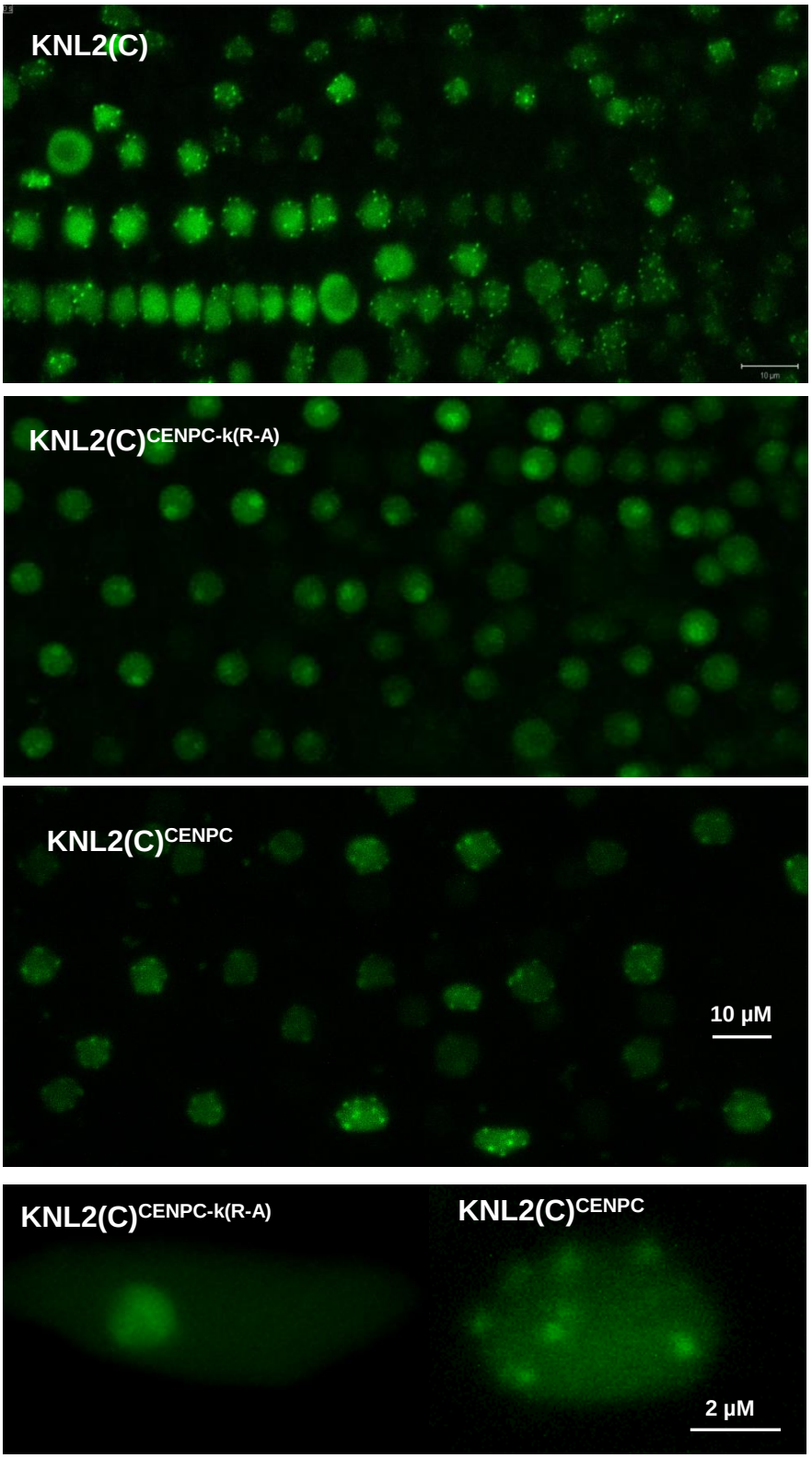
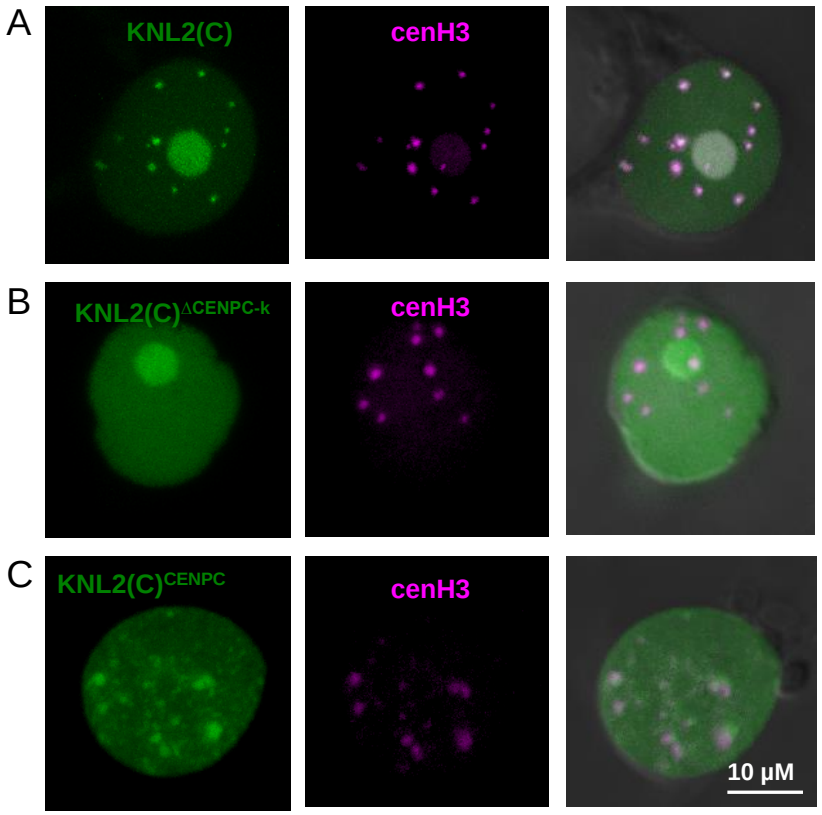
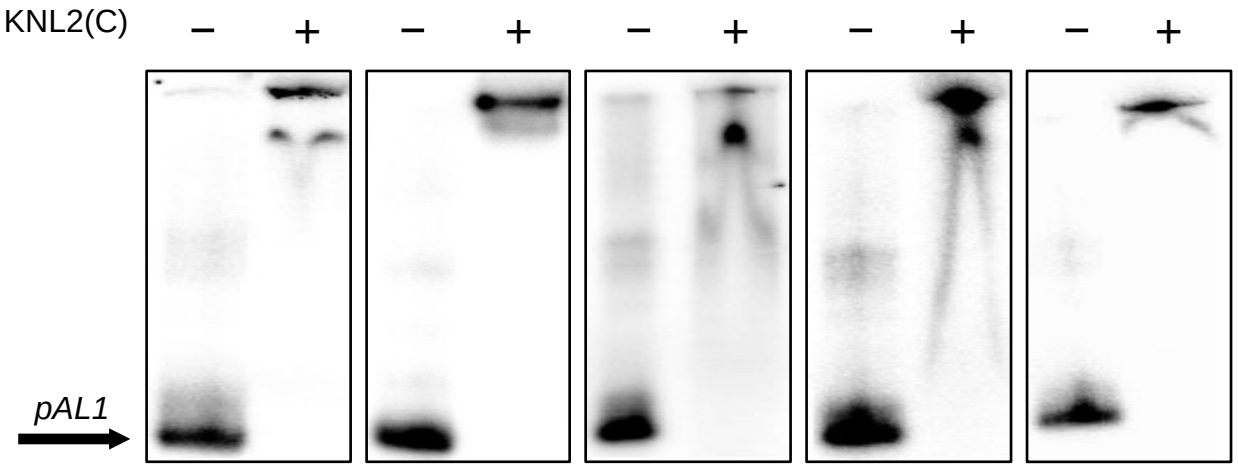


Supplemental Figure 1
Large scale and higher magnification view of localization of KNL2(C), KNL2(C)$^{CENPC\text{-}k(R\text{-}A)}$ and KNL2(C)^{CENPC} fused to EYFP in root tips of stable transformants of *A. thaliana*.
Corresponding small scale view is presented on Figure 2.

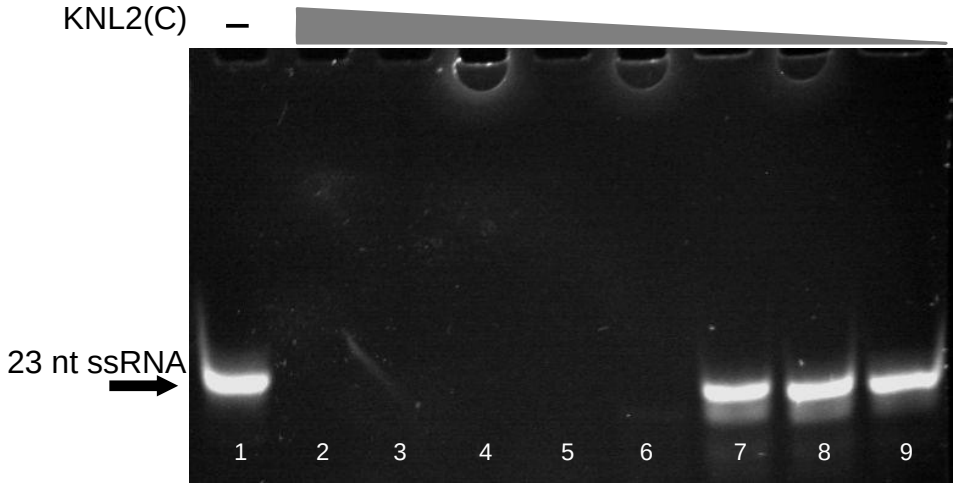


Supplemental Figure 2
The CENPC motif of CENP-C protein restores the ability of KNL2(C)$^{\Delta CENPC}$ to localize at centromeres.
Localization of native KNL2(C) (A), mutagenized KNL2 with deleted CENPC-k motif (KNL2(C)$^{\Delta CENPC\text{-}k}$) (B), and mutagenized KNL2 with CENPC-k motif substituted by the CENPC motif of the CENP-C protein (KNL2(C)^{CENPC}) (C) fused to EYFP in nuclei of *Nicotiana benthamiana* transiently expressing the corresponding constructs. To visualize centromeres, all three constructs were infiltrated together with a construct expressing a cenH3-mCherry fusion protein.

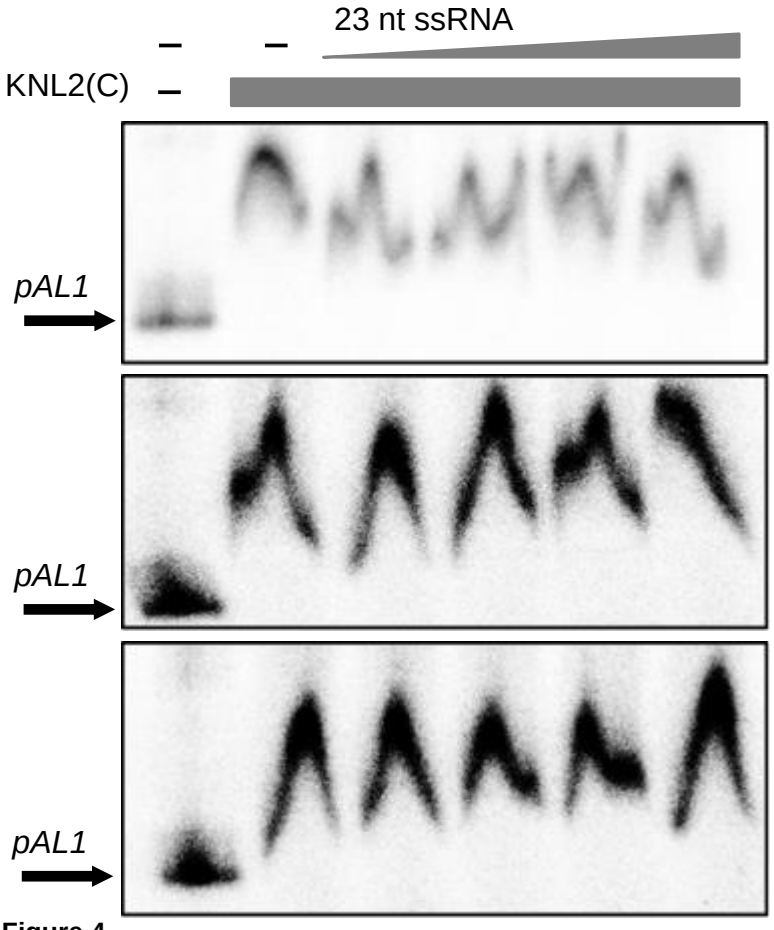


Supplemental Figure 3
The C-terminal part of KNL2 binds the centromeric pAL1 sequences also in radioactive EMSA.
Five different radioactive EMSA experiments with 0,2 pmol labeled *pAL1* (all lanes) without (-) or with (+) 1-2 pmol purified recombinant C-terminal KNL2. Complexes of *pAL1* and KNL2(C) remain close to the slots similar to non-radioactive EMSA experiments.

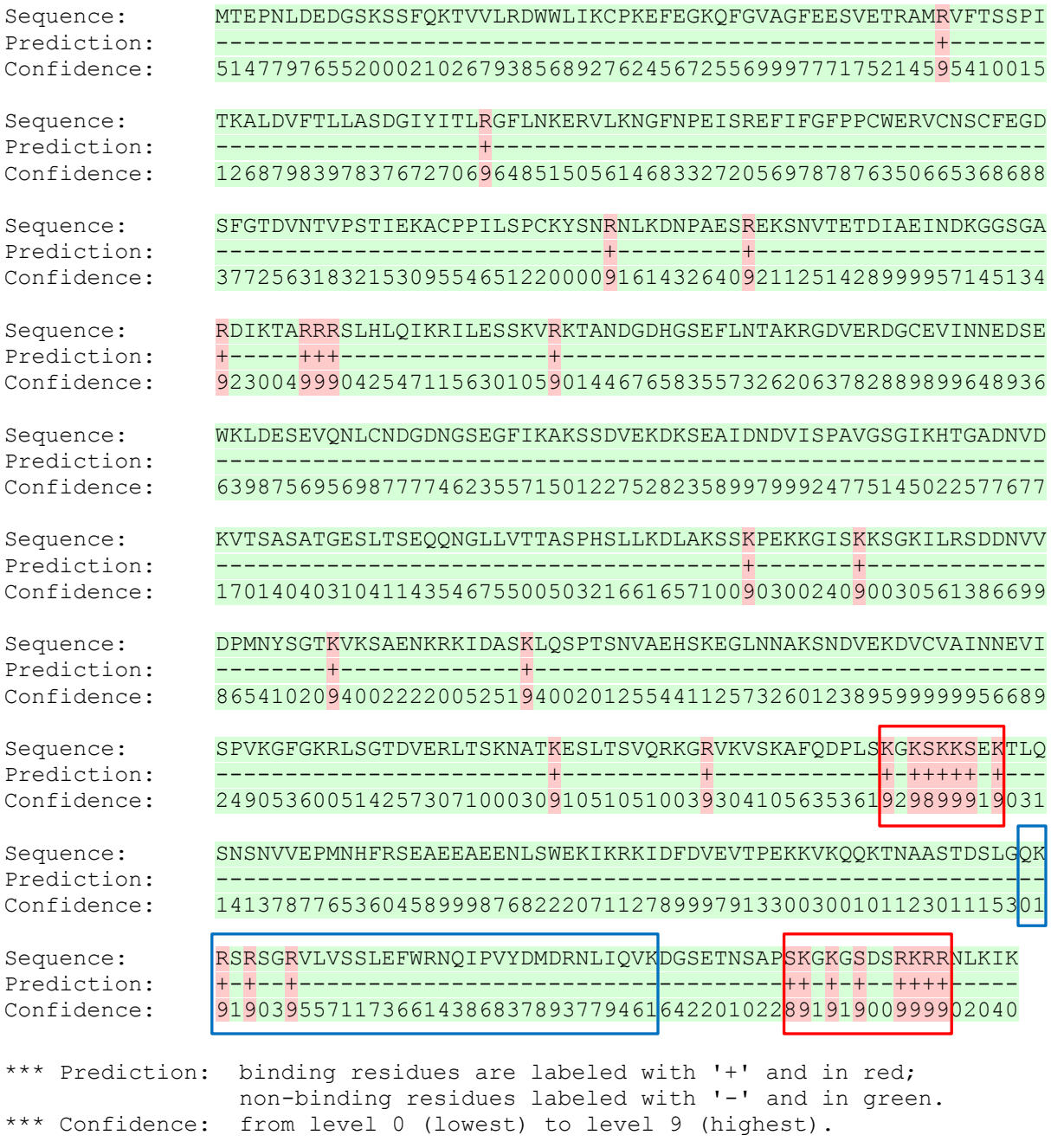
A



B



Supplemental Figure 4
KNL2 DNA binding is not influenced by centromeric 23 nt ssRNA.
(A) EMSA experiment with 1 pmol of 23 nt ssRNA (all lanes) and reduced amounts of purified recombinant C-terminal KNL2 (1; 0.8; 0.6; 0.4; 0.2; 0.1; 0.05 pmol in the lanes 2-9, respectively).
(B) Three different radioactive EMSA experiments with 0.1 pmol labeled *pAL1* (all lanes) and 1 pmol purified recombinant C-terminal KNL2 (lanes 2-6). Increasing amounts of 23 nt ssRNA of *pAL1* were added (lane 3 - 0.1 pmol, lane 4 - 1 pmol. lane 5 - 10 pmol, lane 6 - 100 pmol). ssRNA does not interfere the KNL2(C)-pAL1 complex formation and stability.



*** Prediction: binding residues are labeled with '+' and in red;
non-binding residues labeled with '-' and in green.
*** Confidence: from level 0 (lowest) to level 9 (highest).

Supplemental Figure 5

***In silico* analysis of KNL2 protein by BindN program (Wang and Brown 2006) for DNA binding amino acid residues**

To check for a possible DNA binding region in KNL2 the BindN program was used. Multiple residues with high DNA binding probability (red frame) arise at the C-terminus around the CENPC-k motif (blue frame).

Supplemental Table 1

Primers used in this study.

Primer name	Primer sequence	Reference
Side-directed mutagenesis		
KNL2_R_A_f	GTCAAGATCAGGAGCGGTGCTTGTGTCATC	
KNL2_R_A_r	GTTTCTGTCCCAATGAATCAGTAGACGCCGC	
KNL2_W_R_f	CATCACTAGAGTTTCGGCGTAACCAAATTC	
KNL2_W_R_r	ACACAAGCACCCTTCCTGATCTTGACCGTT	
KNL2_ΔCENPC_f	GATGGTAGTGAGACTAACTCCGCTC	
KNL2_ΔCENPC_r	TCCCAATGAATCAGTAGACGCCGC	
CENP-Csubst_fr1	GCATCCATGAGAGTTTGACTACTGTTGATGGT AGTGAGACTAACTCCGCTC	
CENP-Csubst_rev1	GTCCGTACAAGAATCTTTCACCTCTTCCCAAT GAATCAGTAGACGCCGC	
CENP-Csubst_fr2	TTAAGTCGAGACCGCTCGAATACTGGAGAGGT GAAAGATTCTTGTACGGAC	
CENP-Csubst_rev2	TCCTTGTACTTCGTCTGACACCACCTCCCAAT GAATCAGTAGACGCCGC	
Generation of DNA samples		
Athila_f	ATCACGTTGCCGACATCATAACG	
Athila_r	AGCAACAACGAAAGAGTTGAGAGC	
Telomere_f	TTTAGGGTTTAGGGTTTAGGGTTTAGGG	(Yu et al., 2000)
Telomere_r	AAATCCCAAATCCCAAATCCCAAATCCC	(Yu et al., 2000)
TUA4_f	GCTGTTGGTGGAGGGACTGG	(Ay et al., 2009)
TUA4_r	CCTGTGGAGATGGGTAAACTGTG	(Ay et al., 2009)
pAL1_f	GGTTAGTGTTTTGGAGTCGAATATG	(May et al., 2005)
pAL1_r	TTGCTTCTCAAAGATTTCATGGT	(May et al., 2005)
ChIP and qPCR		
Athila_f (0.1µM.)*	ATCACGTTGCCGACATCATAACG	
Athila_r (0.1µM)	AGCAACAACGAAAGAGTTGAGAGC	
Subtelo_f (0.25µM)	CTAAACTAGTTGTGTTCCCGTCTCTACT	(Gallego and White, 2001)
Subtelo_r (0.25µM)	GGTGGGCGACCTTGTGCTTGCCAAAGTC	(Gallego and White, 2001)
TUA4_f (0.04µM)	GCTGTTGGTGGAGGGACTGG	(Ay et al., 2009)
TUA4_r (0.04µM)	CCTGTGGAGATGGGTAAACTGTG	(Ay et al., 2009)
pAL1_f (0.05µM)	GGTTAGTGTTTTGGAGTCGAATATG	(May et al., 2005)
pAL1_r (0.05µM)	TTGCTTCTCAAAGATTTCATGGT	(May et al., 2005)
ssRNA		
23 bp ssRNA of *pAL1*	ACAAGGAUACAAUUCUUACGCCU	
forward *pAl1* ssRNA	GGUUAUGUUUUGGAGUCGAAUAUGACUUGAUC GAUCUCAUGUGUAUGAUUGAGUAUAAGAACUU AAAACGCAACCGCAUCUUAAAAGCCUAAGUAG UAUUUCCUUGUUAGAAGACACAAAGCCAAGAC UCAUAUGGACUUUGGCUACACCAUGAAAGCUU UGAGAAGCAA	(Zhang et al., 2008)
reverse *pAl1* ssRNA	UUGCUUCUCAAAGCUUUCAUGGUGUAGCCAAA GUCCAUAUGAGUCUUGGCUUUGUGUCUUCUAA CAAGGAAAUACUACUUAGGCUUUUAAGAUGCG GUUGCGUUUUAAGUUCUUAUACUCAAUCAUAC ACAUGAGAUCGAUCAAGUCAUAUUCGACUCCA AAACAUAACC	(Zhang et al., 2008)

***-final concentration of primers**

Supplemental Table 2

PCR programs used for the amplification of DNA probes for EMSA.

DNA fragment	PCR program
Athila	94°C – 5 min / (94°C – 20 s, 54°C – 30 s, 72°C – 1 min)x45 / 72°C – 7 min / 4°C - ∞
TUA4	94°C – 5 min / (94°C – 20 s, 60°C – 30 s, 72°C – 1 min)x35 / 72°C – 7 min / 4°C - ∞
Telomere	94°C – 5 min / (94°C – 20 s, 60°C – 30 s, 72°C – 5 s)x35 / 4°C - ∞
pAL1	94°C – 5 min / (94°C – 20 s, 50°C – 30 s, 72°C – 3 s)x40 / 4°C - ∞

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