoter sequence. The template strands carry two 2′-OMe modifications on the 5′-end, to increase 3′-homogeneity of the transcript. The shorter T7 promoter sequence was annealed to the template by heating to 95 °C at 25 μM concentration, and cooling down on to room temperature.

Sequences:
n: DNA
mN: 2′-OMe RNA

T7 promoter: t t a a t a c g a c t c a c t a t a
Construct 1*: mU mU g c a g t g g g a t g t g g c c c t t a t a g t g a g t c g t a t t a a
Construct 2*: mA mC a a c c a g c t a a g a c a c t g c c a t a t a g t g a g t c g t a t t a a

Table SI 2. Optimized reaction conditions used for T7 IVT from single repeat ssDNA templates

Reagent	Tandem transcripts	Construct 1*	Construct 2*
Tris-Cl pH 8.0	100 mM	100 mM	80 mM
$MgCl_2$	10 mM	30 mM	40 mM
Dithiothreitol	10 mM	3 mM	6 mM
Spermidine	20 mM	2 mM	2 mM
NMP (GMP/AMP/UMP)	5 mM	10 mM	24 mM
NTPs (each)	3 mM	3 mM	3 mM (ATP: 1 mM)
DNA template	2 ng/μl	500 nM	500 nM
T7 RNA polymerase	0.3 mg/ml	0.2 mg/ml	0.3 mg/ml
Inorganic pyrophosphatase	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml
DMSO	-	4 % (v/v)	20 % (v/v)

Obtained yields from HPLC quantification

Table SI 3. Obtained yields for different constructs from HPLC quantification in % as described above

1*	1	2*	2	3	4	5	6
3.77	23.85	2.1	22.8	39.15	14.45	11.2	28.3
	17.91		17.6	14.7	4.53	7	18.8
			17.33				

Original raw images of all gels (red box marks shown region)

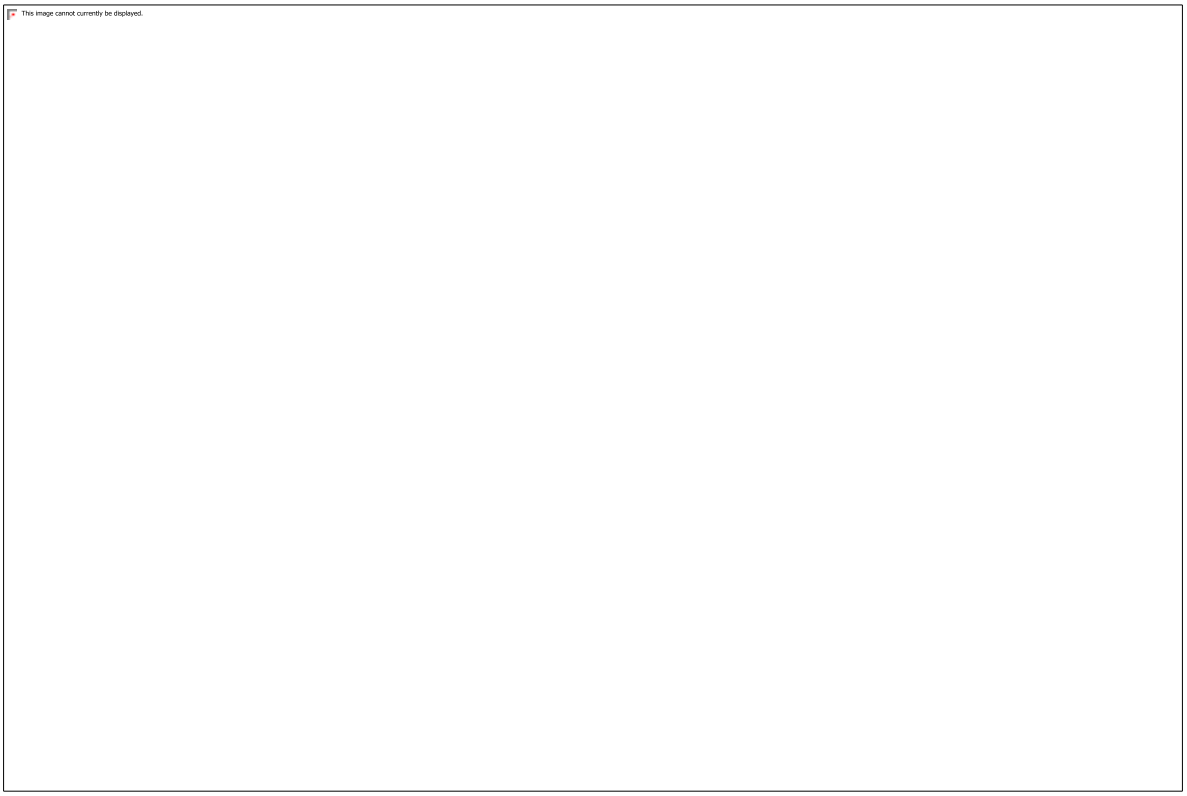

Figure 2A

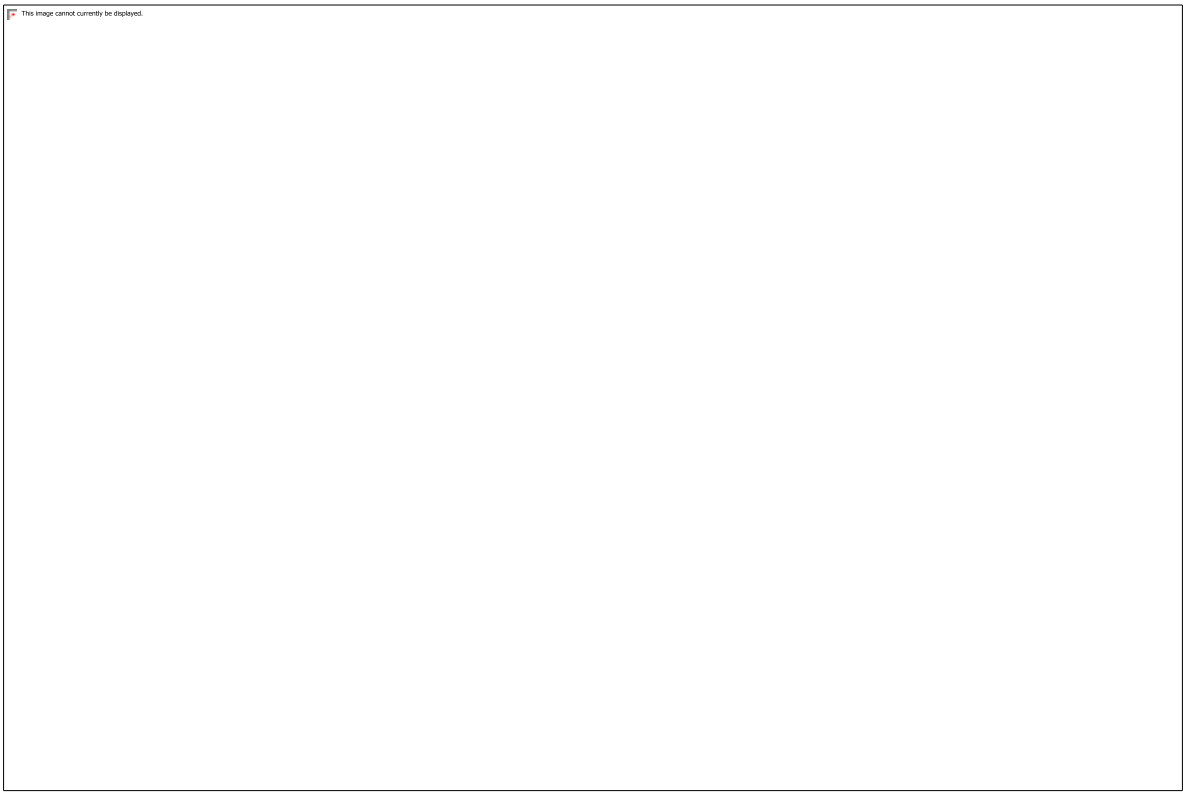

Figure 2B

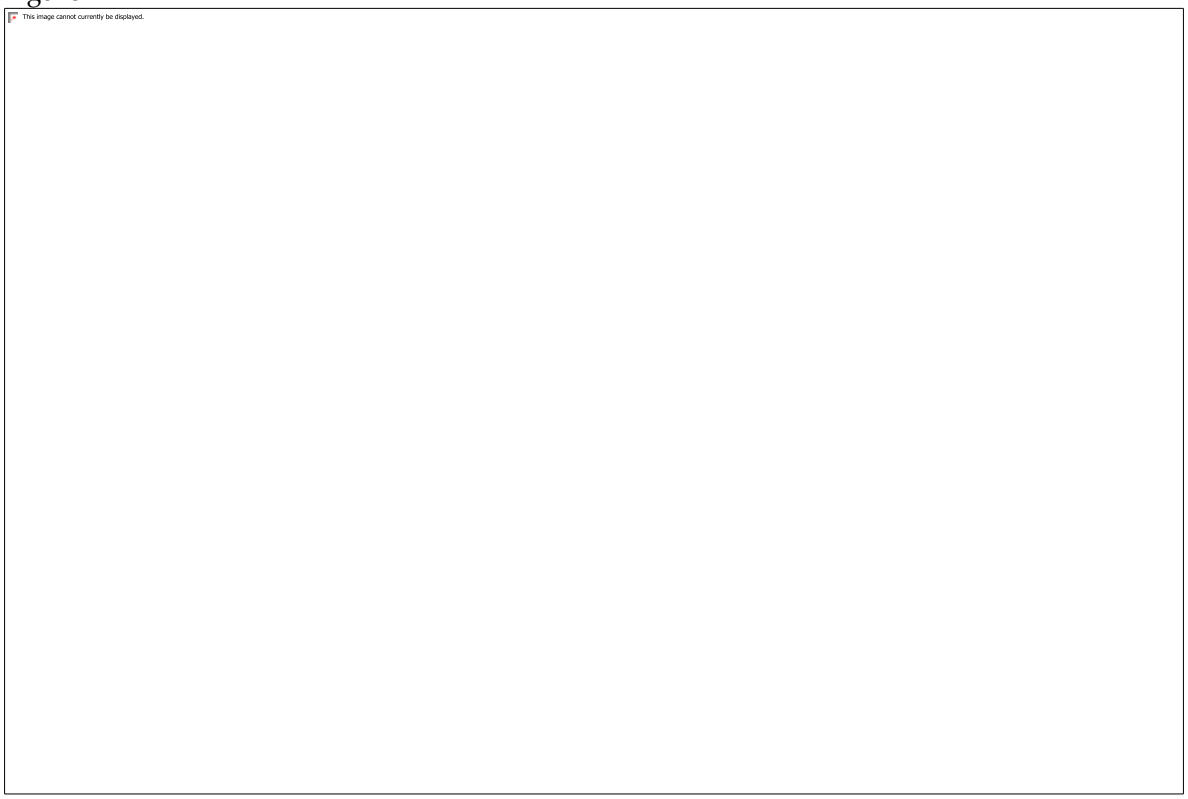

Figure 2C

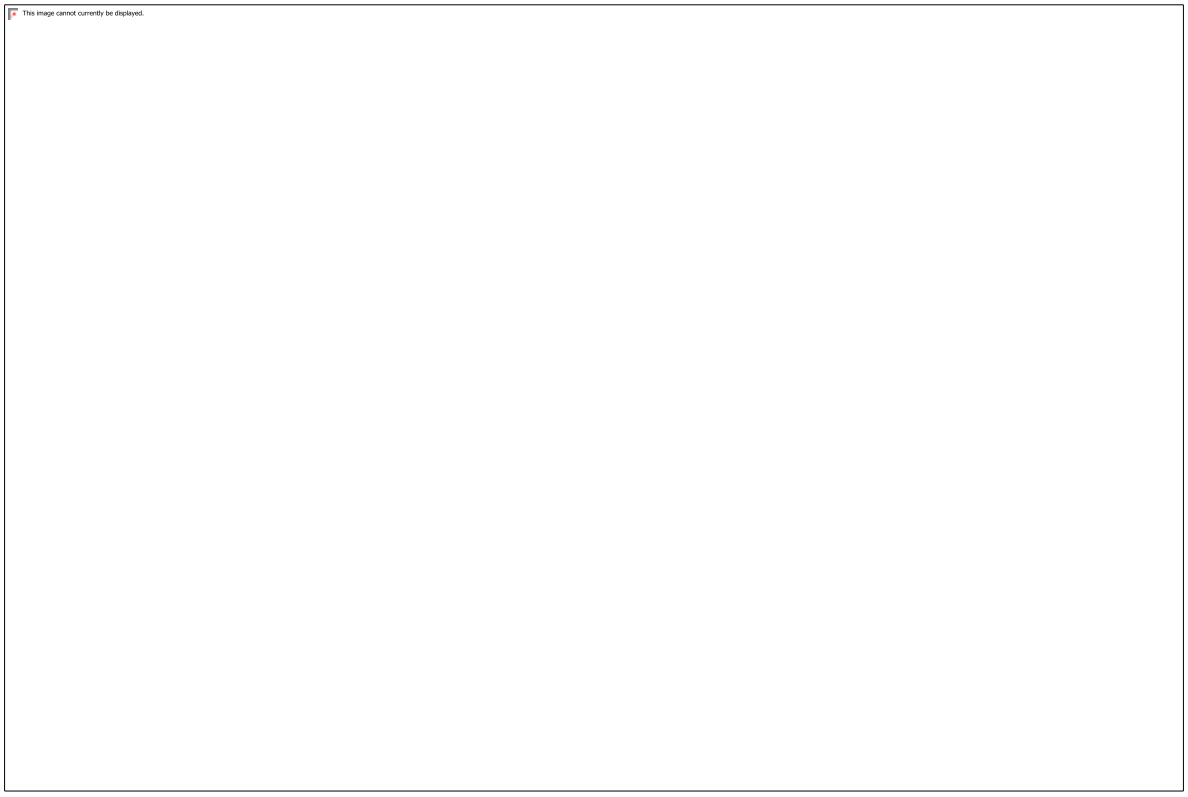

Figure 3A