

Table 1

Protein complexes used for co-expression experiments

Complex	Protein components	Residues	Mr (kDa)	N-terminal his tag	Stoichiometry
Cdt1:Geminin ^a	Geminin	1-209 (fl)	23.6	-	1 : 2
	Cdt1	158-396	29.4	+	
TFIIE α :TFIIE β ^b	TFIIE α	1-439 (fl)	49.5	+	1 : 1
	TFIIE β	1-291 (fl)	33.0	-	
Importin α 5:PB2 ^c	Importin α 5	66-512	49.7	-	1 : 1
	PB2	678-759	8.9	+	
	NFYA	262-347	10.4	-	
NFYA:NFYB:NFYC ^d	NFYB	51-143	10.8	-	1 : 1 : 1
	NFYC	28-120	11.1	+	

The boundaries of each protein (fragment) used for co-expression studies are specified by the residue numbers. Full length proteins are indicated (fl). Mr: Molecular mass in kDa

^a De Marco et al., 2009; ^b Jawhari et al., 2006; ^c Tarendeau et al., 2007; ^d Romier et al., 2006.

Table 2

Co-expression strategies used for complex expression

Partner	Co-expression number	Co-expression vectors	Co-expression strategy ^a
OPPF (Oxford)	1	pOPINF	3
	2	pCoGWA	4
	3	pHGWA + pCo0GWS	2
	4	pHGWA + pCo0GWS	2/3
	5	pCo0GWC + pCoGWA	2/4
	6	pCo0GWS + pCoGWA	3/4
	7	pnEA-tH + pnCS	2
	8	ppEA-tH + ppCS	2
	9	pnEA-tH + pnEK + pnCS	2
	10	ppEA-tH + ppEK + ppCS	2
	11	pnCS + pnEA-tH	2/3
	12	ppCS + ppEA-tH	2/4
	13	pnEA-tH	3
	14	ppEA-tH	4
EMBL (Hamburg)	15	pETM-11 + pCDF-13	2
	16	pETM-13 + pCDF-11	2
ISPC (Rehovot)	17	pET28-TEVH + pACYCDuet-1	2
	18	pACYCDuet-1	4
	19	pET28-TEVH + pACYCDuet-1	2/4
Bijvoet Center (Utrecht)	20	pLICHIS + pCDFLICHIS	2
PSPF (Berlin/Braunschweig)	21	pQLINK	4
Protein Production Laboratory (PPL) (York)	22	pETYSBLIC3C	4
	23	pET21d_LIC	4
	24	pRSF + pET21d_LIC	4
NKI (Amsterdam)	25	pETNKI-his3C-LIC + pET22b	1
	26	pETNKI-his3C-LIC + pETNKIc-LIC	1

^a Strategy 1 represents co-expression using multiple plasmids with incompatible origin of replications.

Strategy 2 represents co-expression using multiple plasmids with compatible origin of replications.

Strategy 3 represents co-expression using a single vector containing multiple genes with one promoter / vector (poly-cistronic).

Strategy 4 represents co-expression using a single vector containing multiple genes with one promoter / gene.

Table 3

His-Cdt1:Geminin co-expression results

Co-expression number	Partner	Construct	Co-expression Strategy	$\mu\text{g his-Cdt1} / \text{g cells}^a$	$\mu\text{g Geminin} / \text{g cells}^a$	Molar Ratio his-Cdt1:Geminin
1	OPPF	pOPINF his-Cdt1:Geminin	3	11	8.0	1 : 0.9
2	IGBMC	pCoGWA his-Cdt1:Geminin	4	112	83	1 : 0.9
3	IGBMC	pHGWA-his-Cdt1 + pCo0GWS-Geminin	2	< 1	< 1	n.d. ^b
7	IGBMC	pnEA-tH-his-Cdt1 + pnCS-Geminin	2	982	327	1 : 0.4
8	IGBMC	ppEA-tH-his-Cdt1 + ppCS-Geminin	2	488	439	1 : 1.1
16	EMBL	pCDF-11-his-Cdt1 + pETM-13-Geminin	2	581	576	1 : 1.2
17	ISPC	pET28-his-Cdt1 + pACYCDuet-Geminin	2	56	38	1 : 0.8
20	Bijvoet Center	pCDFLIC-his-Cdt1 + pLIC-Geminin	2	50	6	1 : 0.2
22	PPL	pET-YSBLIC3C-hisCdt1:Geminin	4	572	675	1 : 1.5
21	PSPF	pQLink-Geminin:his-Cdt1	4	30	16	1 : 0.7
25	NKI	pETNKI-his3C-LIC-his-Cdt1 + pET22b-Geminin	1	280	223	1 : 1.0
26	NKI	pETNKI-his3C-LIC-his-Cdt1 + pETNKIc-LIC-Geminin	1	14	9	1 : 0.6

^a Protein concentrations were determined from the LabChip GXII gelelectrophoresis experiments (supplementary Fig. 1A)^b n.d.: Not determined

Table 4His-TFII α :TFII β co-expression results

Co-expression number	Partner	Construct	Co-expression Strategy	$\mu\text{g his-TFII}\alpha / \text{g cells}^a$	$\mu\text{g TFII}\beta / \text{g cells}^a$	Molar Ratio his-TFII α :TFII β
1	Oxford	pOPINF his-TFII α :TFII β	3	67	30	1 : 0.7
2	IGBMC	pCoGWA his-TFII α :TFII β	4	108	81	1 : 1.1
3	IGBMC	pHGWA-his-TFII α + pCoGWS-TFII β	2	232	104	1 : 0.7
7	IGBMC	pnEA-tH-his-TFII α + pnCS-TFII β	2	366	254	1 : 1.1
8	IGBMC	ppEA-tH-his-TFII α + ppCS-TFII β	2	411	280	1 : 1.0
15	EMBL	pETM-11-his-TFII α + pCDF-13-TFII β	2	361	259	1 : 1.1
16	EMBL	pCDF-11-his-TFII α + pETM-13-TFII β	2	334	211	1 : 1.0
17	ISPC	pET28-his-TFII α + pACYCDuet-TFII β	2	244	172	1 : 1.1
18	ISPC	pACYCDuet-his-TFII α :TFII β	4	175	125	1 : 1.1
23	PPL	pET21d_LIC-his-TFII α :TFII β	4	311	167	1 : 0.8
21	PSPF	pQLink-his-TFII α :TFII β	4	402	204	1 : 0.8
26	NKI	pETNKI-his3C-LIC-his-TFII α + pETNKIc LIC-TFII β	1	105	73	1 : 1.1

^a Protein concentrations were determined from the LabChip GXII gelelectrophoresis experiments (supplementary Fig. 1B)

Table 5His-PB2:Importin $\alpha 5$ co-expression results

Co-expression number	Partner	Construct	Co-expression Strategy	$\mu\text{g his-PB2} / \text{g cells}^a$	$\mu\text{g Importin-}\alpha 5 / \text{g cells}^a$	Molar Ratio His-PB2 : Importin $\alpha 5$
1	OPPF	pOPINF his-PB2:Importin $\alpha 5$	3	12	5	1 : 0.1
2	IGBMC	pCoGWA his-PB2:Importin $\alpha 5$	4	187	149	1 : 0.2
3	IGBMC	pHGWA-his-PB2 + pCoOGWS-Importin $\alpha 5$	2	168	99	1 : 0.1
7	IGBMC	pnEA-tH-his-PB2 + pnCS-Importin $\alpha 5$	2	624	273	1 : 0.1
8	IGBMC	ppEA-tH-his-PB2 + ppCS-Importin $\alpha 5$	2	521	212	1 : 0.1
15	EMBL	pETM-11-his-PB2 + pCDF-13-Importin $\alpha 5$	2	626	240	1 : 0.1
16	EMBL	pCDF-11-his-PB2 + pETM-13-Importin $\alpha 5$	2	337	153	1 : 0.1
17	ISPC	pET28-his-PB2 + pACYCDuet-importin $\alpha 5$	2	179	17	1 : < 0.1
18	ISPC	pACYCDuet-his-PB2/Importin $\alpha 5$	4	121	51	1 : 0.1
20	Bijvoet Center	pCDFLIC-his-PB2 + pLIC-Importin $\alpha 5$	2	412	183	1 : 0.1
21	PSPF	pQLink-his-PB2:Importin $\alpha 5$	4	408	398	1 : 0.2
22	PPL	pYSBLIC3C-his-PB2:Importin $\alpha 5$	4	141	11	1 : < 0.1
26	NKI	pETNKI-his3C-LIC-his-PB2 + pETNKIc-LIC-Importin $\alpha 5$	1	25 ^b	< 0.1 ^b	n.d. ^c

^a Protein concentrations were determined from the LabChip GXII gelelectrophoresis experiments (supplementary Fig. 1C) unless stated otherwise

^b Intensity of protein band on SDS-PAGE was determined using Image J software and the protein concentration was calculated using corresponding bands that have been quantified using the Labchip GXII software (see section 2.4).

^c n.d.: Not determined

Table 6

His-NFYC:NFYB:NFYA co-expression results

Co-expression experiment	Partner	Construct	Co-expression strategy	μg his-NFYC / g cells ^a	μg NFYB / g cells ^a	μg NFYA / g cells ^a	Molar Ratio His-NFYC : NFYB : NFYA
1	OPPF	pOPINF his-NFYC:NFYA:NFYB	3	50	22	19	1 : 0.4 : 0.4
4A	IGBMC	pHGWA-his-NFYC + pCo0GWS-NFYB:NFYA	2/3	< 1	< 1	< 1	n.d. ^c
4B	IGBMC	pHGWA-his-NFYC + pCo0GWS-NFYA:NFYB	2/3	< 1	< 1	< 1	n.d.
5	IGBMC	pCoGWA-his-NFYC:NFYB + pCo0GWC-NFYA	2/4	41	17	12	1 : 0.4 : 0.3
6	IGBMC	pCoGWA-his-NFYC:NFYA + pCo0GWS-NFYB	2/4	< 1	< 1	< 1	n.d.
9	IGBMC	pnEK-NFYA + pnCS-NFYB + pnEA-tH-his-NFYC	2	< 1	< 1	< 1	n.d.
10	IGBMC	ppEK-NFYA + ppCS-NFYB + ppEA-tH-his-NFYC	2	< 1	< 1	< 1	n.d.
11	IGBMC	pnCS- NFYA + pnEA-tH-NFYB:his-NFYC	2/3	121 ^b	109 ^b	107 ^b	1 : 0.8 : 0.9
12	IGBMC	ppCS- NFYA + ppEA-tH-NFYB:his-NFYC	2/4	129 ^b	111 ^b	125 ^b	1 : 0.8 : 1.0
13	IGBMC	pnEA-th- his-NFYC:NFYA:NFYB	3	284	150	260	1 : 0.3 : 1.2
14	IGBMC	ppEA-th- his-NFYC:NFYA:NFYB	4	31	18	35	1 : 0.6 : 0.9
19	ISPC	pET28 his-NFYC + pACYCDuet-NFYB:NFYA	2/4	121	74	103	1 : 0.6 : 0.9
21	PSPF	pQLink-his-NFYC:NFYA:NFYB	4	56	< 1	4.2	1 : <0.1 : 0.1
24	PPL	pET21d_LIC-his-NFYC:NFYA + pRSF-NFYB	2/4	128	79	120	1 : 0.6 : 0.5

^a Protein concentrations were determined from the LabChip GXII gelelectrophoresis experiments (supplementary Fig. 1D) unless stated otherwise.

^b Intensity of protein band on SDS-PAGE was determined using Image J software and the protein concentration was calculated using corresponding bands that have been quantified using the Labchip GXII software (see section 2.4).

^c n.d.: Not determined