In vitro Transcription

Aim of the Experiment

We used *in vitro* transcription (IVT) to synthesize RNA from DNA templates. The first protocol describes IVT from complete double stranded templates. For generation of crRNAs we developed a protocol using incomplete templates to facilitate varyation of target sequences. crRNA templates were completed using DNA polymerase and transcribed in a one-batch reaction.

Materials

- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- $MgCl_2$ (Carl Roth, Germany)
- NaCl (Carl Roth, Germany)
- Reaction buffer (10x RNAPol Reaction Buffer, NEB, Germany)
- T7 polymerase (homemade, provided by host lab)
- Deoxynucleoside triphosphate Mix (dNTP Mix, Thermo Fisher Scientific, Germany)
- Ribonucleotide Solution Mix (rNTP, NEB, Germany)
- Klenow Fragment (3'→5' exo-) (NEB, Germany)
- RNase Inhibitor, Murine (NEB, 40000 U/ml, Germany)
- Pyrophosphatase, Inorganic (IPP, 100 U/ml, NEB, Germany)
- DNaseI (RNAase-free) (NEB, Germany)
- DNaseI buffer (NEB, Germany)
- DNA templates (IDT, USA)

Procedure

1. Prepare the transcription mix

(a) For the complete, double-stranded template, prepare transcription mix as described in table 1.

Table 1: *In vitro* Transcription for target RNA

Concentration	**Chemicals**
1x	10x Reaction buffer
12 mM	$MgCl_2$
4 mM	rNTP
50 nM	template DNA
0.2 µM	T7 polymerase
1 µl	RNase inhibitor
1 µl	IPP

(b) For transcription based on incomplete, partially double-stranded DNA as in the case of crRNA, use the master strand (non-template, NT) and the target strand (template, T) as primers to form a template. Once NT and T bind in a complementary region, the missing 3' segments are filled up with the Klenow DNA polymerase V (figure 1). The completion of the double-stranded DNA allows the T7 polymerase to transcribe it into RNA. With this, targets can be interchanged by changing the T strand. Set up the reaction shown in table 2.

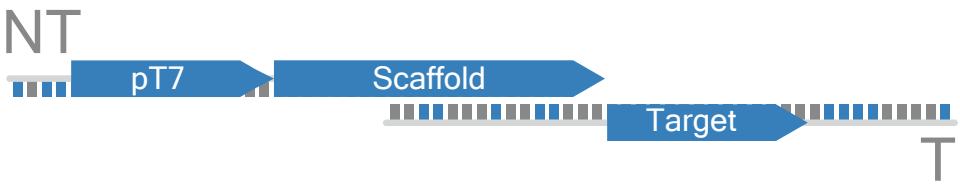

Figure 1: Scheme of NT and T DNA strand, which are designed with complementary sequence parts in the 3' or 5' end, respectively.

Table 2: *In vitro* Transcription for crRNA-incomplete template

Concentration	**Chemicals**
1 x	10x Reaction buffer
12 mM	$MgCl_2$
50 mM	NaCl
4 mM	rNTP
33 µM	dNTP
50 nM	template NT DNA
50 nM	template T DNA
0.2 µM	T7 polymerase
1 U	Klenow
1 µl	RNase inhibitor
1 µl	IPP

2. Incubate the reaction mix at 37 °C for 6h or over night.
3. Add 0.1 volume of 10x DNaseI Buffer and 0.02 volumes of l DNaseI (for 100 µl sample). Incubate at 37 °C for 30 min to 1 h.
4. Continue with the phenol-chloroform purification.