I) Preparation of the plasmids:

1. T7 polymerase plasmid (p70a-T7rnap) provided by *Arbor Biosciences* was cloned in E.*coli* KL740 and then mini-prepped to achieve required concentrations for cell-free expression.

2. Mini-prepped StarCore constructs provided by Design Team for further cell-free expression.

II) Cell-free optimization:
Control Constitutive GFP plasmid (p70a-deGFP) provided by *Arbor Biosciences* were expressed at 5 nM and 2,5 nM plasmid concentration for 15 hours in Lab-made and commercial kit.
See figure 2A in “Cell-free mix calibration” of “Cell-free” part.

Constituents	5nM	2,5nM	Blank
myTXTL	9ul	9ul	9ul
p70a-deGFP	3ul	1,5ul	0
dH2O	0	1,5ul	0

Fluorescence was measured using Teccan M200 plate reader at 55 gain for time interval of 5 mins for 15 hours.

III) Expression optimization for proteins under T7-promoter regulation:

- Plasmids construct at 20nM
- T7 plasmid at 1 nM
- 9 ul of myTXTL mix (as described in sigma70 protocol manual)

myTXTL	9ul
pT70a-deGFP (5nM)	3ul
T70a-T7rnap (1nM) + T7-deGFP (20nM)	1,3ul + 3,75ul
pT7-deGFP	3,75ul

Fluorescence was measured using Teccan M200 plate reader at 55 gain for time interval of 5 mins for 15 hours.

Experimental setup:

The expressions are done under those conditions:

- 29°C expression
- 15 hours
- Fluorescence measurement by Teccan M200 plate reader
- Plasmids at above mentioned concentration
- Proteins storage at -20°C after expression until further use by testing them

Requirements:

- Water
- Teccan plate reader
- 384 well-plates