Tool box: Plasmids for the expression or knockdown of human ARF Family GTPases (ARF/ARL/SAR) and their co-expression in bacteria with N-myristoyltransferases

Shana C Kerr & Richard A Kahn

To cite this article: Shana C Kerr & Richard A Kahn (2015) Tool box: Plasmids for the expression or knockdown of human ARF Family GTPases (ARF/ARL/SAR) and their co-expression in bacteria with N-myristoyltransferases, Cellular Logistics, 5:3, e1090523, DOI: 10.1080/21592799.2015.1090523

To link to this article: http://dx.doi.org/10.1080/21592799.2015.1090523

© 2015 The Author(s). Published with license by Taylor & Francis Group, LLC

Shana C Kerr and Richard A Kahn

Accepted author version posted online: 21 Sep 2015.

Submit your article to this journal

Article views: 66

View related articles

View Crossmark data
Tool box: Plasmids for the expression or knockdown of human ARF Family GTPases (ARF/ARL/SAR) and their co-expression in bacteria with N-myristoyltransferases

Shana C Kerr1 and Richard A Kahn2,*

1School of Biology; Georgia Institute of Technology; Atlanta, GA USA; 2Department of Biochemistry; Emory University School of Medicine; Atlanta, GA USA

Abbreviations: ARF, ADP-ribosylation factor; ARL, ARF-like; MAP, methionine aminopeptidase; NMT, N-myristoyltransferase; ORF, open reading frame

This article is intended to inform researchers about a collection of ~200 plasmids recently made available through Addgene (www.addgene.com), that were generated to facilitate the study of human ARF family GTPases, including all 5 ARF and 2 SAR and an incomplete collection of ARF-like (ARL) proteins. They fall into 3 groups based upon usage; (1) ARF family GTPase expression in mammalian or bacterial cells, (2) N-myristoyltransferase co-expression in bacteria, and (3) pSUPER-based plasmids for siRNA knockdown of human ARF1, ARF3, ARF4, or ARF5. The majority of these plasmids direct the expression of human ARF family GTPases for study in mammalian cells or for purification from bacteria. The constructs are untagged or carry a few commonly used tags such as GFP, HA epitope, or V5-His6. These plasmids were engineered in the Gateway cloning system (Life Technologies) to allow ready insertion of other tags, thus the entry vectors are also provided. A group of 4 plasmids that direct expression of human N-myristoyltransferases, designed for co-expression in bacteria to allow N-myristoylation of recombinant proteins,1,2 is included. We also provide a series of pSUPER-based plasmids,3 proven useful in knockdown of ARF1-ARF5 in human cells.4 A detailed summary of the construction of these plasmids and examples of their use is provided below and can be found in the cited references. Because ARF proteins in particular are very highly conserved (100% amino acid identity among several mammals including rodents), yet differ in DNA sequence, some of these plasmids may be useful in rescue experiments using gene deletion or knockdown.

Arf Family GTPases in Gateway Vectors for Expression of Arf, ARL, And Sar Proteins in Mammalian and Bacterial Cells

With a long-term goal of studying the human ARF family and their functions in cells, we generated a collection of plasmids that direct expression of 21 different members of the human ARF family. The human ARF family today is known to include as many as 30 different members (Jeremy Wideman, Joel Dacks, and R. A. Kahn; manuscript in preparation). We obtained EST clones from public resources that included the entire open reading frames of ARF1, ARF3–6, ARL1–3, ARL4A/C/D, ARL5A/B, ARL6, ARL8A/B, ARL11, ARL14, ARFRP1, and SAR1A/B. These ORFs were amplified by PCR to add appropriate sites for recombination and insertion into the Gateway entry vector pDONR221. Two entry vectors were created for each ORF, one with and another without stop codons, to allow the generation of untagged or C-terminal tagged proteins. Each of these 42 entry vectors were sequence verified. We note in a few instances (ARL4D, ARL11, ARL14) single bp differences from the current NCBI entries, resulting in single missense mutations, but in each case our sequences were present in the EST clones from which they derived.

This set of 21 ARF family members were used to generate a total of 168 Gateway-derived plasmids, including the 2 sets of entry vectors (with and without stop codons), 4 sets for mammalian cell expression (untagged, or tagged at the C-terminus with HA, V5-His6, or GFP), and 2 sets for expression in bacteria (untagged or tagged at the C-terminus with V5-His6). The names and uses of these plasmids, along with gene names, aliases and NCBI Gene ID numbers are summarized in Table 1. Entry clones with stop codons were moved into pDEST47 or pDEST14 to generate plasmids for expression of untagged proteins in mammalian or bacterial cells, respectively. Entry clones lacking a stop codon were moved into (A) pDSHA, for expression of C-terminal HA tagged proteins in mammalian cells, (B) pDEST47, for expression of C-terminal GFP tagged proteins in mammalian cells, (C) pDEST40, for expression in mammalian cells of C-terminal V5-His6 tagged proteins, and (D) pET-DEST42, for expression in bacteria of C-terminal V5-His6 tagged proteins.

© Shana C Kerr and Richard A Kahn
*Correspondence to: Richard A Kahn; Email: rkahn@emory.edu
Submitted: 08/11/2015; Revised: 08/26/2015; Accepted: 08/28/2015
http://dx.doi.org/10.1080/21592799.2015.1090523
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
### Table 1. Summary of the plasmids directing expression of human ARF family GTPases (ARF/ARL/SAR) in mammalian cells or bacteria, using the Gateway cloning system. The GTPase is listed, along with pseudonyms, NCBI Gene ID number, name of the plasmid as it appears in the Addgene collection, short description of the intended use, and reference in which it was first reported. Smaller collections of plasmids used for co-expression in bacteria of proteins of interest with N-myristoyltransferases 1 or 2 (NMT1/2) with or without methionine amino peptidase (MAP). The collection of pSUPER based plasmids directing expression of short hairpin RNAs (shRNAs) that deplete cells of human ARF1–5 are also included.

| GTPase | Pseudonyms | Gene ID | Addgene plasmid name | Description | Use |
|--------|------------|---------|----------------------|-------------|-----|
| ARF1   |            | 375     | pDONR221-ARF1        | Entry vector: ARF1 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARF1-no stop| Entry vector: ARF1 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARF1-HA        | ARF1-HA | Mammalian expression |
|        |            |         | pDEST14-ARF1         | ARF1 | Bacterial expression |
|        |            |         | pDEST47-ARF1-GFP     | ARF1-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARF1-V5-His6| ARF1-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARF1-V5-His6 | ARF1-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARF1         | ARF1 | Mammalian expression |
| ARF3   |            | 377     | pDONR221-ARF3        | Entry vector: ARF3 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARF3-no stop| Entry vector: ARF3 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARF3-HA        | ARF3-HA | Mammalian expression |
|        |            |         | pDEST14-ARF3         | ARF3 | Bacterial expression |
|        |            |         | pDEST47-ARF3-GFP     | ARF3-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARF3-V5-His6| ARF3-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARF3-V5-His6 | ARF3-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARF3         | ARF3 | Mammalian expression |
| ARF4   | ARF2       | 378     | pDONR221-ARF4        | Entry vector: ARF4 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARF4-no stop| Entry vector: ARF4 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARF4-HA        | ARF4-HA | Mammalian expression |
|        |            |         | pDEST14-ARF4         | ARF4 | Bacterial expression |
|        |            |         | pDEST47-ARF4-GFP     | ARF4-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARF4-V5-His6| ARF4-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARF4-V5-His6 | ARF4-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARF4         | ARF4 | Mammalian expression |
| ARF5   |            | 381     | pDONR221-ARF5        | Entry vector: ARF5 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARF5-no stop| Entry vector: ARF5 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARF5-HA        | ARF5-HA | Mammalian expression |
|        |            |         | pDEST14-ARF5         | ARF5 | Bacterial expression |
|        |            |         | pDEST47-ARF5-GFP     | ARF5-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARF5-V5-His6| ARF5-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARF5-V5-His6 | ARF5-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARF5         | ARF5 | Mammalian expression |
| ARF6   |            | 382     | pDONR221-ARF6        | Entry vector: ARF6 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARF6-no stop| Entry vector: ARF6 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARF6-HA        | ARF6-HA | Mammalian expression |
|        |            |         | pDEST14-ARF6         | ARF6 | Bacterial expression |
|        |            |         | pDEST47-ARF6-GFP     | ARF6-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARF6-V5-His6| ARF6-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARF6-V5-His6 | ARF6-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARF6         | ARF6 | Mammalian expression |
| ARL1   | ARF1L      | 400     | pDONR221-ARL1        | Entry vector: ARL1 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL1-no stop| Entry vector: ARL1 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL1-HA        | ARL1-HA | Mammalian expression |
|        |            |         | pDEST14-ARL1         | ARL1 | Bacterial expression |
|        |            |         | pDEST47-ARL1-GFP     | ARL1-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARL1-V5-His6| ARL1-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARL1-V5-His6 | ARL1-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARL1         | ARL1 | Mammalian expression |
| ARL2   | ARFL2      | 402     | pDONR221-ARL2        | Entry vector: ARL2 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL2-no stop| Entry vector: ARL2 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL2-HA        | ARL2-HA | Mammalian expression |
|        |            |         | pDEST14-ARL2         | ARL2 | Bacterial expression |
|        |            |         | pDEST47-ARL2-GFP     | ARL2-GFP | Mammalian expression |
|        |            |         | pET-DEST42-ARL2-V5-His6| ARL2-V5-His6 | Bacterial expression |
|        |            |         | pDEST40-ARL2-V5-His6 | ARL2-V5-His6 | Mammalian expression |
|        |            |         | pDEST47-ARL2         | ARL2 | Mammalian expression |
|        |            |         | pDEST17-HA-ARL2      | HA-ARL2 | Mammalian expression |

(continued on next page)
Table 1. Summary of the plasmids directing expression of human ARF family GTPases (ARF/ARL/SAR) in mammalian cells or bacteria, using the Gateway cloning system. The GTPase is listed, along with pseudonyms, NCBI Gene ID number, name of the plasmid as it appears in the Addgene collection, short description of the intended use, and reference in which it was first reported. Smaller collections of plasmids used for co-expression in bacteria of proteins of interest with N-myristoyltransferases 1 or 2 (NMT1/2) with or without methionine amino peptidase (MAP). The collection of pSUPER based plasmids directing expression of short hairpin RNAs (shRNAs) that deplete cells of human ARF1–5 are also included (Continued)

| GTPase | Pseudonyms | Gene ID | Addgene plasmid name | Description | Use |
|--------|------------|---------|-----------------------|-------------|-----|
| ARL3   | ARFL3      | 403     | pDONR221-ARL3         | Entry vector: ARL3 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL3-no stop | Entry vector: ARL3 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL3-HA         | ARL3-HA     | Mammalian expression |
|        |            |         | pDEST14-ARL3          | ARL3         | Bacterial expression |
|        |            |         | pDEST47-ARL3-GFP      | ARL3-GFP    | Mammalian expression |
|        |            |         | pDEST40-ARL3-V5-His6  | ARL3-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL3          | ARL3         | Mammalian expression |
| ARL4A  | ARL4       | 10124   | pDONR221-ARL4A        | Entry vector: ARL4A w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL4A-no stop| Entry vector: ARL4A w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL4A-HA        | ARL4A-HA    | Mammalian expression |
|        |            |         | pDEST14-ARL4A         | ARL4A        | Bacterial expression |
|        |            |         | pDEST47-ARL4A-GFP     | ARL4A-GFP   | Mammalian expression |
|        |            |         | pDEST40-ARL4A-V5-His6 | ARL4A-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL4A-GFP     | ARL4A        | Mammalian expression |
| ARL4C  | ARL7, LAK  | 10123   | pDONR221-ARL4C        | Entry vector: ARL4C w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL4C-no stop| Entry vector: ARL4C w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL4C-HA        | ARL4C-HA    | Mammalian expression |
|        |            |         | pDEST14-ARL4C         | ARL4C        | Bacterial expression |
|        |            |         | pDEST47-ARL4C-GFP     | ARL4C-GFP   | Mammalian expression |
|        |            |         | pDEST40-ARL4C-V5-His6 | ARL4C-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL4C         | ARL4C        | Mammalian expression |
| ARL4D  | ARL9, ARL4L| 379     | pDONR221-ARL4D        | Entry vector: ARL4D w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL4D-no stop| Entry vector: ARL4D w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL4D-HA        | ARL4D-HA    | Mammalian expression |
|        |            |         | pDEST14-ARL4D         | ARL4D        | Bacterial expression |
|        |            |         | pDEST47-ARL4D-GFP     | ARL4D-GFP   | Mammalian expression |
|        |            |         | pDEST40-ARL4D-V5-His6 | ARL4D-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL4D         | ARL4D        | Mammalian expression |
| ARL5A  | ARL5, ARFLP5| 26225  | pDONR221-ARL5A        | Entry vector: ARL5A w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL5A-no stop| Entry vector: ARL5A w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL5A-HA        | ARL5A-HA    | Mammalian expression |
|        |            |         | pDEST14-ARL5A         | ARL5A        | Bacterial expression |
|        |            |         | pDEST47-ARL5A-GFP     | ARL5A-GFP   | Mammalian expression |
|        |            |         | pDEST40-ARL5A-V5-His6 | ARL5A-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL5A         | ARL5A        | Mammalian expression |
| ARL5B  | ARL8       | 221079  | pDONR221-ARL5B        | Entry vector: ARL5B w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL5B-no stop| Entry vector: ARL5B w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL5B-HA        | ARL5B-HA    | Mammalian expression |
|        |            |         | pDEST14-ARL5B         | ARL5B        | Bacterial expression |
|        |            |         | pDEST47-ARL5B-GFP     | ARL5B-GFP   | Mammalian expression |
|        |            |         | pDEST40-ARL5B-V5-His6 | ARL5B-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL5B         | ARL5B        | Mammalian expression |
| ARL6   | BBS3, RP55 | 84100   | pDONR221-ARL6         | Entry vector: ARL6 w/ stop codon | Gateway entry vector |
|        |            |         | pDONR221-ARL6-no stop | Entry vector: ARL6 w/o stop codon | Gateway entry vector |
|        |            |         | pDSHA-ARL6-HA         | ARL6-HA     | Mammalian expression |
|        |            |         | pDEST14-ARL6          | ARL6         | Bacterial expression |
|        |            |         | pDEST47-ARL6-GFP      | ARL6-GFP    | Mammalian expression |
|        |            |         | pDEST40-ARL6-V5-His6  | ARL6-V5-His6| Bacterial expression |
|        |            |         | pDEST47-ARL6          | ARL6         | Mammalian expression |
| ARL8A  | GIE2, ARL10B| 127829 | pDONR221-ARL8A       | Entry vector: ARL8A w/ stop codon | Gateway entry vector |

(continued on next page)
### Table 1. Summary of the plasmids directing expression of human ARF family GTPases (ARF/ARL/SAR) in mammalian cells or bacteria, using the Gateway cloning system. The GTPase is listed, along with pseudonyms, NCBI Gene ID number, name of the plasmid as it appears in the Addgene collection, short description of the intended use, and reference in which it was first reported. Smaller collections of plasmids used for co-expression in bacteria of proteins of interest with N-myristoyltransferases 1 or 2 (NMT1/2) with or without methionine aminopeptidase (MAP). The collection of pSUPER based plasmids directing expression of short hairpin RNAs (shRNAs) that deplete cells of human ARF1–5 are also included (Continued)

| GTPase  | Pseudonyms  | Gene ID | Addgene plasmid name                        | Description                                      | Use                             |
|---------|-------------|---------|---------------------------------------------|--------------------------------------------------|----------------------------------|
| ARF8    | pDONR221-ARL8A-no stop | pDSHA-ARL8A-HA | Gateway entry vector                         | Mammalian expression                        |
|         | pDEST14-ARL8A | pDEST47-ARL8A-GFP | ARL8A                                          | Bacterial expression                         |
|         | pET-DEST42-ARL8A-V5-His6 | pDEST40-ARL8A-V5-His6 | ARL8A-V5-His6                                  | Mammalian expression                        |
| ARL8B   | GIE1, ARL10C | 55207   | pDONR221-ARL8B                                | Gateway entry vector                         |
|         | pDONR221-ARL8B-no stop | pDSHA-ARL8A-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-ARL8B | pDEST47-ARL8B-GFP | ARL8B                                          | Mammalian expression                         |
|         | pET-DEST42-ARL8A-V5-His6 | pDEST40-ARL8A-V5-His6 | ARL8A-V5-His6                                  | Mammalian expression                        |
|         | pDEST47-ARL8B | pDEST40-ARL8B-V5-His6 | pDEST47-ARL8B  | pDEST14-ARL11 HA | Gateway entry vector                        |
| ARL11   | ARLTS1      | 115761  | pDONR221-ARL11                                | Gateway entry vector                         |
|         | pDONR221-ARL11-no stop | pDSHA-ARL11-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-ARL11 | pDEST47-ARL11-GFP | ARL11                                          | Bacterial expression                         |
|         | pET-DEST42-ARL11-V5-His6 | pDEST40-ARL11-V5-His6 | ARL11-V5-His6                                  | Mammalian expression                        |
|         | pDEST47-ARL11 | pDEST40-ARL14-V5-His6 | pDEST47-ARL14  | pDONR221-ARL14 | Gateway entry vector                         |
| ARL14   | ARF7, ARL10  | 80117   | pDONR221-ARL14                                | Gateway entry vector                         |
|         | pDONR221-ARL14-no stop | pDSHA-ARL14-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-ARL14 | pDEST47-ARL14-GFP | ARL14                                          | Bacterial expression                         |
|         | pET-DEST42-ARL14-V5-His6 | pDEST40-ARL14-V5-His6 | ARL14-V5-His6                                  | Mammalian expression                        |
|         | pDEST47-ARL14 | pDONR221-ARFRP1 | Gateway entry vector                         | Gateway entry vector                        |
| ARFRP1  | ARP, ARP1, ARL18 | 10139   | pDONR221-ARFRP1                                | Gateway entry vector                         |
|         | pDONR221-ARFRP1-no stop | pDSHA-ARFRP1-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-ARFRP1 | pDEST47-ARFRP1-GFP | ARFRP1                                          | Bacterial expression                         |
|         | pET-DEST42-ARFRP1-V5-His6 | pDEST40-ARFRP1-V5-His6 | ARFRP1-V5-His6                                  | Mammalian expression                        |
|         | pDEST47-ARFRP1 | pDONR221-SAR1A | Gateway entry vector                         | Gateway entry vector                        |
| SAR1A   | SAR1, Sara, SARA1, masra2 | 56681   | pDONR221-SAR1A                                | Gateway entry vector                         |
|         | pDONR221-SAR1A-no stop | pDSHA-SAR1A-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-SAR1A | pDEST47-SAR1A-GFP | SAR1A                                          | Mammalian expression                         |
|         | pET-DEST42-SAR1A-V5-His6 | pDEST40-SAR1A-V5-His6 | SAR1A-V5-His6                                  | Mammalian expression                        |
|         | pDEST47-SAR1A | pDONR221-SAR1B | Gateway entry vector                         | Gateway entry vector                        |
| SAR1B   | ANDO, CMRD, GTBPB, SARA2 | 51128   | pDONR221-SAR1B                                | Gateway entry vector                         |
|         | pDONR221-SAR1B-no stop | pDSHA-SAR1B-HA | Gateway entry vector                         | Gateway entry vector                        |
|         | pDEST14-SAR1B | pDEST47-SAR1B-GFP | SAR1B                                          | Mammalian expression                         |
|         | pET-DEST42-SAR1B-V5-His6 | pDEST40-SAR1B-V5-His6 | SAR1B-V5-His6                                  | Mammalian expression                        |
| NMT1    | pMON-HsNMT1  | 56881   | Gateway entry vector                         | Bacterial co-expression                       |
| NMT2    | pMON-HsNMT2  | 51128   | Gateway entry vector                         | Bacterial co-expression                       |

(continued on next page)
We chose not to tag the N-terminus because at least some, perhaps all, ARF family GTPases use the N-terminus as a nucleotide and phospholipid sensitive switch.\(^5\) that may be directly involved in binding to effectors (e.g., see Zhang, et al).\(^6\) In addition, co- or post-translational modifications of the N-termini, including N-myristoylation of ARFs,\(^7\)\(^-\)\(^9\) and ARL1,\(^10\)\(^-\)\(^13\) and acetlylation of ARL3 and ARL8s,\(^14\)\(^-\)\(^16\) have been found to be essential for cellular functions. The one exception to the use of N-terminal fusions is HA-ARL2, as we have found this N-terminal extension inhibits mitochondrial import and facilitates resolution of cytoplasmic and mitochondrial effects of ARL2 (Laura Newman, Cara Schiavon, Richard A. Kahn; manuscript in preparation). Concerns over the use of C-terminal fusions of ARF family members have been reported,\(^17\) and users of these constructs are advised to include whatever controls are possible to protect against artifacts resulting from protein over-expression and/or interference by the tag in protein-protein interactions and functions. Finally, members of the Kahn laboratory have used most of these plasmids over the past few years for a variety of purposes. Our data suggest that the HA tagged proteins express quite poorly and more variability so preference should be given to the GFP or V5-His6 versions before using the HA-tagged constructs. The problem may lie in the vector backbone as we have expressed HA-tagged ARF proteins from pCDNA3-based vectors without this problem.

Vectors for Co-Expression of N-Myristoyltransferase (NMT) With or Without Methionine Aminopeptidase (MAP) in Bacteria

N-myristoylation is the co-translational, covalent attachment of the saturated 14-carbon fatty acid myristate onto the N-terminal glycine of certain proteins, after cleavage of the initiating methionine.\(^18\)\(^-\)\(^21\) Not all proteins with N-terminal glycines are N-myristoylated (e.g., ARL2 is not) yet many of those that do require the modification for function in cells.\(^5\)\(^-\)\(^7\)\(^,\)\(^9\)\(^-\)\(^19\)\(^-\)\(^21\) ARFs use the N-myristate as a critical part of its nucleotide-dependent, and therefore reversible, membrane association mechanism. N-myristoylation of exogenously expressed proteins in mammalian cells is an efficient process; the proteins are completely acylated and the acyl group is thought to persist through the lifetime of the protein.\(^18\)\(^,\)\(^22\)\(^-\)\(^23\) However, bacteria do not express NMTs and have relatively small pools of myristoyl CoA (the other substrate of NMTs). Thus, to generate recombinant, N-myristoylated proteins in bacteria, it is necessary to co-express an NMT with the ARF/ARL protein of interest. Such a system was devised by Duronio, et al,\(^1\) and allows dual selection of the NMT carrying plasmid with kanamycin and the selection of the NMT substrate (e.g., ARF1) with ampicillin. The use of different bacterial promoters also allows for independent induction of the NMT and the ARF/ARL substrate. Some NMT substrates can be purified from bacteria in a nearly completely acylated state by the use of this system. In contrast, we have found that human ARFs are incompletely (as low as a few %) N-myristoylated, resulting in a mixture of acylated and unmodified proteins that can be difficult to resolve. Among the approaches tried in our lab to increase the yield of the acylated species was the co-expression of methionine aminopeptidase (MAP) with the NMT, with the idea that more rapid or complete cleavage of the initiating methionine may result in higher stoichiometry of myristoylation. While we found this to be true, the effects were not as large as hoped.\(^2\) Anyone using bacteria for expression of N-myristoylated proteins should be aware that incomplete acylation is common, though this is highly dependent on the substrate and the NMT used.

Plasmids were generated that direct expression of either human NMT1 or NMT2 and each construct was made with or without the ability to co-express the bacterial MAP, as described in detail in Van Valkenburgh, et al.\(^2\) These 4 plasmids are listed in Table 1. Note that the plasmids in this collection are for expression/co-expression of human NMTs, while the original work from the Gordon lab used the yeast ortholog.\(^1\)\(^-\)\(^24\) Some differences in specificity and efficiency of N-myristoylation in bacterial co-expression systems have been described.\(^2\) While the Gordon lab has done an outstanding job of characterizing substrate specificities of NMTs for their substrates,\(^24\)\(^-\)\(^26\) we recommend empirical testing of the best NMT. The value of co-expressing MAP should also be empirically determined, though we have observed no negative consequences due to MAP co-expression.

### Table 1

| GTPase | Pseudonyms | Gene ID | Addgene plasmid | Description | Use |
|--------|------------|--------|-----------------|-------------|-----|
| NMT1 + Met AP | pMON-NMT1 + MAP | NMT1 + MAP | Bacterial co-expression |
| NMT2 + MetAP | pMON-NMT2 + MAP | NMT2 + MAP | Bacterial co-expression |
| HsARF1 shRNA | pSUPER-ARF1a | ARF1 shRNA | Knockdown in human cells |
| HsARF3 shRNA | pSUPER-ARF3a | ARF3 shRNA | Knockdown in human cells |
| HsARF4 shRNA | pSUPER-ARF4a | ARF4 shRNA | Knockdown in human cells |
| HsARF5 shRNA | pSUPER-ARF5a | ARF5 shRNA | Knockdown in human cells |

---

We chose not to tag the N-terminus because at least some, perhaps all, ARF family GTPases use the N-terminus as a nucleotide and phospholipid sensitive switch.\(^5\) that may be directly involved in binding to effectors (e.g., see Zhang, et al).\(^6\) In addition, co- or post-translational modifications of the N-termini, including N-myristoylation of ARFs,\(^7\)\(^-\)\(^9\) and ARL1,\(^10\)\(^-\)\(^13\) and acetlylation of ARL3 and ARL8s,\(^14\)\(^-\)\(^16\) have been found to be essential for cellular functions. The one exception to the use of N-terminal fusions is HA-ARL2, as we have found this N-terminal extension inhibits mitochondrial import and facilitates resolution of cytosolic and mitochondrial effects of ARL2 (Laura Newman, Cara Schiavon, Richard A. Kahn; manuscript in preparation). Concerns over the use of C-terminal fusions of ARF family members have been reported,\(^17\) and users of these constructs are advised to include whatever controls are possible to protect against artifacts resulting from protein over-expression and/or interference by the tag in protein-protein interactions and functions. Finally, members of the Kahn laboratory have used most of these plasmids over the past few years for a variety of purposes. Our data suggest that the HA tagged proteins express quite poorly and more variability so preference should be given to the GFP or V5-His6 versions before using the HA-tagged constructs. The problem may lie in the vector backbone as we have expressed HA-tagged ARF proteins from pCDNA3-based vectors without this problem.

Vectors for Co-Expression of N-Myristoyltransferase (NMT) With or Without Methionine Aminopeptidase (MAP) in Bacteria

N-myristoylation is the co-translational, covalent attachment of the saturated 14-carbon fatty acid myristate onto the N-terminal glycine of certain proteins, after cleavage of the initiating methionine.\(^18\)\(^-\)\(^21\) Not all proteins with N-terminal glycines are N-myristoylated (e.g., ARL2 is not) yet many of those that do require the modification for function in cells.\(^5\)\(^-\)\(^7\)\(^,\)\(^9\)\(^-\)\(^19\)\(^-\)\(^21\) ARFs use the N-myristate as a critical part of its nucleotide-dependent, and therefore reversible, membrane association mechanism. N-myristoylation of exogenously expressed proteins in mammalian cells is an efficient process; the proteins are completely acylated and the acyl group is thought to persist through the lifetime of the protein.\(^18\)\(^,\)\(^22\)\(^-\)\(^23\) However, bacteria do not express NMTs and have relatively small pools of myristoyl CoA (the other substrate of NMTs). Thus, to generate recombinant, N-myristoylated proteins in bacteria, it is necessary to co-express an NMT with the ARF/ARL protein of interest. Such a system was devised by Duronio, et al,\(^1\) and allows dual selection of the NMT carrying plasmid with kanamycin and the selection of the NMT substrate (e.g., ARF1) with ampicillin. The use of different bacterial promoters also allows for independent induction of the NMT and the ARF/ARL substrate. Some NMT substrates can be purified from bacteria in a nearly completely acylated state by the use of this system. In contrast, we have found that human ARFs are incompletely (as low as a few %) N-myristoylated, resulting in a mixture of acylated and unmodified proteins that can be difficult to resolve. Among the approaches tried in our lab to increase the yield of the acylated species was the co-expression of methionine aminopeptidase (MAP) with the NMT, with the idea that more rapid or complete cleavage of the initiating methionine may result in higher stoichiometry of myristoylation. While we found this to be true, the effects were not as large as hoped.\(^2\) Anyone using bacteria for expression of N-myristoylated proteins should be aware that incomplete acylation is common, though this is highly dependent on the substrate and the NMT used.

Plasmids were generated that direct expression of either human NMT1 or NMT2 and each construct was made with or without the ability to co-express the bacterial MAP, as described in detail in Van Valkenburgh, et al.\(^2\) These 4 plasmids are listed in Table 1. Note that the plasmids in this collection are for expression/co-expression of human NMTs, while the original work from the Gordon lab used the yeast ortholog.\(^1\)\(^-\)\(^24\) Some differences in specificity and efficiency of N-myristoylation in bacterial co-expression systems have been described.\(^2\) While the Gordon lab has done an outstanding job of characterizing substrate specificities of NMTs for their substrates,\(^24\)\(^-\)\(^26\) we recommend empirical testing of the best NMT. The value of co-expressing MAP should also be empirically determined, though we have observed no negative consequences due to MAP co-expression.
psuper-BASED PLASMIDS for siRNA KNOCKDOWN of HUMAN ARF1, ARF3, ARF4, OR ARF5

Brummelkamp, et al. developed the pSUPER vector for use in generating short interfering RNAs to knockdown expression of specific proteins in mammalian cells. This reference includes clear directions for the generation of plasmids that drive expression of double stranded RNA with a hairpin that suppresses expression of genes of interest. We designed into the pSUPER vector different targets directed toward human ARF1, ARF3, ARF4, or ARF5, and examined their effectiveness in depleting cells of specific ARFs. The two best for each ARF were then used in studies examining the consequences of single or dual knockdowns, as described in Volpicelli-Daley, et al. The use of at least 2 sequence-independent targets was used to decrease chances of off-target effects being responsible for the observed phenotypes. The use of synthetic RNAs and more recently of the CRISPR/CAS9 technology appears to have superseded the use of plasmid-based siRNA, but the availability of verified plasmids for knockdown of human ARFs allows the generation of stably transfected cell lines in which the level of each ARF can be experimentally modulated. While the Kahn lab was the source for rabbit polyclonal antibodies specific to each of the human ARF proteins, for many years that were useful in detecting and quantifying knockdowns, unfortunately these reagents are no longer available to the public as a result of depletion in stocks of rabbit sera.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank and acknowledge the many researchers in the Kahn lab and outside who have contributed to the design, construction, and testing of the plasmids described in this collection.

Funding

This work has been supported by several funding sources over the years, most recently R01-GM090158 and R01-GM110663.

References

1. Duronio R, Jackson-Machelski E, Hetuckereth R, Olins P, Devine C, Yonemoto W, Slice L, Taylor S, Gordon J. Protein N-Myristoylation in Escherichia coli: Reconstitution of a Eukaryotic Protein Modification in Bacteria. PNAS 1990; 87:1506-10; PMID:2406721; http://dx.doi.org/10.1073/pnas.87.4.1506
2. Van Valkenburgh HA, Kahn RA. Coexpression of proteins with methionine aminopeptidase and/or N-myristoyltransferase in Escherichia coli to increase acylation and homogeneity of protein preparations. Methods Enzymol 2002; 344:186-93; PMID:11771383; http://dx.doi.org/10.1016/S0076-6879(02)44715-5
3. Brummelkamp TR, Bernards R, Agami R. A system for stable expression of short interfering RNAs in mammalian cells. Science 2002; 296:550-3; PMID:11910072; http://dx.doi.org/10.1126/science.1068999
4. Volpicelli-Daley LA, Li Y, Zhang CJ, Kahn RA. Isoform-selective effects of the depletion of ARF-ribosylation factors I-5 on membrane traffic. Mol Biol Cell 2005; 16:4495-508; PMID:16090262; http://dx.doi.org/10.1091/mbc.E04-12-1042
5. Randazzo PA, Terui T, Sturch S, Fales HM, Ferrige AG, Kahn RA. The myristoylated amino terminus of ARF1-5, and examined their effectiveness in depleting cells of specific ARFs. The two best for each ARF were then used in studies examining the consequences of single or dual knockdowns, as described in Volpicelli-Daley, et al. The use of at least 2 sequence-independent targets was used to decrease chances of off-target effects being responsible for the observed phenotypes. The use of synthetic RNAs and more recently of the CRISPR/CAS9 technology appears to have superseded the use of plasmid-based siRNA, but the availability of verified plasmids for knockdown of human ARFs allows the generation of stably transfected cell lines in which the level of each ARF can be experimentally modulated. While the Kahn lab was the source for rabbit polyclonal antibodies specific to each of the human ARF proteins, for many years that were useful in detecting and quantifying knockdowns, unfortunately these reagents are no longer available to the public as a result of depletion in stocks of rabbit sera.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank and acknowledge the many researchers in the Kahn lab and outside who have contributed to the design, construction, and testing of the plasmids described in this collection.

Funding

This work has been supported by several funding sources over the years, most recently R01-GM090158 and R01-GM110663.