Materials and Method

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Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4139392

SGC Construct ID: DYRK2A-c022

GenBank GI number: gi|4503427

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGGGGAAGGTGAAAGCCACC
CCCATGACACCTGAACAAGCAATGAAGCAA
TACATGCAAAAACTCACAGCCTTCGAACAC
CATGAGATTTTCAGCTACCCTGAAATATAT
TTCTTGGGTCTAAATGCTAAGAAGCGCCAG
GGCATGACAGGTGGGCCCAACAATGGTGGC
TATGATGATGACCAGGGATCATATGTGCAG
GTGCCCCACGATCACGTGGCTTACAGGTAT
GAGGTCCTCAAGGTCATTGGGAAGGGGAGC
TTTGGGCAGGTGGTCAAGGCCTACGATCAC
AAAGTCCACCAGCACGTGGCCCTAAAGATG
GTGCGGAATGAGAAGCGCTTCCACCGGCAA
GCAGCGGAGGAGATCCGAATCCTGGAACAC
CTGCGGAAGCAGGACAAGGATAACACAATG
AATGTCATCCATATGCTGGAGAATTTCACC
TTCCGCAACCACATCTGCATGACGTTTGAG
CTGCTGAGCATGAACCTCTATGAGCTCATC
AAGAAGAATAAATTCCAGGGCTTCAGTCTG
CCTTTGGTTCGCAAGTTTGCCCACTCGATT
CTGCAGTGCTTGGATGCTTTGCACAAAAAC
AGAATAATTCACTGTGACCTTAAGCCCGAG
AACATTTTGTTAAAGCAGCAGGGTAGAAGC
GGTATTAAAGTAATTGATTTTGGCTCCAGT
TGTTACGAGCATCAGCGTGTCTACACGTAC
ATCCAGTCGCGTTTTTACCGGGCTCCAGAA
GTGATCCTTGGGGCCAGGTATGGCATGCCC
ATTGATATGTGGAGCCTGGGCTGCATTTTA
GCAGAGCTCCTGACGGGTTACCCCCTCTTG
CCTGGGGAAGATGAAGGGGACCAGCTGGCC
TGTATGATTGAACTGTTGGGCATGCCCTCA
CAGAAACTGCTGGATGCATCCAAACGAGCC
AAAAATTTTGTGAGCTCCAAGGGTTATCCC
CGTTACTGCACTGTCACGACTCTCTCAGAT
GGCTCTGTGGTCCTAAACGGAGGCCGTTCC
CGGAGGGGGAAACTGAGGGGCCCACCGGAG
AGCAGAGAGTGGGGGAACGCGCTGAAGGGG
TGTGATGATCCCCTTTTCCTTGACTTCTTA
AAACAGTGTTTAGAGTGGGATCCTGCAGTG
CGCATGACCCCAGGCCAGGCTTTGCGGCAC
CCCTGGCTGAGGAGGCGGTTGCCAAAGCCT
CCCACCGGGGAGAAAACGTCAGTGAAAAGG
TGACAGTAAAGGTGGATACGGATCCGAA
```

Final protein sequence (tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfq^smGKVKAT
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PMTPEQAMKQYMQKLTAFEHHEIFSYPEIY
FLGLNAKKRQGMTGGPNNGGYDDDQGSYVQ
VPHDHVAYRYEVLKVIGKGSFGQVVKAYDH
KVHQHVALKMVRNEKRFHRQAAEEIRILEH
LRKQDKDNTMNVIHMLENFTFRNHICMTFE
LLSMNLYELIKKNKFQGFSLPLVRKFAHSI
LQCLDALHKNRIIHCDLKPENILLKQQGRS
GIKVIDFGSSCYEHQRVYTYIQSRFYRAPE
VILGARYGMPIDMWSLGCILAELLTGYPLL
PGEDEGDQLACMIELLGMPSQKLLDASKRA
KNFVSSKGYPRYCTVTTLSDGSVVLNGGRS
RRGKLRGPPESREWGNALKGCDDPLFLDFL
KQCLEWDPAVRMTPGQALRHPWLRRRLPKP
PTGEKTSVKR
^ TEV cleave site

Tags and additions: Cleavable N-terminal His6 tag.

Host:BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Growth medium, induction protocol: 5 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin & 34 µg/ml chloramphenicol were used to inoculate each of two 1 litre cultures of LB containing 50 µg/ml kanamycin & 34 µg/ml chloramphenicol. Cultures were grown at 37°C until the OD_{600} reached ~0.5 then the temperature was adjusted to 18°C. Expression was induced overnight using 0.5 mM IPTG at an OD_{600} of 0.9. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen. **Binding buffer**: 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5 % glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP added to the lysate. Cells were lysed using sonication. The lysate was centrifuged at 16,500 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Column 1 Buffers: **Binding buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% Glycerol; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol (step elution).

Column 1 Procedure: The lysate supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted.

Column 2: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad

Column 2 Buffers: 25 mM HEPES, pH 7.5; 500 mM NaCl, 0.5 mM TCEP

Column 2 Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 25 mM HEPES, pH 7.5; 500 mM NaCl, 0.5 mM TCEP using an ÄKTAexpress system.

Mass spec characterization: LC- ESI -MS TOF showed that the protein was herterogeneously phosphorylated at up to 4 sites in accordance with a mass of 49279 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 7.3 mg/ml using an Amicon 10 kDa cut-off concentrator.

Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 1.26 M $(NH_4)_2SO_4$; 0.2 M Li_2SO_4; 0.1 M Tris pH 8.5.

Data Collection: Crystals were cryo-protected using the well solution supplemented with 2M Li_2SO_4 and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected from a single crystal on Diamond beamline IO3 at a single wavelength of 0.9763 Å and the structure was refined to 2.36 Å.

> **Phasing:** The structure was solved by molecular replacement using the structure of human DYRK1 (PDB ID 2VX3) as a starting model.

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