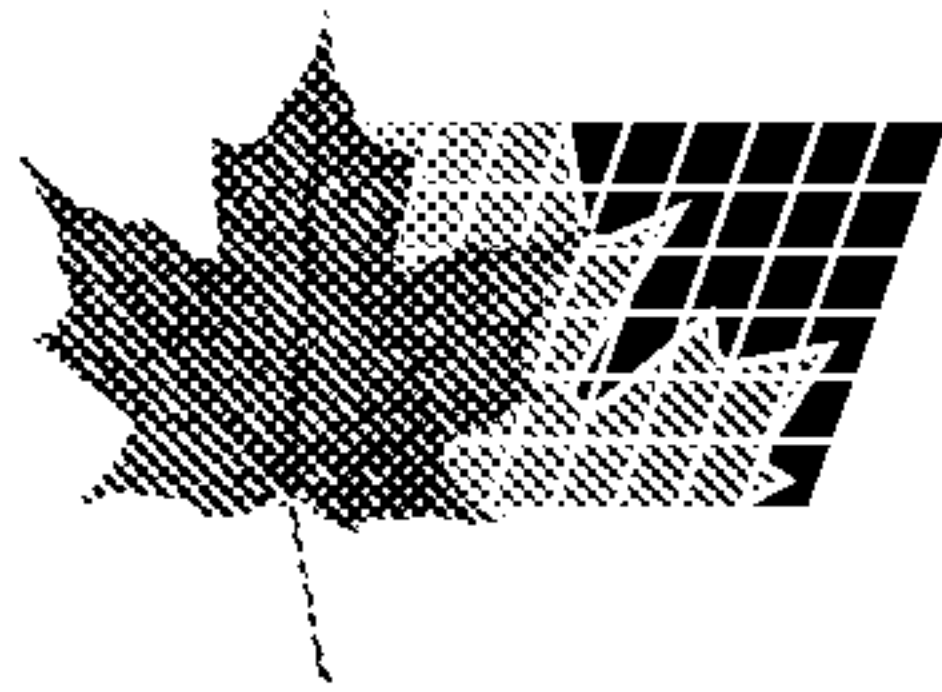




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(30) 1998/04/07 (60/081,057) US

(54) **POLYPEPTIDE SLIT ET PROCEDE DE MODULATION DE
ROBO AVEC DES LIGANDS**

(54) **SLIT POLYPEPTIDE AND METHOD OF MODULATION OF
ROBO WITH LIGANDS**

(57) L'invention concerne des méthodes et des compositions d'identification des agents modulant l'interaction entre Robo et un ligand de Robo et de modulation de l'interaction entre Robo et un ligand de Robo. Les méthodes d'identification des modulateurs de Robo:ligand trouvent une application particulière dans des systèmes de criblage de médicaments de type commercial. Ces méthodes consistent généralement (1) à combiner un polypeptide Robo, un polypeptide Slit et un agent potentiel dans des conditions entraînant, excepté en présence de l'agent, une première interaction entre les polypeptides Robo et Slit, et (2) à déterminer une seconde interaction entre les polypeptides Robo et Slit en présence de l'agent, une différence entre la première et la seconde interaction indiquant que l'agent module l'interaction entre les polypeptides Robo et Slit. Les méthodes de l'invention modulant l'interaction entre Robo et un ligand de Robo consistent à combiner un polypeptide Robo, un polypeptide Slit et un modulateur dans des conditions entraînant, excepté en présence de l'agent, une première interaction entre les polypeptides Robo et Slit, entraînant une seconde interaction entre les polypeptides Robo et Slit différente de la première interaction. Dans un mode de réalisation particulier, le modulateur est une forme négative dominante du polypeptide Robo ou Slit.

(57) Disclosed are methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo: ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interaction indicates that the agent modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

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(21) International Application Number: PCT/US98/24245 (22) International Filing Date: 13 November 1998 (13.11.98) (30) Priority Data: 60/065,544 14 November 1997 (14.11.97) US 60/081,057 7 April 1998 (07.04.98) US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 5th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US). (72) Inventors: GOODMAN, Corey; University of California, Berkeley, Life Sciences Addition #519, Berkeley, CA 94720 (US). KID, Thomas; University of California, Berkeley, Life Sciences Addition #519, Berkeley, CA 94720 (US). BROSE, Katja; University of California, San Francisco, Dept. of Anatomy, Room S-1479, 513 Parnassus Avenue, San Francisco, CA 94143-0452 (US). TESSIER-LAVIGNE, Marc; University of California, San Francisco, Dept. of Anatomy, Room S-1479, 513 Parnassus Avenue, San Francisco, CA 94143-0452 (US). (74) Agent: OSMAN, Richard, Aron; Science & Technology Law Group, 75 Denise Drive, Hillsborough, CA 94010 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: SLIT POLYPEPTIDE AND METHOD OF MODULATION OF ROBO WITH LIGANDS (57) Abstract Disclosed are methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo: ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the present of the agent, wherein a difference between the first and second interaction indicates that the agent modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.		

Modulating Robo:Ligand Interactions

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Field of the Invention

15

In the developing CNS, most growth cones confront the midline at one or multiple times during their journey and make the decision of whether to cross or not to cross. This decision is not a static one but rather changes according to the growth cone's history. For example, in the *Drosophila* ventral nerve cord, about 10% of the interneurons project their axons only on their own side, in some cases extending near the midline without crossing it. The other 90% of the interneurons first project their axons across the midline and then turn to project longitudinally on the other side, often extending near the midline. These growth cones, having crossed the midline once, never cross it again, in spite of their close proximity to the midline and the many commissural axons crossing it. This decision to cross or not to cross is not unique to *Drosophila* but is common to a variety of midline structures in all bilaterally symmetric nervous systems.

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AMENDED SHEET

One approach to find the genes encoding the components of such a system is to screen for mutations in which either too many or too few axons cross the midline. Such a large-scale mutant screen was previously conducted in *Drosophila*, and led to the identification of two key genes: *commissureless* (*comm*) and *roundabout* (*robo*) (Seeger et al., 1993; reviewed by Tear et al., 1993). In *comm* mutant embryos, commissural growth cones initially orient toward the midline but then fail to cross it and instead recoil and extend on their own side. *robo* mutant embryos, on the other hand, display the opposite phenotype in that too many axons cross the midline; many growth cones that normally extend only on their own side instead now project across the midline and axons that normally cross the midline only once instead appear to cross and recross multiple times (Seeger et al, 1993; present disclosure). Double mutants of *comm* and *robo* display a *robo*-like phenotype.

How do *comm* and *robo* function to control midline crossing? Neither the initial paper on these genes (Seeger et al., 1993) nor the cloning of *comm* (Tear et al., 1996) resolved this question. *comm* encodes a novel surface protein expressed on midline cells. In fact, the *comm* paper (Tear et al., 1996) ended with the hope that future work would "... help shed some light on the enigmatic function of Comm."

USSN 08/971,172 (*Robo, A Novel Family of Polypeptides and Nucleic Acids*, by inventors: Corey S. Goodman, Thomas Kidd, Kevin J. Mitchell and Guy Tear) discloses the cloning and characterization of *robo* in various species including *Drosophila*; Robo polypeptides and polypeptide-encoding nucleic acids are also disclosed and their genbank accession numbers referenced in Kidd et al. (1998) Cell 92, 205-215. *robo* encodes a new class of guidance receptor with 5 immunoglobulin (Ig) domains, 3 fibronectin type III domains, a transmembrane domain, and a long cytoplasmic domain. Robo defines a new subfamily of Ig superfamily proteins that is highly conserved from fruit flies to mammals. The Robo ectodomains, and in particular the first two Ig domains, are highly conserved from fruit fly to human, while the cytoplasmic domains are more divergent. Nevertheless, the cytoplasmic domains contain three highly conserved short proline-rich motifs which may represent binding sites for SH3 or other binding domains in linker or signaling molecules.

For those axons that never cross the midline, Robo is expressed on their growth cones from the outset; for the majority of axons that do cross the midline, Robo is expressed at high levels on their growth cones only after they cross the midline. Transgenic rescue experiments

in *Drosophila* reveal that Robo can function in a cell autonomous fashion, consistent with it functioning as a receptor. Thus, in *Drosophila*, Robo appears to function as the gatekeeper controlling midline crossing; growth cones expressing high levels of Robo are prevented from crossing the midline. Robo proteins in mammals function in a similar manner in controlling axon guidance.

USPN 6,065,54 (*Methods for Modulating Nerve Cell Function*, by inventors: Corey S. Goodman, Thomas Kidd, Guy Tear, Claire Russell and Kevin Mitchell) discloses ectopic and overexpression studies revealing that Comm down-regulates Robo expression, demonstrating that Comm functions to suppress the Robo-mediated midline repulsion. These results show that the levels of Comm at the midline and Robo on growth cones are tightly intertwined and dynamically regulated to assure that only certain growth cones cross the midline, that those growth cones that cross do not linger at the midline, and that once they cross they never do so again.

Relevant Literature

Seeger, M., Tear, G., Ferres-Marco, D. and Goodman C.S. (1993) *Neuron* 10, 409 - 426; Tear G., et al. (1996) *Neuron* 16, 501 - 514; Rothberg et al. (1990) *Genes Dev* 4, 2169-2187; Kidd et al. (1998) *Cell* 92, 205-215.

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to vertebrate Slit1 and Slit2, collectively vertebrate Slit) polypeptides, related nucleic acids, polypeptide domains thereof having vertebrate Slit-specific structure and activity, and modulators of vertebrate Slit function. Vertebrate Slit polypeptides can regulate cell, especially nerve cell, function and morphology. The polypeptides may be produced recombinantly from transformed host cells from the subject vertebrate Slit polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated vertebrate Slit hybridization probes and primers capable of specifically hybridizing with natural vertebrate Slit genes, vertebrate Slit-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for vertebrate Slit transcripts), therapy (e.g. to modulate nerve cell growth) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating vertebrate Slit genes and polypeptides,

reagents for screening chemical libraries for lead pharmacological agents, etc.).

The invention also provides methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo:ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the agent modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The subject methods include screens for agents which modulate Robo:ligand interactions and methods for modulating Robo:ligand interactions. Robo activation is found to regulate a wide variety of cell functions, including cell-cell interactions, cell mobility, morphology, etc. Slit polypeptides are disclosed as specific activators and inactivators of Robo polypeptides. Accordingly, the invention provides methods for modulating targeted cell function comprising the step of modulating Robo activation by contacting the cell with a modulator of a Robo:Slit interaction..

The targeted Robo polypeptide is generally naturally expressed on the targeted cells. The nucleotide sequences of exemplary natural cDNAs encoding drosophila 1, drosophila 2, C. elegans, human 1, human 2 and mouse 1 Robo polypeptides and their translates are described in Kidd et al. (1998) Cell 92, 205-215 and USSN 08/971,172. The targeted Robo polypeptides comprise at least a functional Robo domain, which domain has Robo-specific amino acid sequence and binding specificity or function. Preferred Robo domains comprise

at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a natural full length Robo. In a particular embodiment, the domains comprise one or more structural/functional Robo immunoglobulin, fibronectin or cytoplasmic motif domains described herein. The subject domains provide Robo-specific antigens and/or immunogens, especially when coupled to carrier proteins. For example, peptides corresponding to Robo- and human Robo-specific domains are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freund's complete adjuvant. Laboratory rabbits are immunized according to conventional protocol and bled. The presence of Robo-specific antibodies is assayed by solid phase immunosorbent assays using immobilized Robo polypeptides. Generic Robo-specific peptides are readily apparent as conserved regions in aligned Robo polypeptide sequences. In addition, species-specific antigenic and/or immunogenic peptides are readily apparent as diverged extracellular or cytosolic regions in alignments. Human Robo-specific antibodies are characterized as uncross-reactive with non-human Robo polypeptides.

The subject domains provide Robo domain specific activity or function, such as Robo-specific cell, especially neuron modulating or modulating inhibitory activity, Robo-ligand-binding or binding inhibitory activity. Robo-specific activity or function may be determined by convenient *in vitro*, cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Robo regulating protein or other regulator that directly modulates Robo activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Robo specific agent such as those identified in screening assays such as described below. Robo-binding specificity may be assayed by binding equilibrium constants (usually at least about 10^7 M^{-1} , preferably at least about 10^8 M^{-1} , more preferably at least about 10^9 M^{-1}), by the ability of the subject polypeptide to function as negative mutants in Robo-expressing cells, to elicit Robo specific antibody in a heterologous host (e.g. a rodent or rabbit), etc.

Similarly, the Slit polypeptide is conveniently selected from Slit polypeptides which specifically activate or inhibit the activation of the Robo polypeptide. Exemplary suitable Slit polypeptides (a) comprises a vertebrate Slit sequence disclosed herein, especially human Slit-1 (SEQ ID NO:02), or a deletion mutant thereof which specifically modulates Robo

expression or a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence, and/or (b) is encoded by a nucleic acid comprising a natural Slit encoding sequence (such as a natural human Slit-1 encoding sequence, SEQ ID NO:01) or a fragment thereof at least 36, preferably at least 72, more preferably at least 144, most preferably at least 288 nucleotides in length which specifically hybridizes thereto. Suitable deletion mutants are readily screened in Robo binding or activation assays as described herein. Preferred Slit domains/deletion mutants/fragments comprise at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a disclosed vertebrate Slit sequences and provide a Slit specific activity, such as Slit-specific antigenicity and/or immunogenicity, especially when coupled to carrier proteins as described above for Robo above. Suitable natural Slit encoding sequence fragments are of length sufficient to encode such Slit domains. In a particular embodiment, the Slit fragments comprise species specific fragments; such fragments are readily discerned from alignments of the disclosed sequences, see, e.g. shown as unboxed sequences in Tables 1 and 2.

Table 1. Alignment of human Slit-1 (SEQ ID NO:02), human Slit-2 (SEQ ID NOS:03-06), Drosophila Slit-1 (SEQ ID NO:07), C. elegans Slit-1 (SEQ ID NOS:08-09), mouse Slit-2 (SEQ ID NOS:10-11) and mouse Slit-1 (SEQ ID NOS:12-14).

1	M	A	A	P	S	R	T	T	L	M	P	P	P	F	R	L	Q	L	R	L	-	L	I	L	P	I	L	L	L	L	R	H	D	A	V	H	A	E	P	Y	D-Slit		
1	M	R	G	V	G	W	Q	-	-	-	-	-	-	-	M	L	S	L	S	L	G	L	V	L	A	I	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H-Slit1	
40	S	G	G	F	G	S	S	A	V	S	S	G	G	L	G	S	V	G	I	H	I	P	G	G	G	V	G	V	I	T	E	A	R	C	P	R	V	C	S	C	D-Slit		
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	K	V	A	P	Q	A	C	P	A	Q	C	S	C	H-Slit1
80	T	G	L	N	V	D	C	S	H	R	G	L	T	S	V	P	R	K	I	S	A	D	V	E	R	L	E	L	Q	G	N	N	L	T	V	I	Y	E	T	D	D-Slit		
35	S	G	S	T	V	D	C	H	G	L	A	L	R	S	V	P	R	N	I	P	R	N	T	E	R	L	D	L	N	G	N	N	I	T	R	I	T	K	T	D	H-Slit1		
120	F	Q	R	L	T	K	L	R	M	L	Q	L	T	D	N	Q	I	H	T	I	E	R	N	S	F	Q	D	L	V	S	L	E	R	L	-	-	-	-	-	-	D-Slit		
75	F	A	G	L	R	H	L	R	V	L	Q	L	M	E	N	K	I	S	T	I	E	R	G	A	F	Q	D	L	K	E	L	E	R	L	R	L	N	R	N	N	H	H-Slit1	
1	-	-	-	-	-	H	L	R	V	L	Q	L	M	E	N	R	I	S	T	I	E	R	G	A	F	Q	D	L	K	E	L	E	R	L	R	L	N	R	N	N	M	M-Slit1	
154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D-Slit		
115	L	Q	L	F	P	E	L	L	F	L	G	T	A	K	L	Y	R	L	D	L	S	E	N	Q	I	Q	A	I	P	R	K	A	F	R	G	A	V	D	I	K	H-Slit1		
36	L	Q	L	F	P	E	L	L	F	L	G	T	A	R	L	Y	R	L	D	L	S	E	N	Q	I	Q	A	I	P	R	K	A	F	R	G	A	V	D	I	K	M-Slit1		
176	S	L	Q	L	D	N	N	Q	I	T	C	L	D	E	H	A	F	K	G	L	V	E	L	E	I	L	T	L	N	N	N	N	L	T	S	L	P	H	N	I	D-Slit		
155	N	L	Q	L	D	Y	N	Q	I	S	C	I	E	D	G	A	F	R	A	L	R	D	L	E	V	L	T	L	N	N	N	N	I	T	R	L	S	V	A	S	H-Slit1		
76	N	L	Q	L	D	Y	N	Q	I	S	C	I	E	D	G	A	F	R	A	L	R	D	L	E	V	L	T	L	N	N	N	N	I	T	R	L	S	V	A	S	M-Slit1		

216 F G G L G R L R A L R L S D N P F A C D C H L S W L S R F L R S A T R L A P Y T D-Slit
 195 F N H M P K L R T F R L H S N N L Y C D C H L A W L S D W L R K R P R V G L Y T H-Slit1
 116 F N H M P K L R T F R L H S N N L Y C M-Slit1

256 R C Q S P S Q L K G Q N V A D L H D Q E F K C S G L T E - H A P M - - - E C G A D-Slit
 235 Q C M G P S H L R G H N V A E V Q K R E F V C S D E E E G H Q S F M A P S C S V H-Slit1

292 E N S C P H P C R C A D G I V D C R E K S L T S V P V T L P D D T T D V R L E Q D-Slit
 275 L H - C P A A C T C S N N I V D C R G K G L T E I P T N L P E T I T E I R L E Q H-Slit1
 1 - - - - - S P C T C S N N I V D C R G K G L M E I P A N L P E G I V E I R L E Q H-Slit2

332 N F I T E L P P K S F S S F R R L R R I D L S N N N I S R I A H D A L S G L K Q D-Slit
 314 N T I K V I P P G A F S P Y K K L R R I D L S N N Q I S E L A P D A F Q G L R S H-Slit1
 36 N S I K A I P A G A F T Q Y K K L K R I D I S K N Q I S D I A P D A F Q G L K S H-Slit2

372 L T T L V L Y G N K I K D L P S G V F K G L G S L R L L L L N A N E I S C I R K D-Slit
 354 L N S L V L Y G N K I T E L P K S L F E G L F S L Q L L L L N A N K I N C L R V H-Slit1
 76 L T S L V L Y G N K I T E I A K G L F D G L V S L Q L L L L H-Slit2

1 R CE-Slit
 412 D A F R D L H S L S L L S L Y D N N I Q S L A N G T F D A M K S M K T V H L A K D-Slit
 394 D A F Q D L H N L N L L S L Y D N K L Q T I A K G T F S P L R A I Q T M H L A Q H-Slit1

2 N P X I C D C N L Q W L A Q I N L Q K N I E T S G A R C E Q P K R L R K K K F A CE-Slit
 452 N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E D-Slit
 434 N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G H-Slit1

42 T L P P N K F K C K G S E S F V S M Y A D S C F I D S I C P T Q C D C Y G T T V CE-Slit
 492 S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V D-Slit
 474 Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V H-Slit1

82 D C N K R G L N T I P T S I P R F A T Q L L L S G N N I S T V D L N S N I H V L CE-Slit
 531 D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L D-Slit
 514 D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L H-Slit1

122 E N L E X L D L S N N H I T F I N D K S F E K L S K L R E L X L N D - - - - - CE-Slit
 571 P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I D-Slit
 554 P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V H-Slit1
 1 - - - - - E G A F N G A A S V Q E L M L T G N Q L E T V H-Slit2

611 S N K M F - - - - - L G L H Q L K T L N D-Slit
 594 Q H K M F K G - L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S H-Slit1
 24 H G R G F R G G L S G L K T L M L R S N L I G C V S N D T F A G L S S V R L L S H-Slit2

626 L Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W - F D-Slit
 633 L Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W - L H-Slit1
 64 L Y D N R I T T I T P G A F T T L V S L S T I N L L S N P F N C N C H L G A G L H-Slit2

665 A E C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E D-Slit
 672 G E W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G H-Slit1
 104 G K W L R K R R I V S G N P R C Q K P F F L K E I P I Q G V G H P G I H-Slit2

1 S N K N L T S F P S R I P F D CE-Slit
 705 N S E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E D-Slit
 712 N D D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D H-Slit1

16 T T E L Y L D A N Y I N E I P A H D L N R L Y S L T K L D L S H N R L I S L E N CE-Slit
 744 T S E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N D-Slit
 752 V T E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N H-Slit1

56 N T F S N L T R L S T L I I S Y N K L R C L Q P L A F N G L N A L R I L S L H G CE-Slit
 784 Y T F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G D-Slit
 791 Q S F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G H-Slit1

96 N D I S F L P Q S A F S N L T S I T H I A V G S N S L Y C D C N M A W F S K W I CE-Slit
 824 N R I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I D-Slit
 831 N D I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V H-Slit1

136 K S K F I E A G I A R C E Y P N T V S N Q L L L T A Q P Y Q F T C D S K V P T K CE-Slit
 864 K L D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D D-Slit
 871 K S E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N H-Slit1

176 L A T K C D L C L N S P C K N N A I C E T T S S R K Y T C N C T P G F Y G V H C CE-Slit
 904 I L A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C D-Slit
 911 I L A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C H-Slit1

216 E N Q I D A C Y G S P C L N N A T C K V - - A Q A G R F N C Y C N K G F E G D Y CE-Slit
 944 E F M I D A C Y G N P C R N N A T C T V - - L E E G R F S C Q C A P G Y T G A R D-Slit
 951 D V P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N H-Slit1

254 C E K N I D D C V N S - K C E N G G K C V D L V R F C S E E L K N F Q S F Q I N CE-Slit
 982 C E T N I D D C L G E I K C Q N N A T C I D - - - - - G V E D-Slit
 991 C E V N V D D C - E D N D C E N N S T C V D - - - - - G I N H-Slit1

293 S Y R C D C P M E Y E G K H C E D K L E Y C T K K L N P C E N N G K C I P I N G CE-Slit
 1007 S Y K C E C Q P G F S G E F C D T K I Q F C S P E F N P C A N G A K C M D H F T D-Slit
 1015 N Y T C L C P P E Y T G E L C E E K L D F C A Q D L N P C Q H D S K C I L T P K H-Slit1
 D P L P V M-Slit2

333 S Y S C M C S P G F T G N N C E T N I D D C K N V E C Q N G G S C V D G I L S Y CE-Slit
 1047 H Y S C D C Q A G F H G T N C T D N I D D C Q N H M C Q N G G T C V D G I N D Y D-Slit
 1055 G F K C D C T P G Y V G E H C D I D F D D C Q D N K C K N G A H C T D A V N G Y H-Slit1

1 - - - - - N N D D C V G H K C R H G A Q C V D E V N G Y M-Slit1
 1 W P R C E C M P G Y A G D N C S E N Q D D C R D H R C Q N G A Q C M D E V N S Y H-Slit2
 6 H H R C E C M L G Y T G D N C S E N Q D D C K D H K C Q N G A Q C V D E V N S Y M-Slit2

373 D C L C R P G Y A G Q Y C E I P P M M D M E Y Q K T D A C Q Q S A C G Q G - E C CE-Slit
 1087 Q C R C P D D Y T G K Y C E G H N M I S M M Y P Q T S P C Q N H E C K H G V - C D-Slit
 1095 T C I C P E G Y S G L F C E F S P - - P M V L P R T S P C D N F D C Q N G A Q C H-Slit1
 24 T C I C P Q G F S G L F C E H P P - - P M V L L Q T S P C D Q Y E C Q N G A Q C M-Slit1
 41 S C L C A E G Y S G Q L C E I P P - - H L P A P K - S P C E G T E C Q N G A N C H-Slit2
 46 A C L C V E G Y S G Q L C E I P P - - - - - A P R - S S C E G T E C Q N G A N C M-Slit2

[illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible]

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The number of cells in the suspension was 100 million cells per ml. The concentration of the suspension was 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, 10100, 10200, 10300, 10400, 10500, 10600, 10700, 10800, 10900, 11000, 11100, 11200, 11300, 11400, 11500, 11600, 11700, 11800, 11900, 12000, 12100, 12200, 12300, 12400, 12500, 12600, 12700, 12800, 12900, 13000, 13100, 13200, 13300, 13400, 13500, 13600, 13700, 13800, 13900, 14000, 14100, 14200, 14300, 14400, 14500, 14600, 14700, 14800, 14900, 15000, 15100, 15200, 15300, 15400, 15500, 15600, 15700, 15800, 15900, 16000, 16100, 16200, 16300, 16400, 16500, 16600, 16700, 16800, 16900, 17000, 17100, 17200, 17300, 17400, 17500, 17600, 17700, 17800, 17900, 18000, 18100, 18200, 18300, 18400, 18500, 18600, 18700, 18800, 18900, 19000, 19100, 19200, 19300, 19400, 19500, 19600, 19700, 19800, 19900, 20000, 20100, 20200, 20300, 20400, 20500, 20600, 20700, 20800, 20900, 21000, 21100, 21200, 21300, 21400, 21500, 21600, 21700, 21800, 21900, 22000, 22100, 22200, 22300, 22400, 22500, 22600, 22700, 22800, 22900, 23000, 23100, 23200, 23300, 23400, 23500, 23600, 23700, 23800, 23900, 24000, 24100, 24200, 24300, 24400, 24500, 24600, 24700, 24800, 24900, 25000, 25100, 25200, 25300, 25400, 25500, 25600, 25700, 25800, 25900, 26000, 26100, 26200, 26300, 26400, 26500, 26600, 26700, 26800, 26900, 27000, 27100, 27200, 27300, 27400, 27500, 27600, 27700, 27800, 27900, 28000, 28100, 28200, 28300, 28400, 28500, 28600, 28700, 28800, 28900, 29000, 29100, 29200, 29300, 29400, 29500, 29600, 29700, 29800, 29900, 30000, 30100, 30200, 30300, 30400, 30500, 30600, 30700, 30800, 30900, 31000, 31100, 31200, 31300, 31400, 31500, 31600, 31700, 31800, 31900, 32000, 32100, 32200, 32300, 32400, 32500, 32600, 32700, 32800, 32900, 33000, 33100, 33200, 33300, 33400, 33500, 33600, 33700, 33800, 33900, 34000, 34100, 34200, 34300, 34400, 34500, 34600, 34700, 34800, 34900, 35000, 35100, 35200, 35300, 35400, 35500, 35600, 35700, 35800, 35900, 36000, 36100, 36200, 36300, 36400, 36500, 36600, 36700, 36800, 36900, 37000, 37100, 37200, 37300, 37400, 37500, 37600, 37700, 37800, 37900, 38000, 38100, 38200, 38300, 38400, 38500, 38600, 38700, 38800, 38900, 39000, 39100, 39200, 39300, 39400, 39500, 39600, 39700, 39800, 39900, 40000, 40100, 40200, 40300, 40400, 40500, 40600, 40700, 40800, 40900, 41000, 41100, 41200, 41300, 41400, 41500, 41600, 41700, 41800, 41900, 42000, 42100, 42200, 42300, 42400, 42500, 42600, 42700, 42800, 42900, 43000, 43100, 43200, 43300, 43400, 43500, 43600, 43700, 43800, 43900, 44000, 44100, 44200, 44300, 44400, 44500, 44600, 44700, 44800, 44900, 45000, 45100, 45200, 45300, 45400, 45500, 45600, 45700, 45800, 45900, 46000, 46100, 46200, 46300, 46400, 46500, 46600, 46700, 46800, 46900, 47000, 47100, 47200, 47300, 47400, 47500, 47600, 47700, 47800, 47900, 48000, 48100, 48200, 48300, 48400, 48500, 48600, 48700, 48800, 48900, 49000, 49100, 49200, 49300, 49400, 49500, 49600, 49700, 49800, 49900, 50000, 50100, 50200, 50300, 50400, 50500, 50600, 50700, 50800, 50900, 51000, 51100, 51200, 51300, 51400, 51500, 51600, 51700, 51800, 51900, 52000, 52100, 52200, 52300, 52400, 52500, 52600, 52700, 52800, 52900, 53000, 53100, 53200, 53300, 53400, 53500, 53600, 53700, 53800, 53900, 54000, 54100, 54200, 54300, 54400, 54500, 54600, 54700, 54800, 54900, 55000, 55100, 55200, 55300, 55400, 55500, 55600, 55700, 55800, 55900, 56000, 56100, 56200, 56300, 56400, 56500, 56600, 56700, 56800, 56900, 57000, 57100, 57200, 57300, 57400, 57500, 57600, 57700, 57800, 57900, 58000, 58100, 58200, 58300, 58400, 58500, 58600, 58700, 58800, 58900, 59000, 59100, 59

689	E	Q	N	C	C	T	A	V	K	K	K	Q	R	K	V	K	M	I	C	K	N	G	T	T	K	I	S	T	V	H	I	I	R	Q	C	Q	C	E	P	T	CE-Slit		
1440	-	N	Q	C	C	A	A	K	I	V	R	R	R	K	V	R	M	V	C	S	N	N	R	K	Y	I	K	N	L	D	I	V	R	K	C	G	C	-	-	T	D-Slit		
1485	G	Q	-	C	C	G	P	L	R	S	K	R	R	K	Y	S	F	E	C	T	D	G	S	S	F	V	D	E	V	E	K	V	V	K	C	G	C	T	R	-	H-Slit1		
203	G	Q	-	C	C	G	P	L	R	S	K	R	R	K	Y	S	F	E	C	T	D	G	S	S	F	V	D	E	V	E	K	V	V	K	C	G	C	A	R	-	M-Slit1		
64	P	Q	-	C	C	Q	P	T	R	S	K	R	R	K	Y	V	F	Q	C	T	D	G	S	S	F	V	E	E	V	E	R	H	L	E	C	G	C	L	A	-	H-Slit2		
71	T	T	-	C	C	Q	P	I	R	S	K	R	R	K	Y	V	F	Q	C	T	D	G	S	S	F	V	E	E	V	E	R	H	L	E	C	G	C	R	A	-	M-Slit2		
729	K	S	V	L	-	-	S	E	K																																CE-Slit		
1477	K	K	C	Y																																						D-Slit	
1523	-	-	-	-	C	V	S																																				H-Slit1
241	-	-	-	-	C	A	S																																				M-Slit1
102	-	-	-	-	C	-	S																																				H-Slit2
109	-	-	-	-	C	-	S																																				M-Slit2

Table 2. Alignment of human Slit-1 (SEQ ID NO:02) and Drosophila Slit-1 (SEQ ID NO: 07).

1	M	A	A	P	S	R	T	T	L	M	P	P	P	F	R	L	Q	L	R	L	-	L	I	L	P	I	L	L	L	R	H	D	A	V	H	A	E	P	Y	D-Slit		
1	M	R	G	V	G	W	Q	-	-	-	-	-	-	-	M	L	S	L	S	L	G	L	V	L	A	I	L	-	-	-	-	-	-	-	-	-	-	-	-	-	H-Slit1	
40	S	G	G	F	G	S	S	A	V	S	S	G	G	L	G	S	V	G	I	H	I	P	G	G	G	V	G	V	I	T	E	A	R	C	P	R	V	C	S	C	D-Slit	
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	K	V	A	P	Q	A	C	P	A	Q	C	S	C	H-Slit1
80	T	G	L	N	V	D	C	S	H	R	G	L	T	S	V	P	R	K	I	S	A	D	V	E	R	L	E	L	Q	G	N	N	L	T	V	I	Y	E	T	D	D-Slit	
35	S	G	S	T	V	D	C	H	G	L	A	L	R	S	V	P	R	N	I	P	R	N	T	E	R	L	D	L	N	G	N	N	I	T	R	I	T	K	T	D	H-Slit1	
120	F	Q	R	L	T	K	L	R	M	L	Q	L	T	D	N	Q	I	H	T	I	E	R	N	S	F	Q	D	L	V	S	L	E	R	L	-	-	-	-	-	-	D-Slit	
75	F	A	G	L	R	H	L	R	V	L	Q	L	M	E	N	K	I	S	T	I	E	R	G	A	F	Q	D	L	K	E	L	E	R	L	R	L	N	R	N	H	H-Slit1	
154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	I	S	N	N	V	I	T	T	V	G	R	R	V	F	K	G	A	Q	S	L	R	D-Slit		
115	L	Q	L	F	P	E	L	L	F	L	G	T	A	K	L	Y	R	L	D	L	S	E	N	Q	I	Q	A	I	P	R	K	A	F	R	G	A	V	D	I	K	H-Slit1	
176	S	L	Q	L	D	N	N	Q	I	T	C	L	D	E	H	A	F	K	G	L	V	E	L	E	I	L	T	L	N	N	N	N	L	T	S	L	P	H	N	I	D-Slit	
155	N	L	Q	L	D	Y	N	Q	I	S	C	I	E	D	G	A	F	R	A	L	R	D	L	E	V	L	T	L	N	N	N	N	I	T	R	L	S	V	A	S	H-Slit1	
216	F	G	G	L	G	R	L	R	A	L	R	L	S	D	N	P	F	A	C	D	C	H	L	S	W	L	S	R	F	L	R	S	A	T	R	L	A	P	Y	T	D-Slit	
195	F	N	H	M	P	K	L	R	T	F	R	L	H	S	N	N	L	Y	C	D	C	H	L	A	W	L	S	D	W	L	R	K	R	P	R	V	G	L	Y	T	H-Slit1	
256	R	C	Q	S	P	S	Q	L	K	G	Q	N	V	A	D	L	H	D	Q	E	F	K	C	S	G	L	T	E	-	H	A	P	M	-	-	-	E	C	G	A	D-Slit	
235	Q	C	M	G	P	S	H	L	R	G	H	N	V	A	E	V	Q	K	R	E	F	V	C	S	D	E	E	E	G	H	Q	S	F	M	A	P	S	C	S	V	H-Slit1	
292	E	N	S	C	P	H	P	C	R	C	A	D	G	I	V	D	C	R	E	K	S	L	T	S	V	P	V	T	L	P	D	D	T	T	D	V	R	L	E	Q	D-Slit	
275	L	H	-	C	P	A	A	C	T	C	S	N	N	I	V	D	C	R	G	K	G	L	T	E	I	P	T	N	L	P	E	T	I	T	E	I	R	L	E	Q	H-Slit1	
332	N	F	I	T	E	L	P	P	K	S	F	S	S	F	R	R	L	R	R	I	D	L	S	N	N	N	I	S	R	I	A	H	D	A	L	S	G	L	K	Q	D-Slit	
314	N	T	I	K	V	I	P	P	G	A	F	S	P	Y	K	K	L	R	R	I	D	L	S	N	N	Q	I	S	E	L	A	P	D	A	F	Q	G	L	R	S	H-Slit1	
372	L	T	T	L	V	L	Y	G	N	K	I	K	D	L	P	S	G	V	F	K	G	L	G	S	L	R	L	L	L	N	A	N	E	I	S	C	I	R	K	D-Slit		
354	L	N	S	L	V	L	Y	G	N	K	I	T	E	L	P	K	S	L	F	E	G	L	F	S	L	Q	L	L	L	N	A	N	K	I	N	C	L	R	V	H-Slit1		
412	D	A	F	R	D	L	H	S	L	S	L	L	S	L	Y	D	N	N	I	Q	S	L	A	N	G	T	F	D	A	M	K	S	M	K	T	V	H	L	A	K	D-Slit	
394	D	A	F	Q	D	L	H	N	L	N	L	L	S	L	Y	D	N	K	L	Q	T	I	A	K	G	T	F	S	P	L	R	A	I	Q	T	M	H	L	A	Q	H-Slit1	

452 N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E D-Slit
 434 N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G H-Slit1

 492 S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V D-Slit
 474 Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V H-Slit1

 531 D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L D-Slit
 514 D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L H-Slit1

 571 P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I D-Slit
 554 P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V H-Slit1

 611 S N K M F L G L H Q L K T L - - - - - N L D-Slit
 594 Q H K M F K G L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S L H-Slit1

 627 Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W F A E D-Slit
 634 Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W L G E H-Slit1

 667 C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E N S D-Slit
 674 W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G N D H-Slit1

 707 E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E T S D-Slit
 714 D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D V T H-Slit1

 746 E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N Y T D-Slit
 754 E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N Q S H-Slit1

 786 F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G N R D-Slit
 793 F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G N D H-Slit1

 826 I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I K L D-Slit
 833 I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V K S H-Slit1

 866 D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D I L D-Slit
 873 E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N I L H-Slit1

 906 A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C E F D-Slit
 913 A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C D V H-Slit1

 946 M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R C E D-Slit
 953 P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N C E H-Slit1

 984 T N I D D C L G E I K C Q N N A T C I D G V E S Y K C E C Q P G F S G E F C D T D-Slit
 993 V N V D D C - E D N D C E N N S T C V D G I N N Y T C L C P P E Y T G E L C E E H-Slit1

 1024 K I Q F C S P E F N P C A N G A K C M D H F T H Y S C D C Q A G F H G T N C T D D-Slit
 1032 K L D F C A Q D L N P C Q H D S K C I L T P K G F K C D C T P G Y V G E H C D I H-Slit1

 1064 N I D D C Q N H M C Q N G G T C V D G I N D Y Q C R C P D D Y T G K Y C E G H N D-Slit
 1072 D F D D C Q D N K C K N G A H C T D A V N G Y T C I C P E G Y S G L F C E F S P H-Slit1

 1104 M I S M M Y P Q T S P C Q N H E C K H G V - C F Q P N A Q G S D Y L C R C H P G D-Slit
 1112 - - F M V L P R T S P C D N F D C Q N G A Q C I - - - V R I N E P I C Q C L P G H-Slit1

1143 Y T G K W C E Y L T S I S F V H N N S F V E L E P L R T R P E A N V T I V F S S D-Slit
 1147 Y Q G E K C E K L V S V N F I N K E S Y L Q I P S A K V R P Q T N I T L Q I A T H-Slit1
 1183 A E Q N G I L M Y D G Q D A H L A V E L F N G R I R V S Y D V G N H P V S T M Y D-Slit
 1187 D E D S G I L L Y K G D K D H I A V E L Y R G R V R A S Y D T G S H P A S A I Y H-Slit1
 1223 S F E M V A D G K Y H A V E L L A I K K N F T L R V D R G L A R S I I N E G S N D-Slit
 1227 S V E T I N D G N F H I V E L L A L D Q S L S L S V D G G N P K I I T N L S K Q H-Slit1
 1263 D Y L K L T T P M F L G G L P V D P A Q Q A Y K N W Q I R N L T S F K G C M K E D-Slit
 1267 S T L N F D S P L Y V G G M P G K S N V A S L R Q A P G Q N G T S F H G C I R N H-Slit1
 1303 V W I N H K L V D F G N A Q R Q Q K I T P G C A L - - - - L E G E Q Q E E E D D D-Slit
 1307 L Y I N S E L Q D F Q K V P M Q T G I L P G C E P C H K K V C A H G T C Q P S S H-Slit1
 1339 E Q D F M D E - - - - - T P H I K E E P V D P C L E N K C R R G S R C V P N S D-Slit
 1347 Q A G F T C E C Q E G W M G P L C D Q R T N D P C L G N K C V H G T - C L P I N H-Slit1
 1373 N A R D G Y Q C K C K H G Q R G R Y C D Q G E G S T E P - - - - - D-Slit
 1386 A F - - S Y S C K C L E G H G G V L C D E E E D L F N P C Q A I K C K H G K C R H-Slit1
 1401 - - - - - P T V T A A S - - - - - T C R K E Q V R E Y Y T E N D - D-Slit
 1424 L S G L G Q P Y C E C S S G Y T G D S C D R E I S C R G E R I R D Y Y Q K Q Q G H-Slit1
 1423 - - - C R S R Q P L K Y A K C V G G C - G N Q C C A A K I V R R R K V R M V C S D-Slit
 1464 Y A A C Q T T K K V S R L E C R G G C A G G Q C C G P L R S K R R K Y S F E C T H-Slit1
 1459 N N R K Y I K N L D I V R K C G C T K K C Y D-Slit
 1504 D G S S F V D E V E K V V K C G C T R - C V S H-Slit1

Exemplary such human Slit-1 immunogenic and/or antigenic peptides are shown in Table 3.

20 Table 3. Immunogenic human Slit-1 polypeptides eliciting Slit-1 specific rabbit polyclonal antibody: Slit polypeptide-KLH conjugates immunized per protocol described above.

	<u>Slit Polypeptide</u>		<u>Immunogenicity</u>	
25	SEQ ID NO:02, res. 1-10		+++	
	SEQ ID NO:02, res. 29-41		+++	
	SEQ ID NO:02, res. 75-87		+++	
	SEQ ID NO:02, res. 92-109		+++	
	SEQ ID NO:02, res. 132-141		+++	
30	SEQ ID NO:02, res. 192-205		+++	
	SEQ ID NO:02, res. 258-269		+++	
	SEQ ID NO:02, res. 295-311		+++	
	SEQ ID NO:02, res. 316-330		+++	
	SEQ ID NO:02, res. 373-382		+++	
	SEQ ID NO:02, res. 403-422		+++	
	SEQ ID NO:02, res. 474-485		+++	
	SEQ ID NO:02, res. 561-576		+++	
	SEQ ID NO:02, res. 683-697		+++	
	SEQ ID NO:02, res. 768-777		+++	
	SEQ ID NO:02, res. 798-813		+++	
	SEQ ID NO:02, res. 882-894		+++	
	SEQ ID NO:02, res. 934-946		+++	
	SEQ ID NO:02, res. 1054-1067		+++	
	SEQ ID NO:02, res. 1181-1192		+++	
	SEQ ID NO:02, res. 1273-1299		+++	
	SEQ ID NO:02, res. 1383-1397		+++	
	SEQ ID NO:02, res. 1468-1477		+++	
	SEQ ID NO:02, res. 1508-1517		+++	

35 The subject domains provide Slit domain specific activity or function, such as Slit-

specific cell, especially neuron modulating or modulating inhibitory activity, Slit-ligand-binding or binding inhibitory activity. Slit-specific activity or function may be determined by convenient *in vitro*, cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Slit regulating protein or other regulator that directly modulates Slit activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Slit specific agent such as those identified in screening assays such as described below. Slit-binding specificity may be assayed by binding equilibrium constants (usually at least about 10^7 M^{-1} , preferably at least about 10^8 M^{-1} , more preferably at least about 10^9 M^{-1}), by the ability of the subject polypeptide to function as negative mutants in Slit-expressing cells, to elicit Slit specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

In one embodiment, the Slit polypeptides are encoded by a nucleic acid comprising SEQ ID NO:01 or a fragment thereof which hybridizes with a full-length strand thereof, preferably under stringent conditions. Such nucleic acids comprise at least 36, preferably at least 72, more preferably at least 144 and most preferably at least 288 nucleotides of SEQ ID NO:01. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO_4 , pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE (Conditions I); preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C (Conditions II). Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 are shown in Table 4.

Table 4. Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 under Conditions I and/or II.

	<u>Slit Nucleic Acid</u>	<u>Hybridization</u>	<u>Slit Nucleic Acid</u>	<u>Hybridization</u>
	SEQ ID NO:01, nucl. 1-47	+	SEQ ID NO:01, nucl. 1258-1279	+
5	SEQ ID NO:01, nucl. 58-99	+	SEQ ID NO:01, nucl. 1375-1389	+
	SEQ ID NO:01, nucl. 95-138	+	SEQ ID NO:01, nucl. 1581-1595	+
	SEQ ID NO:01, nucl. 181-220	+	SEQ ID NO:01, nucl. 1621-1639	+
	SEQ ID NO:01, nucl. 261-299	+	SEQ ID NO:01, nucl. 1744-1755	+
	SEQ ID NO:01, nucl. 274-315	+	SEQ ID NO:01, nucl. 1951-1969	+
10	SEQ ID NO:01, nucl. 351-389	+	SEQ ID NO:01, nucl. 2150-2163	+
	SEQ ID NO:01, nucl. 450-593	+	SEQ ID NO:01, nucl. 2524-2546	+
	SEQ ID NO:01, nucl. 524-546	+	SEQ ID NO:01, nucl. 2761-2780	+
	SEQ ID NO:01, nucl. 561-608	+	SEQ ID NO:01, nucl. 2989-2999	+
	SEQ ID NO:01, nucl. 689-727	+	SEQ ID NO:01, nucl. 3108-3117	+
15	SEQ ID NO:01, nucl. 708-737	+	SEQ ID NO:01, nucl. 3338-3351	+
	SEQ ID NO:01, nucl. 738-801	+	SEQ ID NO:01, nucl. 3505-3514	+
	SEQ ID NO:01, nucl. 805-854	+	SEQ ID NO:01, nucl. 3855-3867	+
	SEQ ID NO:01, nucl. 855-907	+	SEQ ID NO:01, nucl. 4010-4025	+
	SEQ ID NO:01, nucl. 910-953	+	SEQ ID NO:01, nucl. 4207-4219	+
20	SEQ ID NO:01, nucl. 1007-1059	+	SEQ ID NO:01, nucl. 4333-4345	+
	SEQ ID NO:01, nucl. 1147-1163	+	SEQ ID NO:01, nucl. 4521-4529	+

A wide variety of cell types express Robo polypeptides subject to regulation by the disclosed methods, including many neuronal cells, transformed cells, infected (e.g. virus) cells, etc. Ascertaining Robo binding or activation is readily effected by binding assays or cells function assays as disclosed herein or in the cited copending applications. Accordingly, indications for the subject methods encompass a wide variety of cell types and function, including axon outgrowth, tumor cell invasion or migration, etc. The target cell may reside in culture or in situ, i.e. within the natural host. For in situ applications, the compositions are added to a retained physiological fluid such as blood or synovial fluid. For CNS administration, a variety of techniques are available for promoting transfer of the therapeutic across the blood brain barrier including disruption by surgery or injection, drugs which transiently open adhesion contact between CNS vasculature endothelial cells, and compounds which facilitate translocation through such cells. Slit polypeptides may also be amenable to direct injection or infusion, topical, intratracheal/nasal administration e.g. through aerosol, intraocularly, or within/on implants e.g. fibers e.g. collagen, osmotic pumps, grafts comprising appropriately transformed cells, etc. A particular method of administration involves coating, embedding or derivatizing fibers, such as collagen fibers, protein polymers,

etc. with therapeutic polypeptides. Other useful approaches are described in Otto et al. (1989) J Neuroscience Research 22, 83-91 and Otto and Unsicker (1990) J Neuroscience 10, 1912-1921. Generally, the amount administered will be empirically determined, typically in the range of about 10 to 1000 $\mu\text{g/kg}$ of the recipient and the concentration will generally be in the range of about 50 to 500 $\mu\text{g/ml}$ in the dose administered. Other additives may be included, such as stabilizers, bactericides, etc. will be present in conventional amounts.

In one embodiment, the invention provides administering the subject Slit polypeptides in combination with a pharmaceutically acceptable excipient such as sterile saline or other medium, gelatin, an oil, etc. to form pharmaceutically acceptable compositions. The compositions and/or compounds may be administered alone or in combination with any convenient carrier, diluent, etc. and such administration may be provided in single or multiple dosages. Useful carriers include solid, semi-solid or liquid media including water and non-toxic organic solvents. In another embodiment, the invention provides the subject compounds in the form of a pro-drug, which can be metabolically converted to the subject compound by the recipient host. A wide variety of pro-drug formulations for polypeptide-based therapeutics are known in the art. The compositions may be provided in any convenient form including tablets, capsules, troches, powders, sprays, creams, etc. As such the compositions, in pharmaceutically acceptable dosage units or in bulk, may be incorporated into a wide variety of containers. For example, dosage units may be included in a variety of containers including capsules, pills, etc. The compositions may be advantageously combined and/or used in combination with other therapeutic or prophylactic agents, different from the subject compounds. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents, see e.g. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., 1996, McGraw-Hill.

In another aspect, the invention provides methods of screening for agents which modulate Robo-Slit interactions. These methods generally involve forming a mixture of a Robo-expressing cell, a Slit polypeptide and a candidate agent, and determining the effect of the agent on the amount of Robo expressed by the cell. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and

human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Cell and animal based neural guidance/repulsion assays are described in detail in the experimental section below.

5 The amino acid sequences of the disclosed vertebrate Slit polypeptides are used to back-translate Slit polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural Slit-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group,
10 Inc, Madison WI). Slit-encoding nucleic acids used in Slit-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with Slit-modulated cell function, etc.

 The invention also provides nucleic acid hybridization probes and replication /
15 amplification primers having a vertebrate Slit cDNA specific sequence comprising a fragment of a disclosed vertebrate cDNA sequence, and sufficient to effect specific hybridization thereto. Such primers or probes are at least 12, preferably at least 24, more preferably at least 36 and most preferably at least 96 nucleotides in length. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer
20 comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO₄, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. Slit nucleic acids can also be distinguished using alignment
25 algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410). In addition, the invention provides nucleic acids having a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural
30 drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence. In a particular embodiment, the Slit polynucleotide fragments comprise species specific

fragments; such fragments are readily discerned from alignments of the disclosed sequences.

The subject nucleic acids are of synthetic/non-natural sequences and/or are recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. The subject recombinant nucleic acids comprising the nucleotide sequence of disclosed vertebrate Slit nucleic acids, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, more preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of Slit genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional Slit homologs and structural analogs. In diagnosis, Slit hybridization probes find use in identifying wild-type and mutant Slit alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic Slit nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active Slit. Exemplary human Slit-1 probes and primers are shown in Table 5 and Table 6.

Table 5. Hybridization Probes for Regions of Human Slit-1.

Hybridization probe for first leucine rich repeat region	SEQ ID NO:01, nucleotides 82-828
Hybridization probe for second leucine rich repeat region	SEQ ID NO:01, nucleotides 829-1503
Hybridization probe for third leucine rich repeat region	SEQ ID NO:01, nucleotides 1504-2166
Hybridization probe for fourth leucine rich repeat region	SEQ ID NO:01, nucleotides 2167-2751

	Hybridization probe for EGF repeats one to five	SEQ ID NO:01, nucleotides 2752-3327
	Hybridization probe for the sixth EGF repeat and preceding spacer region	SEQ ID NO:01, nucleotides 3328-3461
5	Hybridization probe for the 99aa spacer/G-loop region	SEQ ID NO:01, nucleotides 3462-3987
	Hybridization probe for EGF repeats seven to nine	SEQ ID NO:01, nucleotides 3988-4341
10	Hybridization probe for the cysteine knot region	SEQ ID NO:01, nucleotides 4342-4575

Table 6. PCR Primers for regions of Human Slit.

	PCR Primers for first leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 82-111 Reverse: reverse complement of SEQ ID NO:01, nucleotides 799-828
15	PCR Primers for second leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 829-858 Reverse: reverse complement of SEQ ID NO:01, nucleotides 1474-1503
	PCR Primers for third leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 1504-1533 Reverse: reverse complement of SEQ ID NO:01, nucleotides 2137-2166
20	PCR Primers for fourth leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 2167-2196 Reverse: reverse complement of SEQ ID NO:01, nucleotides 2722-2751
	PCR Primers for EGF repeats one to five	Forward: SEQ ID NO:01, nucleotides 2752-2781 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3298-3327
25	PCR Primers for the sixth EGF repeat and preceding spacer region	Forward: SEQ ID NO:01, nucleotides 3328-3357 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3432-3461

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	PCR Primers for the 99aa spacer/G-loop region	Forward: SEQ Inucleotides 3462-3491 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3958-3987
	PCR Primers for EGF repeats seven to nine	Forward: SEQ ID NO:01, nucleotides 3988-4017 Reverse: reverse complement of SEQ ID NO:01, nucleotides 4312-4341
5	PCR Primers for the cysteine knot region	Forward: SEQ ID NO:01, nucleotides 4342-4371 Reverse: reverse complement of SEQ ID NO:01, nucleotides 4546-4575

Leucine rich repeats (LRRs) are predicted by comparison with known proteins and by the presence of the core sequence: xxxFxxLxxLxxLxLxxNxLxxL, where x is any amino acid.

10 In slit proteins, the LRRs are flanked by conserved sequences referred to as the amino- and carboxy- flanking regions. These flanking regions are found in other known proteins, but only in a few instances are both the amino- and carboxy- flank regions present in a single protein.

The amino flank region is defined by the consensus:

CPxxCxC[1-6x]GxxVDCxxxGL[2-4x] α Pxx α Pxdttx where x is any amino acid, [x]

15 represents a variable number of amino acids and α is a hydrophobic residue. Lower case indicates a residue is not highly conserved at a particular position. The carboxy flank region is defined by the consensus:

P β xC γ Cx α [1-5x]W α [14-26x]RCxxPxxxxxxxx α xx α xxxxxF[1-3x]Cs[3-17x] where β is W or a hydrophobic residue, γ is D or N and α is a hydrophobic residue. Epidermal growth factor

20 (EGF) repeats are predicted by the consensus:

CxxxxCxngxC[6-9x] α CxCxxG α xGxxCxxxxxx. The so called "99aa spacer" is actually ~200 amino acids in the Drosophila protein and 174 amino acids in Human Slit-1. This region shows homology to the G-loops of laminin A chains.

25 Cysteine knots are dimerisation domains defined by the presence of six cysteine residues between which disulphide bridges form. The only absolutely conserved residues are the six cysteines, and spacing between them is highly variable, apart from between cysteines 2 and 3, and 5 and 6: C[x]C[1-3x]GxC[x]C[x]CxC. The glycine between cysteines 2 and 3 is only present in a subset of cysteine knots. Drosophila slit and Human slit-1 both have an

extra cysteine after cysteines 5 and 6: this may serve as an intermolecular bond. Human Slit-1 gene displays the overall structure of the Drosophila gene, and amino acid conservation is found along the entire length of the protein (48% homology at the amino acid sequence excluding the signal sequence; see below). The Human gene has an extra LRR between LRR2 and LRR3 of the first set of LRRs; in the third set, the Human gene has an extra LRR between LRR3 and LRR4. The Human gene has two extra EGF repeats, on either side of the seventh EGF repeat in Drosophila slit.

Isolation of Human slit-1

Searching of the EST database revealed an EST, ab16g10.r1, with homology to the 99aa spacer region of Drosophila slit. This EST was used to probe a Human fetal brain library (Stratagene), and clones for Human slit-1 were isolated.

Features of Human Slit Predicted Protein

15	Signal sequence	SEQ ID NO:02, residues 7-24
	First amino-flanking sequence	SEQ ID NO:02, residues 28-59
	First set of Leucine Rich Repeats	SEQ ID NO:02, residues 60-179 (6 repeats)
	First carboxy-flanking sequence	SEQ ID NO:02, residues 180-276
	Second amino-flanking sequence	SEQ ID NO:02, residues 277-308
20	Second set of Leucine Rich Repeats	SEQ ID NO:02, residues 309-434 (5 repeats)
	Second carboxy-flanking sequence	SEQ ID NO:02, residues 435-501
	Third amino-flanking sequence	SEQ ID NO:02, residues 502-533
	Third set of Leucine Rich Repeats	SEQ ID NO:02, residues 534-560 (5 repeats)
	Third carboxy-flanking sequence	SEQ ID NO:02, residues 661-722
25	Fourth amino-flanking sequence	SEQ ID NO:02, residues 723-754
	Fourth set of Leucine Rich Repeats	SEQ ID NO:02, residues 755-855 (4 repeats)
	Fourth carboxy-flanking sequence	SEQ ID NO:02, residues 856-917
	First EGF repeat	SEQ ID NO:02, residues 918-952
	Second EGF repeat	SEQ ID NO:02, residues 953-993
30	Third EGF repeat	SEQ ID NO:02, residues 994-1031

	Fourth EGF repeat	SEQ ID NO:02, residues 1032-1071
	Fifth EGF repeat	SEQ ID NO:02, residues 1072-1109
	Spacer	SEQ ID NO:02, residues 1110-1116
	Sixth EGF repeat	SEQ ID NO:02, residues 1117-1153
5	"99aa spacer"	SEQ ID NO:02, residues 1155-1329
	Seventh EGF repeat	SEQ ID NO:02, residues 1330-1366
	Eighth EGF repeat	SEQ ID NO:02, residues 1367-1404
	Nineth EGF repeat	SEQ ID NO:02, residues 1405-1447
	Cysteine knot motif	SEQ ID NO:02, residues 1448-1525

10

Amino acid identity between Drosophila and Human Slit-1

	First amino-flanking sequence	53%
	First set of Leucine Rich Repeats	52% (54%, 67%, NA, 38%, 54%, 50%)
	First carboxy-flanking sequence	42%
15	Second amino-flanking sequence	50%
	Second set of Leucine Rich Repeats	60% (54%, 58%, 67%, 71%, 50%)
	Second carboxy-flanking sequence	62%
	Third amino-flanking sequence	56%
	Third set of Leucine Rich Repeats	49% (46%, 46%, 42%, NA, 58%)
20	Third carboxy-flanking sequence	36%
	Fourth amino-flanking sequence	53%
	Fourth set of Leucine Rich Repeats	48% (25%, 58%, 46%, 63%)
	Fourth carboxy-flanking sequence	63%
	First EGF repeat	34%
25	Second EGF repeat	46%
	Third EGF repeat	46%
	Fourth EGF repeat	35%
	Fifth EGF repeat	47%
	Spacer	22%

11 07 09 09

Sixth EGF repeat	40%
"99aa spacer"	38%
Seventh EGF repeat	11% /NA
Eighth EGF repeat	44%
5 Ninth EGF repeat	29% /NA
Cysteine knot motif	34%

NA: not applicable due to absence of homologous repeat.

Figures for individual LLRs are shown in brackets.

The following exemplary assay is offered by way of illustration and not by way of limitation:

EXAMPLES

Protocol for Ligand Screening of Transfected COS cells.

I. Prepare the Ligand

Expression Construct: cDNAs encoding targeted Slit polypeptides are tagged with the Fc portion of human IgG and subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected (CaPO₄ method) with the Slit expression constructs. After 24 h recovery, transfected cells are selected with G418 (geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Prepare Truncated Receptor (Positive Control)

Expression Construct: cDNA encoding a corresponding Robo C-terminal deletion mutant comprising the extracellular domain (truncated immediately N-terminal to the transmembrane region) is subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected (CaPO₄ method) with the receptor mutant expression construct. After 24 h recovery, transfected cells are selected with G418

(geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Transfect COS Cells

Seed COS cells (250,000) on 35 mm dishes in 2 ml DME/10% FCS.

18-24 h later, dilute 1 ug of Robo-encoding DNA (cDNA cloned into pMT21 expression vector) into 200 ul serum-free media and add 6 ul of Lipofectamine (Gibco). Incubate this solution at room temperature for 15-45 min.

Wash the cells 2X with PBS. Add 800 ul serum-free media to the tube containing the lipid-DNA complexes. Overlay this solution onto the washed cells.

Incubate for 6 h. Stop the reaction by adding 1 ml DMA/20% FCS. Refeed cells. Assay cells 12 hr later.

III. Ligand Binding Assay

Wash plates of transfected COS cells 1X with cold PBS (plus Ca/Mg)/1% goat serum. Add 1 ml conditioned media neat and incubate 90 min at room temp.

Wash plates 3X with PBS (plus Ca/Mg). On the 4th wash, add 1 ml 50% methanol to 1 ml PBS. Then add 1 ml methanol. Evacuate and add 1 ml methanol.

Wash 1X with PBS. Wash 1X PBS/1% goat serum.

Add secondary antibody (1-to-2,000 anti-human Fc conjugated to alkaline phosphatase (Jackson Lab)) in PBS/1% goat serum. Incubate 30-40 min room temp.

Wash 3X with PBS. Wash 1X alkaline phosphatase buffer (100 mM Tris-Cl, pH 9.5, 100 mM NaCl, 5 mM MgCl₂). Prepare alkaline phosphatase reagents: 4.5 ul/ml NBT and 3.5 ul/ml BCIP (Gibco) in alkaline phosphatase buffer.

Incubate 10-30 min, quench with 20 mM EDTA in PBS. Cells that have bound Slit polypeptides are visible by the presence of a dark purple reaction product.

In parallel incubations, positive controls are provided by titrating Slit binding with serial dilutions of the mutant receptor conditioned medium.

IV. Results: Binding of Slit to Robo

Cell expressing mammalian Slit polypeptides were shown to bind Robo. No reactivity was observed with control COS cells or with receptor-expressing COS cells in the presence of the secondary antibody but in the absence of the Slit-Fc fusion. Binding was observed to receptor-expression cells using a construct in which a Slit polypeptide is fused directly to alkaline phosphatase, for which a secondary antibody is not required. Receptor deletion mutants titrate the Slit-Robo binding, serving as a positive control for inhibition assays.

10 Protocol for high throughput Robo-Slit binding assay.

A. Reagents:

- Neutralite Avidin: 20 µg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM MgCl₂, 1% glycerol, 0.5% NP-40, 50 mM β-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.
- ³³P Robo polypeptide 10x stock: 10⁻⁸ - 10⁻⁶ M "cold" Robo polypeptide specific Robo domain supplemented with 200,000-250,000 cpm of labeled Robo (Beckman counter). Place in the 4°C microfridge during screening.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM NaVO₃ (Sigma # S-6508) in 10 ml of PBS.
- Slit: 10⁻⁷ - 10⁻⁵ M biotinylated Slit in PBS.

B. Preparation of assay plates:

- Coat with 120 µl of stock N-Avidin per well overnight at 4°C.
- Wash 2 times with 200 µl PBS.
- Block with 150 µl of blocking buffer.
- Wash 2 times with 200 µl PBS.

C. Assay:

- Add 40 µl assay buffer/well.
- Add 10 µl compound or extract.

- Add 10 μ l ^{33} P-Robo (20-25,000 cpm/0.1-10 pmoles/well = 10^{-9} - 10^{-7} M final conc).
- Shake at 25°C for 15 minutes.
- Incubate additional 45 minutes at 25°C.
- Add 40 μ M biotinylated Slit (0.1-10 pmoles/40 μ l in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200 μ M PBS.
- Add 150 μ M scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

- a. Non-specific binding
- b. Soluble (non-biotinylated Slit) at 80% inhibition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQ ID NO:02, res. 1-10;
 SEQ ID NO:02, res. 29-41;
 SEQ ID NO:02, res. 75-87;
 SEQ ID NO:02, res. 92-109;
 5 SEQ ID NO:02, res. 132-141;
 SEQ ID NO:02, res. 192-205;
 SEQ ID NO:02, res. 258-269;
 SEQ ID NO:02, res. 295-311;
 SEQ ID NO:02, res. 316-330;
 10 SEQ ID NO:02, res. 373-382;
 SEQ ID NO:02, res. 403-422;
 SEQ ID NO:02, res. 474-485;

SEQ ID NO:02, res. 561-576;
 SEQ ID NO:02, res. 683-697;
 SEQ ID NO:02, res. 768-777;
 SEQ ID NO:02, res. 798-813;
 SEQ ID NO:02, res. 882-894;
 SEQ ID NO:02, res. 934-946;
 SEQ ID NO:02, res. 1054-1067;
 SEQ ID NO:02, res. 1181-1192;
 SEQ ID NO:02, res. 1273-1299;
 SEQ ID NO:02, res. 1383-1397;
 SEQ ID NO:02, res. 1468-1477; and
 SEQ ID NO:02, res. 1508-1517;

6. An isolated vertebrate Slit polypeptide according to claim 4, comprising a peptide selected from the group consisting of:

15 SEQ ID NO:02, residues 7-24;
 SEQ ID NO:02, residues 28-59;
 SEQ ID NO:02, residues 60-179;
 SEQ ID NO:02, residues 180-276;
 SEQ ID NO:02, residues 277-308;
 20 SEQ ID NO:02, residues 309-434;
 SEQ ID NO:02, residues 435-501;
 SEQ ID NO:02, residues 502-533;
 SEQ ID NO:02, residues 534-560;
 SEQ ID NO:02, residues 661-722;
 25 SEQ ID NO:02, residues 723-754;
 SEQ ID NO:02, residues 755-855;
 SEQ ID NO:02, residues 856-917;

SEQ ID NO:02, residues 918-952;
 SEQ ID NO:02, residues 953-993;
 SEQ ID NO:02, residues 994-1031;
 SEQ ID NO:02, residues 1032-1071;
 SEQ ID NO:02, residues 1072-1109;
 SEQ ID NO:02, residues 1110-1116;
 SEQ ID NO:02, residues 1117-1153;
 SEQ ID NO:02, residues 1155-1329;
 SEQ ID NO:02, residues 1330-1366;
 SEQ ID NO:02, residues 1367-1404;
 SEQ ID NO:02, residues 1405-1447 and
 SEQ ID NO:02, residues 1448-1525;

7. A recombinant nucleic acid encoding a vertebrate Slit polypeptide according to claim 4.

8. A recombinant Slit nucleic acid comprising a sequence selected from the group consisting of:

- | | | |
|----|--------------------------------|------------------------------------|
| 5 | SEQ ID NO:01, nucl. 1-47; | SEQ ID NO:01, nucl. 1258-1279; |
| | SEQ ID NO:01, nucl. 58-99; | SEQ ID NO:01, nucl. 1375-1389; |
| | SEQ ID NO:01, nucl. 95-138; | SEQ ID NO:01, nucl. 1581-1595; |
| | SEQ ID NO:01, nucl. 181-220; | SEQ ID NO:01, nucl. 1621-1639; |
| | SEQ ID NO:01, nucl. 261-299; | SEQ ID NO:01, nucl. 1744-1755; |
| | SEQ ID NO:01, nucl. 274-315; | SEQ ID NO:01, nucl. 1951-1969; |
| | SEQ ID NO:01, nucl. 351-389; | SEQ ID NO:01, nucl. 2150-2163; |
| 10 | SEQ ID NO:01, nucl. 450-593; | SEQ ID NO:01, nucl. 2524-2546; |
| | SEQ ID NO:01, nucl. 524-546; | SEQ ID NO:01, nucl. 2761-2780; |
| | SEQ ID NO:01, nucl. 561-608; | SEQ ID NO:01, nucl. 2989-2999; |
| | SEQ ID NO:01, nucl. 689-727; | SEQ ID NO:01, nucl. 3108-3117; |
| | SEQ ID NO:01, nucl. 708-737; | SEQ ID NO:01, nucl. 3338-3351; |
| 15 | SEQ ID NO:01, nucl. 738-801; | SEQ ID NO:01, nucl. 3505-3514; |
| | SEQ ID NO:01, nucl. 805-854; | SEQ ID NO:01, nucl. 3855-3867; |
| | SEQ ID NO:01, nucl. 855-907; | SEQ ID NO:01, nucl. 4010-4025; |
| | SEQ ID NO:01, nucl. 910-953; | SEQ ID NO:01, nucl. 4207-4219; |
| | SEQ ID NO:01, nucl. 1007-1059; | SEQ ID NO:01, nucl. 4333-4345; and |
| 20 | SEQ ID NO:01, nucl. 1147-1163; | SEQ ID NO:01, nucl. 4521-4529, |

9. A recombinant Slit nucleic acid according to claim 7 comprising a sequence selected from the group consisting of:

- | | | |
|----|--------------------------------------|--|
| | SEQ ID NO:01, nucleotides 82-828; | SEQ ID NO:01, nucleotides 3328-3461; |
| | SEQ ID NO:01, nucleotides 829-1503; | SEQ ID NO:01, nucleotides 3462-3987; |
| 25 | SEQ ID NO:01, nucleotides 1504-2166; | SEQ ID NO:01, nucleotides 3988-4341; and |
| | SEQ ID NO:01, nucleotides 2167-2751; | SEQ ID NO:01, nucleotides 4342-4575. |
| | SEQ ID NO:01, nucleotides 2752-3327; | |

SEQUENCE LISTING

5 <110> Goodman, Corey
 Kid, Thomas
 Brose, Katja
 Tessier-Lavigne, Marc

10 <120> Modulating Robo: Ligand Interactions
 <130> B98-031-3
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15 <150> 60/065,544
 <151> 1997-11-14
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 <151> 1998-04-07

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 1 5 10 15

40 ctg gcg atc ctg aac aag gtg gca ccg cag gcg tgc ccg gcg cag tgc 96
 Leu Ala Ile Leu Asn Lys Val Ala Pro Gln Ala Cys Pro Ala Gln Cys
 20 25 30

45 tct tgc tcg ggc agc aca gtg gac tgt cac ggg ctg gcg ctg cgc agc 144
 Ser Cys Ser Gly Ser Thr Val Asp Cys His Gly Leu Ala Leu Arg Ser
 35 40 45

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 Val Pro Arg Asn Ile Pro Arg Asn Thr Glu Arg Leu Asp Leu Asn Gly
 50 55 60

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 Asn Asn Ile Thr Arg Ile Thr Lys Thr Asp Phe Ala Gly Leu Arg His
 65 70 75 80

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 Leu Arg Val Leu Gln Leu Met Glu Asn Lys Ile Ser Thr Ile Glu Arg
 85 90 95

60 gga gca ttc cag gat ctt aaa gaa cta gag aga ctg cgt tta aac aga 336
 Gly Ala Phe Gln Asp Leu Lys Glu Leu Glu Arg Leu Arg Leu Asn Arg
 100 105 110

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10	gcc aac aag ata aac tgc ctt cgg gta gat gct ttt cag gat ctc cac Ala Asn Lys Ile Asn Cys Leu Arg Val Asp Ala Phe Gln Asp Leu His 385 390 395 400			1200
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30	ctc cat acc aac ccg att gag acc agt ggt gcc cgt tgc acc agc ccc Leu His Thr Asn Pro Ile Glu Thr Ser Gly Ala Arg Cys Thr Ser Pro 450 455 460			1392
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 REPRODUCED FROM
 ORIGINAL DOCUMENT

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Cys Asp Gln Arg Thr Asn Asp Pro Cys Leu Gly Asn Lys Cys Val His
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Cys Asp Arg Glu Ile Ser Cys Arg Gly Glu Arg Ile Arg Asp Tyr Tyr
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Arg Leu Glu Cys Arg Gly Gly Cys Ala Gly Gly Gln Cys Cys Gly Pro
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55 Leu Arg Ser Lys Arg Arg Lys Tyr Ser Phe Glu Cys Thr Asp Gly Ser
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60 Ser Phe Val Asp Glu Val Glu Lys Val Val Lys Cys Gly Cys Ala Arg
 225 230 235 240

Cys Ala Ser

1. A method of identifying agents which modulate the interaction of Robo and a Robo ligand, said method comprising the steps of:

determining a second interaction of the Robo and Slit polypeptides in the presence of the agent,

2. A method of modulating the interaction of Robo and a Robo ligand, said method comprising the step of

whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction.

4. An isolated Slit polypeptide comprising a species-specific Slit fragment of a Slit polypeptide selected from the group consisting of: human Slit-1 (SEQ ID NO:02), human Slit-2 (SEQ ID NOS:03-06), Drosophila Slit-1 (SEQ ID NO:07), C. elegans Slit-1 (SEQ ID NOS:08-09), mouse Slit-2 (SEQ ID NOS:10-11) and mouse Slit-1 (SEQ ID NOS:12-14).

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AMENDED SHEET