

YEAR	#	MILESTONE TITLE	FREQUENCY	GO-NO-GO PLAN	ALTERNATIVE STRATEGY	PROJECT DELIVERABLE	COMMENTS	MILESTONE DESCRIPTION
Year 1		Project start aggregates team	monthly	NA	NA	Scheduled events	meetings will be held monthly until project end (year 6)	Formalize the interaction and communication structure. At the project start, we plan to aggregate a collective team and establish monthly meetings to promote communication and transparency. We will make one hire (Project Manager) who will help coordinate these events and oversee the below milestone workflow.
Month 6		COLLABORATIVE OPPORTUNITIES				collaborative endeavors with other IDG teams	Success is approval of project(s) by NIH program staff	Collaborative opportunities. This 6-month milestone is to have worked with other IDG components to define collaborative projects.
Month 6		PRIORITIZATION				address initial prioritization and schema	Success is approval by NIH staff.	Assess prioritization. This 6-month milestone is to address initial prioritization and schema to tackle the understudied on channels.
Month 6		INFORMATICS				expanded informatics plan	Success is approval of plans by NIH staff.	Assess prioritization. This 6-month milestone is for developing an expanded informatics plan, possibly involving collaborations within the IDG or expansion of existing components.
Month 6		MI-YEAR UPDATE FOR THE FIRST SIX MONTHS				our six month deliverables are outlined in the progress report.	Success is approval by the NIH staff.	Mid-year year revisions/updates. As we expected, we re-shuffled some of our priorities to achieve maximal success.
Year 1	1	Create tagged channel overexpression cell line	every 6 months beginning in Y1Q3	sequence verification failure	re-synthesize the construct	20 tagging targeting constructs	Homologous targeting cell modification will continue at the rate of 20 knock-in month intervals until milestone completion (in year 3)	Tag ion channel cell lines. Our goal is to create tagged overexpression cell lines (approx. 100 on channels) with a reporter construct, allowing evaluation of expression patterns in cells. Key Criteria for Success includes cloning the full-length cDNA in an expression cassette. For each gene, a go-no-go decision point is failed detection of expression after three attempts. We have defined a two-year timeline for tagging all target genes, and completion of this milestone yields a panel of dark matter ion channels well suited to assays (direct measurement of ion channel function, RNAi expression analysis by HD). These lines will be amplified and banked. Est. 2 years for completion.
Year 1	2	Synthesize validated sgRNAs for all ion channels	every 6 months beginning in Y1Q2	failure to achieve protein (>95%) knockdown	re-synthesize and test 5 new sgRNAs for the failed gene	1-2 potent sgRNAs for each ion channel gene	CRISPR centric cell modification will continue at the rate of 20 knock-in month intervals until milestone completion (in year 3)	Create validated sgRNAs. In parallel to the tagging milestone above, we are creating CRISPR lines which each dark matter ion channel genes are individually targeted with CRISPR sgRNAs. Key Criteria for Success includes developing potent sgRNAs capable of efficiently knocking down each ion channel gene and development of quantitative assays (Western Blot, quantitative RT-PCR, etc.) to evaluate residual ion channel gene expression. For each gene, a go-no-go decision point is failed knockdown (>95%) after testing 10-15 sgRNAs, in which case the alternative options include wCRISPR and sgRNAs. Success of this milestone yields a panel of validated sgRNAs that are banked. Est. 3 years.
Year 1	3a	map cell expression profiles	every 6 months beginning in Y1Q4	failure to detect expression in sequencing datasets	evaluate expression in primary tissues	high resolution expression profile single cell datasets, associating each ion channel with a cell type	this aim is divided into two deliverables: 1) analysis of single cell data sets to ascertain the identity of the cell types that express the channel and 2) image data for intracellular localization and in vivo expression patterns. Intracellular localization data will be collected in year 1 (consists for the tagged cell lines (milestone 1), tissue distribution will be collected in year 1-2 onwards with a completion date in year 6.	Map expression profiles. Using deep sequence analysis of single cell datasets, and use antibodies, X, gel, and/or fluorescence, this milestone will be to collect expression profiles for all targeted ion channels in both single cells and tissues. This goal will be accomplished throughout the project period, starting year one, and the Key Criteria for Success is based on our ability to successfully accomplish the above Milestones 1 and 4. When intractable challenges occur, a go-no-go decision point will be based when the expression level is undetectable using the reagents above, in which case we will accomplish this Milestone using alternative strategies such as quantitative RT-PCR across tissues. As these represent independent approaches, this Milestone has a split dependency which bolsters our chances of success. The deliverables will constitute high-resolution datasets and images which will be supplied to the relevant data Portals as described in the Sharing Plan. Est. 5 years.
Year 1	3b	map tissue expression profiles	every 6 months beginning in Y1Q4	failure to detect expression in tissues	use monoclonal antibodies to assess expression	high resolution expression profile images in tissues, for 20 different tagged genes		Generate mouse lines. We have already begun tackling this Milestone in year one, two years ahead of schedule. That said, we expect the major workflow will be accomplished during years 3-6. Key Criteria for Success includes our ability to genetically modify mice using 1) the established technologies and 2) identify potent sgRNAs against protein targets. As outlined in the Research Plan, we anticipate creating single and double mutant mice. The Milestones table lists the accumulating number of unique lines per year, with at least 4-6 animals per cohort size. At the highest point, we may generate more than 100 lines in year 5. For each gene, a go-no-go decision point is made when no genetically modified mice are made using any of the alternative options (wCRISPR and conventional gene targeting). The deliverables for this Milestone constitute a battery of genetically modified mice that will be banked via cryopreservation of embryos and/or sperm. These reagents will be distributed to the MIRC as appropriate.
Year 1	4a	generate knock/knockdown mice for 20 ion channels	every 6 months beginning in Y1Q4	failure to produce germline lines using CRISPR	use wCRISPR approaches as an alternative	20 mouse lines	We have moved this milestone higher in the priority queue; the milestone is split into two deliverables: 1) single gene mouse lines and 2) double genetic CRISPR mouse lines. These deliverables will be accomplished in years 1-6.	
Year 1	4b	generate double knock/knockdown mice for 20 ion channels	every 6 months beginning in Y1Q4	failure to produce germline lines using CRISPR	use wCRISPR approaches as an alternative	20 mouse lines		
Year 1	8	Collect phenotypes	every 6 months beginning in Y1Q2	failure to produce a cohort of animals to a phenotype	use wCRISPR approaches as an alternative	20 mouse lines	We have elevated this pipeline higher in the Milestone Plan, given that we have already realized success in mouse production and have identified a cohort of animals having interesting phenotypes.	Phenotype mouse lines. This aim uses MxPro and Lycop produced mouse lines and the Lycop (zygotically) produced mice described in the proposal body. These data will be cross-examined with bioinformatic datasets and evaluated in the context of human disease datasets (when available). Data are currently being assembled for a publication that may be submitted in Y1Q4, or perhaps early Y2.
Month 18		RIGOR, RELIABILITY, AND REPRODUCIBILITY				globally evaluate cumulative data quality	Success is acceptance of report and any revised (perturbations) by the NIH	Rigor, reliability, and reproducibility. This 18-month milestone is for addressing rigor, reliability, and reproducibility of the experiments from Y1 1 and emerging data in Y2.
Year 1		GLOBAL ASSESSMENT				completion of milestone deliverables	Success is acceptance of report and any revised (perturbations) by the NIH	Global assessment. At the end of the year, we will formally assess our overall success rates and therefore our expected throughput. A needed we will modify milestones and approaches to achieve maximal efficiency.
Year 1		REVIEW AND UPDATE				refine the official list and revise as needed	Success is approval by the IDG Steering Committee and NIH staff	Review and update. At the end of the year, we will formally review the criteria and update the official undrafted list.
Year 1		HARMONIZE				a harmonized list of deliverables	Success is approval by the IDG Steering Committee and NIH staff	Minimize and harmonize. This year 1 milestone is to work with the IDG Steering Committee to minimize and harmonize, as much as possible, the list of depositors for data and reagents.
Year 1		END OF YEAR MILESTONE UPDATE				year 1 cumulative deliverables: 40 tagged ion channel cDNAs; 40 CRISPR on channel cell lines; 20 potent validated sgRNAs	Success is approval by the NIH staff	End of the year revisions/updates. We fully expect that the milestones will change as we encounter experimental challenges and accelerated success. The Project Deliverables, Milestones, Decision Tree, Endpoints, and Timeline will be updated annually.
Year 2	5	Create/validate antibodies (WB and/or IP)	every 6 months beginning in Y1Q4	failure to achieve specificity as measured by flow cytometry or Western blot	apply an alternative strategy (polyclonal, Phages, etc)	10 high titer validated monoclonal antibody producing cell lines	antibodies created at the NeuroMab facility at UC Davis; an existing panel of targets are already available	Create/validate antibodies. One of the milestones outlined in the proposal body is to establish a resource of high quality antibodies. Key Criteria for Success includes developing protocols capable of quantifying specific binding of the antibody on Western Blot, IHC, and/or immunoprecipitation. For each gene, a go-no-go decision point is failure to identify specific high-titer antibodies, in which case alternative options include derivation of new monoclonal antibodies, polyclonal antibodies, or apply Phages or other technologies. Notably, the tagged and CRISPR cell lines developed in Milestones 1 and 2 will be instrumental for success. The tangible deliverable will constitute monoclonal cell lines that will be banked and distributed. This milestone includes an estimated failure rate of approximately 50%. Est. 5 years.
Year 2	6a	Optimize IP conditions for tagged cells	Y2Q3	high background interactions or poor signal	evaluate different conditions (salt/ligands, binding time, cross-linking, elution etc)	a turnkey SOP readily applicable to all tagged cell lines	We expect the SOPs to be established before the end of year one. The proteomic pipeline will begin in the first quarter of year 2 and continue at a rate of 10 cell line/month intervals until milestone completion (in year 3)	Capture proteomic data. This project will capture a vast amount of Proteomic data using advanced MS protocols, using the tagged line developed in year one. The Research Plan outlines a streamlined workflow where specific tagged cells will be immediately generated onto the MS platform, allowing us to capture proteomic data throughout the first three years, beginning in year one. Key Criteria for Success includes the ability to efficiently and cleanly immunoprecipitate tagged genes. For each gene, a go-no-go decision point is the failure to detect specific proteomic interactions, assessed by co-IP, in which case the alternative strategy includes optimize tagging on the NeuroMab internally. The deliverable goal will be to use antibodies to immunoprecipitate the ion channel (see below). The deliverable constitutes proteomic data that will be cataloged as the data are captured and deposited into the relevant data Portals as described in the Sharing Plan. Est. ~3 years
Year 2	6b	Capture proteomic profiles for 20 ion channel genes	every 6 months beginning in Y2Q4	high background interactions or poor signal	place tag at another position; evaluate other tags; evaluate monoclonal antibodies	10 proteomic datasets, each containing validated interactions		
Year 2	7	Directly analyze channel activity	beginning Y2, we are figuring this out	limited sensitivity and/or slow kinetics	use a Ca ²⁺ assay, or conduct electrophysiology	direct assessment of each ion channel activity in cells; project initiated in year 1 and a target ion channels analyzed by year 3	We have developed a plan to use the published GCaMP fluorescent assay, although we are revisiting this in light of assessing other matter technologies that will make use of our overexpression lines	Directly analyze channel activity. This revised milestone centers on our goal to analyze ion channel activity in cells using the published Ca ²⁺ assay, although we may turn to RT electrophysiology. We are re-estimating this milestone to be completed earlier than the set deadline (Year 3), this plan is to provide a comprehensive analysis of all channels in parallel (>100 channels in year 3). As we garner success in this aim, we may further study specific channel genes using in vivo electrophysiology in the Jan laboratory, measuring specific ion flux in cells and tissues. Est. 3 years.
Year 2		END OF YEAR MILESTONE UPDATE				year 2 cumulative deliverables: 40 tagged ion channel cDNAs; 80 ion channel overexpression cell lines; 80 potent validated sgRNAs; 10 Ab producing cell lines or Ab cDNA; 40 mouse lines with expression analyzed for 20 or a comparable subset analysis from single cell datasets	Success is approval by the NIH staff	End of the year revisions/updates. We fully expect that the milestones will change as we encounter experimental challenges and accelerated success. The Project Deliverables, Milestones, Decision Tree, Endpoints, and Timeline will be updated annually.
Year 2		DEMONSTRATED RELEASE OF DELIVERABLES				release of all deliverables will be formally demonstrated	Success is to have all deliverables formally released	Release of Milestones. At the end of the year 2, we will formally demonstrate release of initial reagents, reagents, informatics tools, protocols, etc. in accordance to IDG policies and approved by the RDCO, and MIRC, as appropriate. Note that this is a formally, as we have developed monthly data and reagents, we expect where validated materials are immediately transferred.
Year 2		COLLABORATIVE OPPORTUNITIES				collaborative endeavors with other IDG teams	Success is approval of project(s) by NIH program staff	Collaborative opportunities. This Year 2 milestone is to have worked with other IDG components to define collaborative projects.
Year 2		GLOBAL ASSESSMENT				completion of milestone deliverables	Success is acceptance of report and any revised (perturbations) by the NIH	Global assessment. At the end of the year, we will formally assess our overall success rates and therefore our expected throughput. A needed we will modify milestones and approaches to achieve maximal efficiency.
Year 3	1	Create tagged channel overexpression cell line	every 6 months beginning in Y3Q1	sequence verification failure	re-synthesize the construct	20 tagging targeting constructs	Homologous targeting cell modification will continue at the rate of 20 knock-in month intervals until milestone completion (in year 3)	Tag ion channel cell lines. Our goal is to create tagged overexpression cell lines (approx. 100 on channels) with a reporter construct, allowing evaluation of expression patterns in cells. Key Criteria for Success includes cloning the full-length cDNA in an expression cassette. For each gene, a go-no-go decision point is failed detection of expression after three attempts. We have defined a two-year timeline for tagging all target genes, and completion of this milestone yields a panel of dark matter ion channels well suited to assays (direct measurement of ion channel function, RNAi expression analysis by HD). These lines will be amplified and banked. Est. 3 years.
Year 3	2	Synthesize validated sgRNAs for all ion channels	every 6 months beginning in Y3Q2	failure to achieve protein (>95%) knockdown	re-synthesize and test 5 new sgRNAs for the failed gene	1-2 potent sgRNAs for each ion channel gene	CRISPR centric cell modification will continue at the rate of 20 knock-in month intervals until milestone completion (in year 3)	Create validated sgRNAs. In parallel to the tagging milestone above, we are creating CRISPR lines which each dark matter ion channel genes are individually targeted with CRISPR sgRNAs. Key Criteria for Success includes developing potent sgRNAs capable of efficiently knocking down each ion channel gene and development of quantitative assays (Western Blot, quantitative RT-PCR, etc.) to evaluate residual ion channel gene expression. For each gene, a go-no-go decision point is failed knockdown (>95%) after testing 10-15 sgRNAs, in which case the alternative options include wCRISPR and sgRNAs. Success of this milestone yields a panel of validated sgRNAs that are banked. Est. 3 years.
Year 3	7a	map cell expression profiles	every 6 months beginning in Y3Q4	failure to detect expression in sequencing datasets	evaluate expression in primary tissues	high resolution expression profile images in single cells, for each ion channel	this aim is divided into two deliverables: 1) analysis of single cell data sets to ascertain the identity of the cell types that express the channel and 2) image data for intracellular localization and in vivo expression patterns. Intracellular localization data will be collected in year 1 (consists for the tagged cell lines (milestone 1), tissue distribution will be collected in year 1-2 onwards with a completion date in year 6.	Map expression profiles. Using deep sequence analysis of single cell datasets, and use antibodies, X, gel, and/or fluorescence, this milestone will be to collect expression profiles for all targeted ion channels in both single cells and tissues. This goal will be accomplished throughout the project period, starting year one, and the Key Criteria for Success is based on our ability to successfully accomplish the above Milestones 1 and 4. When intractable challenges occur, a go-no-go decision point will be based when the expression level is undetectable using the reagents above, in which case we will accomplish this Milestone using alternative strategies such as quantitative RT-PCR across tissues. As these represent independent approaches, this Milestone has a split dependency which bolsters our chances of success. The deliverables will constitute high-resolution datasets and images which will be supplied to the relevant data Portals as described in the Sharing Plan. Est. 5 years.
Year 3	7b	map tissue expression profiles	every 6 months beginning in Y3Q4	failure to detect expression in tissues	use monoclonal antibodies to assess expression	high resolution expression profile images in tissues, for 20 different tagged genes		
Year 3	8a	generate knock/knockdown mice for 20 ion channels	every 6 months beginning in Y3Q4	failure to produce germline lines using CRISPR	use wCRISPR approaches as an alternative	20 mouse lines	We have moved this milestone higher in the priority queue; the milestone is split into two deliverables: 1) single gene mouse lines and 2) double genetic CRISPR mouse lines. These deliverables will be accomplished in years 1-6.	
Year 3	8b	generate double knock/knockdown mice for 20 ion channels	every 6 months beginning in Y3Q4	failure to produce germline lines using CRISPR	use wCRISPR approaches as an alternative	20 mouse lines		
Year 6		END OF YEAR MILESTONE UPDATE				year 6 cumulative deliverables: 120 tagged ion channel cell lines; 120 CRISPR on channel cell lines; direct measurements of 120 on channels; 120 potent validated sgRNAs; 50 Ab producing cell lines or Ab cDNA; 500 salt/size based expression profiles; 300 mouse lines; 300 lines phenotyped	Success is approval by the NIH staff	End of the year revisions/updates. We fully expect that the milestones will change as we encounter experimental challenges and accelerated success. The Project Deliverables, Milestones, Decision Tree, Endpoints, and Timeline will be updated annually.
All Years	9a	prioritize genes	every 6 months beginning in year 1	NA	NA	deep bioinformatic analysis performed on all dark matter ion channel genes		Disease associated data. An important element of the planned workflow is to continually assess the relationships between mouse on channel data and human disease data. This includes expression data and potential therapeutic data. We anticipate that this Milestone represents the remaining effort that will be accomplished for each ion channel as it moves through the production pipeline. Based on our Key Criteria for Success, 1 month is needed to complete at least 20 separate analyses per year, although we anticipate as some measurements are more efficiently accomplished in mass (e.g. for example, quantitative gene analysis on all 120 on channels will be completely finished during the first year). The deliverables constitute digital data and as such will be pipelined to the MIRC via our bioinformatic team led by Anil Butts and Ben Chen. This will be an ongoing milestone.
All Years	9b	associate disease data with target genes	every 6 months beginning in year 1	NA	NA	deep bioinformatic analysis performed on all dark matter ion channel genes		
All Years	10	solidify catalog and distribution pipeline	every 6 months beginning in year 1	NA	NA	all on channel data and reagents will be transferred from our Center on a monthly or frequency appropriate for each recipient	this milestone will be accomplished monthly, localized every month, and all material deposited by project end (year 6)	Catalog and distribute. Accomplishment of this Milestone is described in all previous Milestones but we wish to emphasize it as a separate point. The major deliverable (objective) of project end is to utilize an assortment of suitable technology platforms to characterize the functions of understudied on channels revealing the molecular, cellular, and physiological role in cells and in vivo contexts. Our DRDC incorporates suitable technology platforms via streamlined experimental workflows, with an emphasis on reproducibility in analyses of protein functions in cellular models and complex biological tissues in mouse models. As such, cataloging and distributing the technologies, data, and tangible resources in testament to our success. As described above, this Milestone will be accomplished throughout the project period, leading to year one, and continue as data and resources are produced.

DARK MATTER ION CHANNELS

PROJECT TIMELINE & CUMULATIVE DELIVERABLES																									
#		YEAR 1				YEAR 2				YEAR 3				YEAR 4				YEAR 5				YEAR 6			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	Create tagged channel overexpression cell line				40		80																		
2	Develop validated potent sgRNAs				20		40		60		80		100												
3	Map expression profiles				20				40				80				120				160				200
4	Generate mouse lines				20				40				80				120				160				200
5	Create/Validate antibodies (WB and/or IP)						10				20				30				40				50		
6	Capture proteomic data (from tagged channels)								10		20		30		40		60		80						
7	Analyze channel activity																		80						
8	Collect phenotypes				20				40				80				120				160				200
9	Disease associated data	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS
10	Catalog and distribute	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS

- KEY:**
- initiate project and fill gaps (plan details, coordinate with team members, order reagents, schedule activities, complete residuals, etc)
 - development project workflow (optimize conditions, develop SOPsetc)
 - major workflow effort; data and reagent production
 - 20** numerical values correspond to the number of accumulated reagents for that milestone row, e.g. 20 tagged reporter cell lines, etc.
 - DS** Data Sweep: quarterly sweep of all validated data into repositories; bioinformatic re-analysis of all emerging data from the field and within our program
 - RS** Reagent Sweep: monthly sweep of all validated materials into physical repositories

102 Dark Matter Ion Channels

KEY	MILESTONE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Genes	Cells	CRISPR	Expression	Mouse	Antibody	Proteomic	Activity	Phenotype	Disease	Distribute
AS1C4		✓		✓				✓		
BEST4		✓								
CACNA2D2		✓								
CACNA2D3		✓								
CACNA2D4	✓	✓		✓				✓		
CACNB1	✓	✓			✓					
CACNB2		✓		✓				✓		
CACNB3		✓			✓					
CACNB4		✓		✓	✓			✓		
CACNG1	✓	✓								
CACNG3	✓	✓								
CACNG4	✓	✓								
CACNG5		✓								
CACNG6		✓								
CACNG7	✓	✓								
CACNG8		✓								
CATSPER2	✓	✓								
CHRNA10		✓								
CHRNA2		✓								
CHRNA1	✓	✓								
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CHRNA102	✓	✓								
TOTAL	56	102	0	28	26	0	0	19	0	0
	Cells	CRISPR	Expression	Mouse	Antibody	Proteomic	Activity	Phenotype	Disease	Distribute
NOTES (recorded April 17, 2018)	DNA available: cell lines in progress	sgRNA designed; cloning in progress	expression data identified	mouse produced or in the pipeline	antibody available (not yet validated)	proteomic data identified	direct activity identified	phenotype identified	disease association identified	reagent distributed