CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Selected References for Wheat Germ Cell-Free Protein Expression

The wheat germ cell-free protein expression system has been used for many years as a basic tool to drive life science research and to support product development in industry. Below some selected references are provided to give examples and guidance on how the system can be used to address specific protein needs.

Table of Contents

CellFree Sciences Co., Ltd., Yokohama Bio Industry Center, 1-6 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
Tel: +81-(0)45-345-2625 Fax: +81-(0)45-345-2626 Web: http://cfsciences.com/eg/index.html

Overview

The wheat germ cell-free protein expression system offered by CFS was originally developed at Ehime University and technology was described in detail in the following publications:

A cell-free protein synthesis system for high-throughput proteomics.
Sawasaki T. et al.: Proc Natl Acad Sci U S A. 2002 Nov 12;99(23):14652-7.

- Original publication of the wheat germ cell-free expression system developed by the Endo laboratory
- Split-PCR protocol to prepare linear expression templates by two-step PCR method for direct use in cell-free protein expression

PMID: 12409616, PMCID: PMC137474

Practical cell-free protein synthesis system using purified wheat embryos.
Takai K., Sawasaki T., Endo Y.: Nat Protoc. 2010 Feb;5(2):227-38.

- Detailed protocol on how to prepare highly active wheat germ extracts for translation experiments

PMID: 20134421

More information on the widespread use of the wheat germ cell-free protein expression systems can be found in the following review articles:

The wheat-germ cell-free expression system.
Takai K, Sawasaki T, Endo Y.: Curr Pharm Biotechnol. 2010 Apr;11(3):272-8.

PMID: 20210744

Wheat germ systems for cell-free protein expression.
Harbers M.: FEBS Lett. 2014 Aug 25;588(17):2762-73.

PMID: 24931374

Template Preparation

In cell-free protein expression systems, expression vectors and linear DNA templates can be used to drive protein expression. For the wheat germ system, a special enhancer was developed that induces CAP-independent translation without need for a Kozak consensus sequence. *The E01 enhancer is used in all expression vectors from CFS:*

Selection of 5'-untranslated sequences that enhance initiation of translation in a cell-free protein synthesis system from wheat embryos.
Kamura N. et al.: Bioorg Med Chem Lett. 2005 Dec 15;15(24):5402-6.

- Screening random libraries of mRNA 5'-leader sequences to find new translation initiation site for wheat germ system
- Comparing activity to Omega sequence from tobacco mosaic virus

PMID: 16213724

Additional expression vectors using the E01 enhancer have been described in the literature for working with different affinity tags:

A novel family of expression vectors with multiple affinity tags for wheat germ cell-free protein expression.
Nagy S.K. et al.: BMC Biotechnol. 2020 Mar 14;20(1):17.

- pEU3-NII vector with rare-cutting NotI restriction enzyme cleavage site for vector linearization
- Vectors for using His_{12}, FLAG, and Halo-tag
- GST-His_6 and GST-biotin double-tagging vectors

PMID: 32169064, PMCID: PMC7071761

Bilayer Reaction Format

To increase the protein yields for cell-free protein expression reactions, Ehime University developed the bilayer reaction format for the

wheat germ cell-free expression system. *This reaction format is used in several of our reagent kits:*

A bilayer cell-free protein synthesis system for high-throughput screening of gene products.
Sawasaki T. et al.: FEBS Lett. 2002 Mar 6;514(1):102-5.

- Diffusion system in bilayer setup enables the continuous supply of substrates
- Prolonged reaction times yield in more than 10 times more protein than in similar batch-mode reactions

PMID: 11904190

Protein MS and Proteomics

Protein mass spectrometry (MS) has become a powerful method to analyze individual proteins or even complex protein mixtures from clinical or biological samples. Protein quantification in such samples is often done by adding a reference peptide or reference protein having a higher mass than the native protein. This mass-shift is commonly achieved by incorporation of isotope-labeled amino acid(s) or a chemical modification. The wheat germ cell-free protein expression system was used to prepare such protein standards by direct incorporation of labeled amino acids, or chemical modification after completion of protein synthesis. *CFS supports the use of our system in protein MS by providing dedicated reagents to prepare $^{13}C/^{15}N$ lysine and arginine-labeled reference proteins*:

High-throughput synthesis of stable isotope-labeled transmembrane proteins for targeted transmembrane proteomics using a wheat germ cell-free protein synthesis system.
Takemori N. et al.: Mol Biosyst. 2015 Feb;11(2):361-5.

- High throughout expression of membrane proteins into proteoliposomes
- High incorporation rates for added isotope-labeled amino acids
- Use as internal standard library for targeted transmembrane proteomics

PMID: 25431973

A large-scale targeted proteomics assay resource based on an in vitro human proteome.
Matsumoto M. et al.: Nat Methods. 2017 Mar;14(3):251-258.

- Expression of > 18,000 human recombinant proteins from AIST and ORFeome clone sets
- Digestion and mTRAQ△4 labeling
- MRM assay with mass tag (mTRAQ) labeled peptides
- Genome-wide peptide resource: database comprised 216,476 unique peptides for 17,973 proteins, corresponding to 86.3% of all (20,819) human protein-coding genes

PMID: 28267743

High-throughput production of a stable isotope-labeled peptide library for targeted proteomics using a wheat germ cell-free synthesis system.
Takemori N. et al.: Mol Biosyst. 2016 July 19;12(8):2389-93.

- High-throughput production of reference peptide library from 2201 selected peptides
- Designed 162 His-tagged QconCATs with on average 14 peptides
- Incorporation of $^{13}C/^{15}N$ labeled Lys and Arg during cell-free protein expression (up to 99% labeling efficiency for incorporating [^{13}C, ^{15}N]-L-Lys and [^{13}C, ^{15}N]-L-Arg)
- Gel-separated QconCATs were digested with trypsin
- 71% of all peptides within the QconCATS identified during MS/MS analysis

PMID: 27203355

MEERCAT: Multiplexed Efficient Cell Free Expression of Recombinant QconCATs For Large Scale Absolute Proteome Quantification.

Takemori N. et al.: Mol Cell Proteomics. 2017 Dec;16(12):2169-2183.

- Use of wheat germ system to prepare QconCATs, artificial proteins comprising concatemers of multiple standard peptides, on high throughput for use in proteomics studies

PMID: 29055021, PMCID: PMC5724179

A practical guide to the FLEXIQuant method.
Singh S. et al.: Methods Mol Biol. 2012;893:295-319.

- Detailed protocol for using FLEXIQuant method for indirect protein quantification
- Isotope-labeled FLEX-tag quantified by reference to peptide
- Because of indirect quantification using FLEX-tag, no need for highly purified protein standards
- Commonly multiple peptides per target protein for better quantification/coverage
- FLEXIQuant method used in medical research
- The related FLEXIQinase method is using FLEXIQuant to study protein modification (PTM)

PMID: 22665308

FLEXIQinase, a mass spectrometry-based assay, to unveil multikinase mechanisms.
Singh S.A. et al.: Nat Methods. 2012 Apr 8;9(5):504-8.

- Mass spectrometry-based method that provides residue-resolved quantitative information about protein phosphorylation
- Combination of full-length stable isotope-labeled protein (FLEXIQuant) with traditional kinase assay to determine multikinase substrate phosphorylation
- Examples c-Jun N-terminal kinase (JNK)-dependent glycogen synthase kinase 3β (GSK3β) activity

PMID: 22484849 PMCID: PMC3595540

FLEXITau: Quantifying Post-translational Modifications of Tau Protein in Vitro and in Human Disease.
Mair W. et al.: Anal Chem. 2016 Apr 5;88(7):3704-14.

- Use of FLEXIQuant to study phosphorylation of the Tau protein related to Alzheimer's disease (AD)
- FLEXITau is used to measure phosphorylation stoichiometry obtaining an unbiased quantitative view of the tau post-translational modification (PTM)
- FLEXITau further defined the tau PTM landscape in AD post-mortem brain

PMID: 26877193, PMCID: PMC5808556

CFS supports FLEXIQuant by providing dedicated reagents, the expression vector, and the reference peptide.

Working with Biotinylated Proteins

The extraordinarily strong non-covalent binding of biotin to avidin and streptavidin has been widely used in analytical assays, detection, and protein purification. Adding the biotin ligase BirA from *E. coli* to wheat germ cell-free protein expression reactions allows for specific mono-biotinylation of proteins having a short recognition sequence for the BirA ligase:

Simple screening method for autoantigen proteins using the N-terminal biotinylated protein library produced by wheat cell-free synthesis.
Matsuoka K. et al..: Proteome Res. 2010 Aug 6;9(8):4264-73.

- Direct preparation of biotinylated proteins by adding BirA to translation reaction
- Development of HTP screening assay on PerkinElmer AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay)
- Background free detection without need of protein purification

- Use of biotinylated proteins to screen serum samples for autoimmune antibodies

PMID: 20575507, PMCID: PMC2917173

Specific in situ visualization of plasma cells producing antibodies against Porphyromonas gingivalis in gingival radicular cyst: application of the enzyme-labeled antigen method.
Tsuge S. et al.: J Histochem Cytochem. 2011 Jul;59(7):673-89.

- Biotinylated protein used in different detection assays
- Biotinylated protein used in PerkinElmer AlphaScreen™ experiments (see above)

PMID: 21525188, PMCID: PMC3201162

Profiling of autoantibodies in sera of pancreatic cancer patients.
Nagayoshi Y. et al.: Ann Surg Oncol. 2014 Jun;21 Suppl 3:S459-65.

- Preparation of biotinylated protein library covering 2,183 human genes
- Screening of serum antibodies by PerkinElmer AlphaScreen™ method (see above)
- Identified potential biomarkers for pancreas cancer

PMID: 24585405

Identification of new abscisic acid receptor agonists using a wheat cell-free based drug screening system.
Nemoto K. et al.: Sci Rep. 2018 Mar 9;8(1):4268.

- Abscisic acid (ABA) is main phytohormone involved plant stress response
- Using wheat germ system and PerkinElmer AlphaScreen™ method in a drug screening system for biochemical validation ABA receptors
- Successful use of the system for screening compound library to identify ABA receptor agonists that are candidate agrichemicals

PMID: 29523814, PMCID: PMC5844987

CFS is providing protein expression services for the preparation of mono-biotinylated proteins using the BirA biotin ligase.

Studying Protein-Protein Interactions

Easy access to modified proteins using a cell-free expression system also provides a great tool to better study protein-protein interactions on different platforms including the PerkinElmer AlphaScreen™ technology or Surface Plasmon Resonance (SPR) that can use biotinylated proteins as mentioned above and for example combines them with tagged proteins to confirm binding of the partners. Protein-protein interactions have been studied with the wheat germ system in plant, animal, and viral research:

Viral Research

Involvement of the 3' Untranslated Region in Encapsidation of the Hepatitis C Virus.
Shi G. et al.: PLoS Pathog. 2016 Feb 11;12(2):e1005441.

- Study on selective packaging of the HCV genome into viral particles
- Identification of 3' UTR element that acts in cis for encapsidation of viral genome
- Using wheat germ system to express FLAG-tagged HCV Core and its mutants to perform RNA binding assays with biotinylated RNA on AlphaScreen™

PMID: 26867128 PMCID: PMC4750987

HTLV-1 Tax Induces Formation of the Active Macromolecular IKK Complex by Generating Lys63- and Met1-Linked Hybrid Polyubiquitin Chains.
Shibata Y. et al.: PLoS Pathog. 2017 Jan 19;13(1):e1006162.

- Study on Tax protein of human T-cell leukemia virus type 1 (HTLV-1)

- Tax recruits linear (Met1-linked) ubiquitin chain assembly complex (LUBAC) to the IKK complex
- Data suggest that Tax could lead to trans-autophosphorylation-mediated IKK activation
- GST, GST-HOIL-1L, GST-HOIP and GST-Sharpin were prepared by wheat germ cell-free protein synthesis during this study

PMID: 28103322 PMCID: PMC5283754

PIM kinases facilitate lentiviral evasion from SAMHD1 restriction via Vpx phosphorylation.
Miyakawa K. et al.: Nat Commun. 2019 Apr 23;10(1):1844.

- PIM family of serine/threonine protein kinases phosphorylate Vpx protein
- Using proteomics and functional analysis to show that PIM family kinases, PIM1 and PIM3, phosphorylate HIV-2 Vpx at Ser13
- This study used the AlphaScreen™ system with proteins made in the wheat germ system to study protein–protein interactions
- Initial screen used a set of 412 human protein kinases

PMID: 31015445 PMCID: PMC6479052

Plant Research

Members of the Plant CRK Superfamily Are Capable of Trans- and Autophosphorylation of Tyrosine Residues.
Nemoto K. et al.: J Biol Chem. 2015 Jul 3;290(27):16665-77

- Arabidopsis calcium-dependent protein kinase (CDPK/CPK)-related PKs (CRKs) have high Tyr-autophosphorylation activity and can phosphorylate Tyr residue(s) on substrate proteins in Arabidopsis
- Examined autophosphorylation activity of 759 protein kinases using an Arabidopsis protein array and wheat cell-free expression system
- Substrate screening for CRK3 by pulldown assay using protein expressed in wheat cell-free system and protein extracts prepared from cultured Arabidopsis cells
- Found Tyr-autophosphorylation activity for 38 protein kinases included into the screen

PMID: 25969537 PMCID: PMC4505418

Tyrosine phosphorylation of the GARU E3 ubiquitin ligase promotes gibberellin signalling by preventing GID1 degradation.
Nemoto K. et al.: Nat Commun. 2017 Oct 17;8(1):1004.

- Gibberellin (GA) is a major hormone for plant growth and development
- Study shows that GARU (GA receptor RING E3 ubiquitin ligase) mediates ubiquitin-dependent degradation of GID1
- TAGK2 plant Tyr-kinase is a target of genistein and inhibits GARU-GID1A interactions by phosphorylation of GARU at Tyr321
- This study used the AlphaScreen™ system with proteins made in the wheat germ system to study protein–protein interactions

PMID: 29042542 PMCID: PMC5645313

OsMYC2, an essential factor for JA-inductive sakuranetin production in rice, interacts with MYC2-like proteins that enhance its transactivation ability.
Ogawa S. et al.: Sci Rep. 2017 Jan 9;7:40175.

- Jasmonic acid (JA) signaling induces basic helix-loop-helix transcriptional factor OsMYC2 and enhanced the activity of the OsNOMT promoter
- OsMYC2 interacts with OsMYL1 and OsMYL2 to further enhance transactivation activity of OsMYC2
- OsMYL1 and OsMYL2 support JA signaling via OsMYC2 and play role in production of sakuranetin in rice

- This study used the AlphaScreen™ system with proteins made in the wheat germ system to study protein–protein interactions

PMID: 28067270 PMCID: PMC5220304

Animal Research

Bach2-Batf interactions control Th2-type immune response by regulating the IL-4 amplification loop.
Kuwahara M. et al.: Nat Commun. 2016 Sep 1;7:12596.

- Study shows that Bach2 associates with Batf and binds to the regulatory regions of the Th2 cytokine gene loci
- This study used the AlphaScreen™ system with proteins made in the wheat germ system to study protein–protein interactions

PMID: 27581382 PMCID: PMC5025763

Ubiquitin E3 Ligases and DUB Proteins

The wheat germ cell-free protein expression system has been used very successfully in several studies on protein ubiquitination and E3 ubiquitin ligases:

Wheat germ-based protein libraries for the functional characterisation of the Arabidopsis E2 ubiquitin conjugating enzymes and the RING-type E3 ubiquitin ligase enzymes.
Ramadan A. et al.: BMC Plant Biol. 2015 Nov 10;15:275.

- Use of RIKEN Arabidopsis full-length cDNA library in combination with template preparation by PCR to make protein libraries of Arabidopsis E2 and RING E3 enzymes
- In total 35 E2s and 204 RING proteins from Arabidopsis made for functional characterization
- Thioester assays using dithiothreitol (DTT) showed DTT-sensitive ubiquitin thioester formation for all E2s expressed
- All the 27 RING E3s tested showed ubiquitin ligase activity

PMID: 26556605 PMCID: PMC4641371

Establishment of a Wheat Cell-Free Synthesized Protein Array Containing 250 Human and Mouse E3 Ubiquitin Ligases to Identify Novel Interaction between E3 Ligases and Substrate Proteins.
Takahashi H. et al.: PLoS One. 2016 Jun 1;11(6):e0156718.

- Preparation of protein array containing 227 human and 23 mouse recombinant E3 ligases
- Using PerkinElmer AlphaScreen™ method (see above) for high-throughput binding assay
- Using MDM2 and p53 as target proteins for E3 ligases

PMID: 27249653, PMCID: PMC4889105

The E3 ubiquitin ligase MIB2 enhances inflammation by degrading the deubiquitinating enzyme CYLD.
Uematsu A. et al.: Nat Commun. J Biol Chem. 2019 Sep 20;294(38):14135-14148.

- Study uses wheat germ cell-free expression together with AlphaScreen™ assays to detect protein-protein interactions
- results suggest that MIB2 enhances NF-κB signaling in inflammation by promoting the ubiquitin-dependent degradation of tumor suppressor CYLD

PMID: 31366726 PMCID: PMC6755803

The ubiquitin ligase STUB1 regulates stability and activity of RUNX1 and RUNX1-RUNX1T1.
Yonezawa T. et al.: J Biol Chem. 2017 Jul 28;292(30):12528-12541

- Preparation of 287 E3s in wheat germ cell-free expression system
- Identification of several RUNX1-interacting E3 ubiquitin ligases

- Out of those, STUB1 bound to RUNX1 and induced its ubiquitination and degradation mainly in the nucleus

PMID: 28536267, PMCID: PMC5535027

Ubiquitin-proteasome system controls ciliogenesis at the initial step of axoneme extension.
Kasahara K. et al.: Nat Commun. 2014 Oct 1;5:5081.

- Using a two-stepped global E3 screening process
- 1,172 E3 ligase proteins (including putative E3s) were prepared from the human proteome expression resource library (HuPEX) using wheat germ cell-free expression system
- E3 ligase proteins involve in trichoplein polyubiquitylation and ciliogenesis were screened using a special protein array platform ("Protein Active Array")

PMID: 25270598, PMCID: PMC4205846

In addition to the expression and screening of E3 ligases, the wheat germ system was also used to prepare a comprehensive set of active human deubiquitinating enzymes (DUBs):

A Human DUB Protein Array for Clarification of Linkage Specificity of Polyubiquitin Chain and Application to Evaluation of Its Inhibitors.
Takahashi A. et al.: Biomedicines. 2020 Jun 4;8(6):152.

- 88 full-length recombinant human DUB proteins prepared
- 80 showed DUB activities, and their linkage specificities were determined
- DUB screening assay based on AlphaScreen™ technology

PMID: 32512835, PMCID: PMC7344921

Membrane Proteins

Membrane proteins are exceedingly difficult to handle, and their expression in many expression systems had failed due to interactions with the cell host. The problems can be avoided by using cell-free protein expression, where special reaction formats are available to conduct protein expression in the presence of detergents, nanodiscs, or add lipid vesicles to directly prepare proteoliposomes. *CFS supports the expression of membrane proteins in our system by offering ready-to-use lyophilized liposomes in combination with the BD reaction format:*

Production of monoclonal antibodies against GPCR using cell-free synthesized GPCR antigen and biotinylated liposome-based interaction assay.
Takeda H. et al.: Sci Rep. 2015 Jun 10;5:11333.

- Expression of GPCRs in the presence of liposomes
- New BD protein expression reaction format combining bilayer with dialysis method
- Direct use of proteoliposomes in antibody production

PMID: 26061673, PMCID: PMC4462149

Production and partial purification of membrane proteins using a liposome-supplemented wheat cell-free translation system.
Nozawa A. et al.: BMC Biotechnol. 2011 Apr 11;11:35.

- Used 40 mammalian membrane proteins having one to 14 transmembrane domains and five soluble proteins to test expression of membrane proteins in wheat germ cell-free expression system
- Their results indicate that the wheat cell-free system is a highly productive method for proteoliposome formation: At least 29 of the membrane proteins, as judged by their higher productivity compared to GFP, might be suitable for a large-scale preparation

PMID: 21481249, PMCID: PMC3090341

A cell-free method for expressing and reconstituting membrane proteins enables functional characterization of the plant receptor-like protein kinase FERONIA.
Minkoff B.B.: J Biol Chem. 2017 Apr 7;292(14):5932-5942.

- Plant receptor-like kinases RLK FERONIA is a peptide receptor
- Cell-free wheat germ expression system was used to co-translate mRNA encoding FERONIA mRNA together with mRNA encoding membrane scaffold protein variant MSP1D1
- The addition of the lipid cardiolipin allowed for assembly of both proteins into nanodiscs
- FERONIA protein kinase activity in nanodiscs was higher than that of soluble protein and comparable with other heterologously expressed protein kinases

PMID: 28235802, PMCID: PMC5392584

Modifications of wheat germ cell-free system for functional proteomics of plant membrane proteins.
Nozawa A., Tozawa Y.: Methods Mol Biol. 2014;1072:259-72.

- They describe three methods for membrane protein production utilizing a wheat germ cell-free protein expression system
- Supplementation of liposomes or detergents allows the synthesis of functional integral membrane proteins
- Supplementation of myristic acid enables synthesis of N-myristylated peripheral membrane proteins

PMID: 24136528

Wheat Germ Cell-Free Overexpression for the Production of Membrane Proteins.
Fogeron M.L. et al.: Methods Mol Biol. 2017;1635:91-108.

- Method to produce a viral integral membrane protein for structural studies by solid-state NMR in a native-like lipid environment

PMID: 28755365

Functional G-Protein-Coupled Receptor (GPCR) Synthesis: The Pharmacological Analysis of Human Histamine H1 Receptor (HRH1) Synthesized by a Wheat Germ Cell-Free Protein Synthesis System Combined with Asolectin Glycerosomes.
Suzuki Y. et al.: Front Pharmacol. 2018 Feb 6;9:38.

- Glycerosomes (liposomes containing high concentrations of glycerol) have greater morphological stability than liposomes
- Use of glycerosomes instead of regular liposomes to express two GPCRs into proteoglycerosomes using the wheat germ system
- Functional analysis of expressed GPCRs to show feasibility for working with proteoglycerosomes

PMID: 29467651, PMCID: PMC5808195

Engineered membrane protein antigens successfully induce antibodies against extracellular regions of claudin-5.
Hashimoto Y. et al.: Sci Rep. 2018 May 30;8(1):8383.

- Approach to make antibodies against the extracellular regions (ECR) of membrane proteins
- Normalizing mRNA GC content improved expression of CLDN-5 protein in the cell-free system
- Antigen synthesized as proteoliposomes
- Successful monoclonal antibody developed targeting difficult-to-produce membrane proteins

PMID: 29849184

Characterization of mitochondrial carrier proteins of malaria parasite Plasmodium falciparum based on in vitro translation and reconstitution.
Nozawa A. et al.: Parasitol Int. 2020 Jun 20;79:102160.

- Expression of mitochondrial carrier (MC) membrane transporters form malaria parasite *Plasmodium falciparum* and *Saccharomyces cerevisiae*
- Functional properties of reconstituted MCs could reflect lipid content of their native membranes, where some *P. falciparum* proteins showed cardiolipin-dependent transport activities

PMID: 32574727

Protein Structure and Folding

The wheat germ cell-free protein expression system has been used in different projects to determine information on protein folding and protein structures including the Protein Structural Initiative (PSI) in the US. Labeled proteins for NMR studies have been made successfully using the open nature of a cell-free expression reaction. *CFS provides amino acid free versions of our expression system to prepare labeled proteins for use in NMR:*

A wheat germ cell-free system is a novel way to screen protein folding and function.
Morita E.H.: Protein Sci. 2003 Jun;12(6):1216-21.

- Expression of two (^{15})N-labeled proteins in E. coli and wheat germ cell-free protein expression system
- Obtained (^{1})H-(^{15})N HSQC spectra from those proteins to obtain structural information
- Comparing the spectra, they showed that proteins synthesized with a wheat germ cell-free system have the proper protein folding and enough biological activity

PMID: 12761392, PMCID: PMC2323893

Cell-free protein synthesis for functional and structural studies.
Makino S. et al.: Methods Mol Biol. 2014;1091:161-78.

- Overview based on the experience learned using the wheat germ cell-free expression system during the Protein Structure Initiative (PSI) project for high-throughput protein production

PMID: 24203331

Expression platforms for producing eukaryotic proteins: a comparison of E. coli cell-based and wheat germ cell-free synthesis, affinity and solubility tags, and cloning strategies.
Aceti D.J. et al.: J Struct Funct Genomics. 2015 Jun;16(2):67-80.

- Use of 21 well-characterized eukaryotic proteins used as controls within the context of a structural genomics pipeline
- Steps included cloning, small-scale expression trials, large-scale growth or synthesis, and purification
- Successfully purified proteins were either crystallized or used in (^{1})H-(^{15})N HSQC NMR analyses

PMID: 25854603, PMCID: PMC4430420

Wheat-germ cell-free production of prion proteins for solid-state NMR structural studies.
Noirot C. et al.: N Biotechnol. 2011 Apr 30;28(3):232-8.

- Use of wheat germ cell-free expression to produce recombinant proteins for solid-state NMR studies
- Productions of the prions Ure2p and HET-s for structural studies

PMID: 20609396

NMR assignment method for amide signals with cell-free protein synthesis system.
Kohno T.: Methods Mol Biol. 2010;607:113-26.

- They describe a method to produce dual amino acid-selective (^{13})C-(^{15})N labeled proteins for NMR using wheat germ cell-free expression system
- Method enables sequence-specific assignments of amide signals even for very large proteins

PMID: 20204853

Cell-free protein production and labeling protocol for NMR-based structural proteomics.
Vinarov D.A. et al.: Nat Methods. 2004 Nov;1(2):149-53. Epub 2004 Oct 21.

- They describe a wheat germ cell-free platform for protein production to support efficient NMR structural studies on eukaryotic proteins
- Expression of At3g01050.1 from Arabidopsis thaliana using a semicontinuous cell-free translation reaction to incorporate (^{15})N-labeled or (^{13})C, (^{15})N-labeled amino acids
- They obtained three-dimensional (3D) structure of At3g01050.1 showing that this protein is an unusual member of the beta-grasp protein family

PMID: 15782178

Structural Studies of Self-Assembled Subviral Particles: Combining Cell-Free Expression with 110 kHz MAS NMR Spectroscopy.
David G. et al.: Angew Chem Int Ed Engl. 2018 Apr 16;57(17):4787-4791

- Made milligram amounts of the small envelope protein of the duck hepatitis B virus (DHBV) in wheat germ system
- Protein used in NMR structural analysis

PMID: 29457857

Protein sample preparation for solid-state NMR investigations.
Lacabanne D. et al.: Prog Nucl Magn Reson Spectrosc. 2019 Feb 110:20-33.

- Review on aspects of solid-state NMR sample preparation
- Describes use of wheat germ cell-free expression system

PMID: 30803692

Protein structural biology using cell-free platform from wheat germ.
Novikova I.V. et al.: Adv Struct Chem Imaging. 2018;4(1):13.

- They are using a wheat germ cell-free expression platform for obtaining functional proteins for structural biology
- Very good advice on template testing and scaling protein expression
- First example for using the wheat germ cell-free expression system for cryo-EM

PMID: 30524935, PMCID: PMC6244559

Automation

The wheat germ cell-free protein expression system can be fully automated as for example done in the CFS Protemist DTII instrument that can perform all reaction steps from RNA synthesis to protein purification. For large-scale production, the translation reaction can be automated for repeated reagent supply as done in the CFS Protemist XE instrument. Both CFS instruments have been successfully used in protein production as given in the following examples:

Automated cell-free protein production methods for structural studies.
Beebe E.T. et al.: Methods Mol Biol. 2014;1140:117-35.

- Overview on using different CFS protein synthesizers during structural genomics project

PMID: 24590713

Development of oligomannose-coated liposome-based nasal vaccine against human parainfluenza virus type 3.
Senchi K. et al.: Front Microbiol. 2013 Nov 26;4:346.

- Example for using Protemist XE for large-scale protein production during vaccine development

PMID: 24324462, PMCID: PMC3840497

Working with Genomic Resources

The wheat germ cell-free expression system has been used to study cDNA clones derived from large genomic resources. These applications have often used large clone numbers, a PCR method for direct template preparation, and fully automated protein expression. In some cases, proteins were later used for protein array preparation. *CFS can provide protocols to prepare expression templates by PCR:*

Functional genomics using RIKEN Arabidopsis thaliana full-length cDNAs.
Seki M., Shinozaki K.: J Plant Res. 2009 Jul;122(4):355-66.

- Review on the use and analysis of the RIKEN Arabidopsis thaliana full-length cDNA collection including protein studies using wheat germ cell-free expression system

PMID: 19412652

An integrated immunoproteomics and bioinformatics approach for the analysis of Schistosoma japonicum tegument proteins.
Chen J.H. et al.: J Proteomics. 2014 Feb 26;98:289-99.

- They combined immunoproteomics and bioinformatics approaches to profile the tegument of the human blood fluke Schistosoma japonicum
- Full-length tegument proteins were cloned and expressed at high-throughput using wheat germ cell-free expression system
- They screened sera from S. japonicum-infected patients and normal subjects using protein arrays

PMID: 24448400

Human protein factory for converting the transcriptome into an in vitro-expressed proteome.
Goshima N. et al.: Nat Methods. 2008 Dec;5(12):1011-7.

- They generated 33,275 human Gateway entry clones for protein synthesis
- They expressed 13,364 human proteins using wheat germ cell-free expression system
- Functional assessment of 75 tested phosphatases showed biological activity for 58 (77%) proteins
- Several cytokines containing disulfide bonds were produced in an active form in a nonreducing wheat germ cell-free expression system
- They manufactured protein microarrays by direct printing of unpurified in vitro-synthesized proteins

PMID: 19054851

Drug Development

Protein expression is an essential part of many drug development projects to verify drug targets, find new binders, or to elucidate underlying mechanisms of drugs. Here are few examples on how the wheat germ system had been used in such projects:

DANFIN functions as an inhibitor of transcription factor NF-κB and potentiates the antitumor effect of bortezomib in multiple myeloma
Uematsu A. et al.: Biochem Biophys Res Commun. 2018 Jan 15;495(3):2289-2295.

- Study shows that DANFIN (N,N'-bis-(2,4-dimethyl-phenyl)-ethane-1,2-diamine)

functions as an inhibitor of the p65 family proteins and induces chemosensitization to bortezomib in multiple myeloma

PMID: 29284118

Pyrrothiogatain acts as an inhibitor of GATA family proteins and inhibits Th2 cell differentiation in vitro
Nomura S. et al.: Sci Rep. 2019 Nov 22;9(1):17335.

- New high-throughput screening system for an inhibitor of DNA-binding proteins like transcription factors
- Identified pyrrothiogatain as a novel inhibitor of GATA3 DNA-binding activity
- Pyrrothiogatain inhibited the DNA-binding activity of GATA3 and other members of the GATA family
- System is efficient for doing drug screening to develop small compounds that inhibit the DNA-binding activity of transcription factors

PMID: 31758034 PMCID: PMC6874683

Subquinocin, a small molecule inhibitor of CYLD and USP-family deubiquitinating enzymes, promotes NF-kB signaling
Yamanaka S. et al.: Biochem Biophys Res Commun. 2020 Mar 26;524(1):1-7.

- Using the wheat cell-free protein synthesis system in combination with the AlphaScreen™ assay, the study identified Subquinocin to inhibit the deubiquitinating activity of recombinant CYLD

PMID: 31898971

Structural bases of IMiD selectivity that emerges by 5-hydroxythalidomide.
Furihata H et al.: Nat Commun. 2020 Sep 14;11(1):4578. doi: 10.1038/s41467-020-18488-4.

- Thalidomide is used as an effective anti-cancer drug for treatment of multiple myeloma.
- However, it exhibits side effects that cause developmental disorders in children during pregnancy.
- This study provides results that can help to design better immunomodulatory drugs in the future that mitigate those side effects.

PMID: 32929090

Antibody Development

The wheat germ cell-free protein expression system is used in research and commercial projects to produce individual antigens for antibody development and analysis. Because of the high throughput of automated protein production, our system is also well suited to prepare protein sets for antibody specificity testing:

Cell-Free Production of Proteoliposomes for Functional Analysis and Antibody Development Targeting Membrane Proteins.
Zhou W, Takeda H.: J Vis Exp. 2020 Sep 22;(163). doi: 10.3791/61871.

- Using a "bilayer-dialysis method" and wheat germ cell-free system with liposomes to efficiently synthesize membrane proteins
- Membrane protein are obtained as purified proteoliposomes
- High success rate to produce several milligrams of GPCRs, ion channels, transporters, and tetraspanins proteins
- These proteoliposomes can be used for antibody development

PMID: 33044457

AGIA Tag System Based on a High Affinity Rabbit Monoclonal Antibody against Human Dopamine Receptor D1 for Protein Analysis.
Yano T. et al.: PLoS One. 2016 Jun 6;11(6):e0156716.

- Study describes novel tag system with an anti-Ra48 antibody and its epitope
- Antibody binds to EEAAGIARP sequence in the C-terminal region of DRD1

- The new affinity tag was named "AGIA"
- This tag can be used in combination with the wheat germ system

PMID: 27271343 PMCID: PMC4894603

CP5 system, for simple and highly efficient protein purification with a C-terminal designed mini tag.
Takeda H. et al.: PLoS One. 2017 May 25;12(5):e0178246.

- New protein purification system using short CP5 tag composed of 5 amino acids
- System allows for rapid protein preparation with high yield and purity
- This tag can be used in combination with the wheat germ system

PMID: 28542437 PMCID: PMC5444806

Enzyme Discovery and Engineering

The wheat germ cell-free protein expression system has been used in enzyme discovering to improve for example biofuel production and to develop a new biotinylation enzyme for protein-protein interaction analysis:

Cell-free translation of biofuel enzymes.
Takasuka T.E. et al.: Methods Mol Biol. 2014;1118:71-95.

- Use of Protemist DTII to screen for new enzymatic activities

PMID: 24395410, PMCID: PMC5820533

AirID, a novel proximity biotinylation enzyme, for analysis of protein-protein interactions.
Kido K. et al.: Elife. 2020 May 11;9:e54983.

- Reconstitution of ancestral BirA enzyme from metagenomic data
- Enzymatic characterization of newly designed BirA enzymes
- Biochemical characterization of AirID enzyme

PMID: 32391793, PMCID: PMC7302878

Special Applications and Additives

A cell-free protein expression system uses an open *in vitro* reaction format that allows for modifying the reaction conditions to better match with protein requirements. In the following, some examples are given where additives like for example detergents had been used during the translation reaction:

Cell-free protein synthesis of membrane (1,3)-β-d-glucan (curdlan) synthase: co-translational insertion in liposomes and reconstitution in nanodiscs.
Periasamy A. et al.: Biochim Biophys Acta. 2013 Feb;1828(2):743-57.

- The publication offers detailed information on the use of detergents and lipids in wheat germ cell-free expression reactions

PMID: 23063656

Efficient production and purification of functional bacteriorhodopsin with a wheat-germ cell-free system and a combination of Fos-choline and CHAPS detergents.
Genji T., Nozawa A., Tozawa Y.: Biochem Biophys Res Commun. 2010 Oct 1;400(4):638-42.

- Example for use of detergents in translation reaction

PMID: 20807510

Wheat germ cell-free translation, purification, and assembly of a functional human stearoyl-CoA desaturase complex.
Goren M.A. and Fox B.G.: Protein Expr Purif. 2008 Dec;62(2):171-8.

- Expression of human stearoyl-CoA desaturase in the presence of unilamelar liposomes
- Co-translation of the desaturase along with human cytochrome b(5) led to transfer of both membrane proteins into the liposomes

- After *in vitro* reconstitution of non-heme iron and heme active sites, the function of reconstituted enzyme complex was demonstrated by conversion of stearoyl-CoA to oleoyl-CoA
- Publication provides information on element and lipid analysis of wheat germ extract

PMID: 18765284, PMCID: PMC2586813

Apoglobin Stability Is the Major Factor Governing both Cell-free and in Vivo Expression of Holomyoglobin.
Samuel P.P. et al.: J Biol Chem. 2015 Sep 25;290(39):23479-95.

- They used wheat germ cell-free protein expression system to examine quantitatively the factors that govern expression of holoMb
- Their results demonstrated that the cell-free transcription/translation system can be used as a high throughput platform to screen for apoglobin stability

PMID: 26205820, PMCID: PMC4583012

The quiescin sulfhydryl oxidase (hQSOX1b) tunes the expression of resistin-like molecule alpha (RELM-α or mFIZZ1) in a wheat germ cell-free extract.
Gad W. et al.: PLoS One. 2013;8(1):e55621.

- Example for expressing in the wheat germ cell-free expression system proteins having disulfide bonds by adding cofactor to expression reaction

PMID: 23383248, PMCID: PMC3561318

Wheat germ in vitro translation to produce one of the most toxic sodium channel specific toxins.
Gad W. et al.: Biosci Rep. 2014 Jul 29;34(4). pii: e00122.

- They developed a wheat germ cell-free expression system for the expression of the highly toxic Aah (Androctonus australis hector) II protein that requires the proper formation of four disulfide bonds
- Soluble, recombinant GST-tagged AahII toxin was obtained, and the purified rAahII was highly toxic after i.c.v. (intracerebroventricular) injection in Swiss mice
- An LD50 (median lethal dose)-value of 10 ng (or 1.33 pmol), close to that of the native toxin (LD50 of 3 ng) indicated that the wheat germ cell-free expression system produces properly folded and biological active rAahII

PMID: 24924257, PMCID: PMC4114062

Functional expression, purification, characterization, and membrane reconstitution of non-structural protein 2 from hepatitis C virus.
Fogeron M.L. et al.: Protein Expr Purif. 2015 Dec;116:1-6.

- Non-structural protein 2 (NS2) of hepatitis C virus (HCV) is an integral membrane protein that contains a cysteine protease
- Using a wheat germ cell-free expression system, they produced and purify milligram amounts of a detergent-solubilized full-length NS2 exhibiting the expected secondary structure features

PMID: 26325423

Wheat germ cell-free expression: Two detergents with a low critical micelle concentration allow for production of soluble HCV membrane proteins.
Fogeron M.L. et al.: Protein Expr Purif. 2015 Jan;105:39-46.

- Use of wheat germ cell-free protein expression system in the presence of various detergents to produce the non-structural membrane proteins 2, 4B and 5A of the hepatitis C virus (HCV)

- They showed that lauryl maltose neopentyl glycol (MNG-3) and dodecyl octaethylene glycol ether (C12E8) detergents can yield essentially soluble membrane proteins at detergent concentrations that do not inhibit the cell-free protein expression reaction

PMID: 25306874

Rational optimization of amber suppressor tRNAs toward efficient incorporation of a non-natural amino acid into protein in a eukaryotic wheat germ extract.
Ogawa A., Namba Y., Gakumasawa M.: Org Biomol Chem. 2016 Mar 7;14(9):2671-8.

- Amber suppression can be used for genetically incorporating a non-natural amino acid (NAA) into a protein during translation by utilizing an NAA-charged amber suppressor tRNA (sup-tRNA)
- They optimized amber sup-tRNAs to efficiently incorporate a model NAA, p-acetyl-phenylalanine (AcPhe), into a protein using the wheat germ cell-free protein expression system

PMID: 26832824

Wheat germ cell-free expression system as a pathway to improve protein yield and solubility for the SSGCID pipeline.
Guild K. et al.: Acta Crystallogr Sect F Struct Biol Cryst Commun. 2011 Sep 1;67(Pt 9):1027-31.

- The SSGCID program used wheat germ cell-free protein expression system as a rescue pathway for proteins that are either not expressed or insoluble when produced in E. coli
- Testing indicates that the system is a valuable tool for these protein targets
- Protein solubility could be increased by the addition of the NVoy polymer to the reaction mixture
- These data indicate that wheat germ cell-free protein expression system has a high success rate and that the addition of specific reagents can increase the yield of soluble protein

PMID: 21904045, PMCID: PMC3169397

Extent and Origins of Functional Diversity in a Subfamily of Glycoside Hydrolases.
Glasgow E.M. et al.: J Mol Biol. 2019 Mar 15;431(6):1217-1233.

- Template preparation by gene synthesis followed by expression in wheat germ system
- Glycoside hydrolase family 5 members were screened for activity of the catalytic core domains from subfamily 4 (GH5_4) and closely related enzymes on four substrates: lichenan, xylan, mannan, and xyloglucan
- Discussing several possibilities for the ongoing evolutionary specialization of GH5_4 enzymes

PMID: 30685401

Preparation of a Millimeter-Sized Supergiant Liposome That Allows for Efficient, Eukaryotic Cell-Free Translation in the Interior by Spontaneous Emulsion Transfer.
Takahashi H. and Ogawa A.: ACS Synth Biol. 2020 Jul 17;9(7):1608-1614.

- Preparation millimeter-sized supergiant unilamellar vesicles (SGUVs) by spontaneous emulsion transfer to encapsulate cell-free protein expression system
- Encapsulated wheat germ translation system allowed for protein synthesis with a high efficiency comparable to that outside a liposome

PMID: 32559381

Preparation and Use of Human Protein Bead Array

CFS uses the wheat germ cell-free protein expression system to prepare our human protein bead array. We selected this format to keep

proteins always in solution during the entire experiment and to avoid protein denaturation. This array holds ~20,000 human full-length proteins and can be used for studying protein-protein interactions. In the following example it had been used to characterize antibodies for their binding specificity:

CF-PA [2] Vtech: a cell-free human protein array technology for antibody validation against human proteins.
Morishita R. et al.: Sci Rep. 2019 Dec 18;9(1):19349.

- Presenting ~20,000 proteins on 1536-well plate with up to 14 proteins per well
- Use plate with multiple targets to screen antibodies for binding to human proteins
- Re-array proteins from positive wells to identify individual antibody targets
- Mapping of binding sites combining protein alignments with mutation screening

PMID: 31852950, PMCID: PMC6920144

Biomarker Discovery, Viral Proteins, and Development of Diagnostic Assays

The wheat germ cell-free protein expression system is a valuable tool to prepare proteins for the discovery of new biomarkers, antibody validation, and rapid development of diagnostic assays. Additional examples for the expression of viral proteins are already listed above:

Development and validation of serological markers for detecting recent *Plasmodium vivax* infection.
Longley R.J.; White M.T. et al.: Nat Med. 2020 May;26(5):741-749.

- Screening for markers to identify patients with recent *P. vivax* malaria infection
- Marker set for serological screening of patients developed which could be used for screen and treat companies to prevent relapses of the disease

PMID: 32405064

In vitro translation of virally-encoded replication polyproteins to recapitulate polyprotein maturation processes.
Habersetzer J. et al.: Protein Expr Purif. 2020 Jul 15;105694.

- Expression of viral polyproteins from human norovirus and plant tymovirus
- Proteins had fully functional active protease domain and underwent spontaneous auto-cleavage
- Introduction of mutation to block proteolytic maturation

PMID: 32681958

Whole nucleocapsid protein of SARS-CoV-2 may cause false positive results in serological assays.
Yamaoka Y. et al.: Clin Infect Dis. 2020 May 23;ciaa637.

- Expression of nucleocapsid protein (NP) of SARS-CoV-2 as full-length protein and with an N-terminal deletion
- Proteins used in development of commercial SARS-CoV-2 tests

PMID: 32445559, PMCID: PMC7314131

A cell-free enzymatic activity assay for the evaluation of HIV-1 drug resistance to protease inhibitors.
Matsunaga S. et al.: Front Microbiol. 2015 Oct 31;6:1220.

- Novel in vitro method for monitoring phenotypic information regarding the drug resistance of HIV-1 protease (PR)
- Using wheat germ cell-free protein production system to synthesize enzymatically active HIV-1 PRs directly from PCR products amplified from HIV-1 molecular clones or clinical isolates in a rapid one-step procedure
- Enzymatic activity of PRs measured by AlphaScreen™ in the presence or absence of clinically used protease inhibitors

PMID: 26583013 PMCID: PMC4628118

Development of Monoclonal Antibody and Diagnostic Test for Middle East Respiratory Syndrome Coronavirus Using Cell-Free Synthesized Nucleocapsid Antigen.
Yamaoka Y. et al.: Front Microbiol. 2016 Apr 20;7:509.

- Production of MERS-CoV NP antigen
- Production of monoclonal antibodies for use in commercial MERS-CoV test

PMID: 27148198, PMCID: PMC4837155

Use in Malaria Research

The wheat germ cell-free protein expression system proved immensely powerful to express malaria proteins for biochemical studies. The following publications give some examples on its use in malaria research:

Leveraging the wheat germ cell-free protein synthesis system to accelerate malaria vaccine development. (review article)
Kanoi BN et al.: Parasitol Int. 2020 Oct 30;80:102224.

- The wheat germ cell-free protein synthesis system has a record for identifying and evaluating targets for malaria vaccine development
- Progresses for novel antigen discovery benefit from the throughput and scalability of the wheat germ system serving screening and evaluation steps in serology and immunization tests.

PMID: 33137499

Malaria transmission-blocking vaccines: wheat germ cell-free technology can accelerate vaccine development. (review article)
Miura K. et al.: Expert Rev Vaccines
. 2019 Oct;18(10):1017-1027.

- Review on progress and prospect of malaria transmission blocking vaccines (TBV) research and development
- Applying wheat germ cell-free protein synthesis to accelerate TBV development

PMID: 31566026

Identification of domains within Pfs230 that elicit transmission blocking antibody responses.
Tachibana M. et al.: Vaccine. 2019 Mar 22;37(13):1799-1806.

- Pfs230 is a promising candidate to develop a transmission blocking vaccine for *P. falciparum* malaria
- Expression of protein fragments to identify transmission blocking domain
- Protein fragments used for antibody production and later analysis
- Antibodies against protein fragments including a CM domain 1 showed transmission blocking activity

PMID: 30824357 PMCID: PMC6708081

Discovery of Novel *Plasmodium falciparum* Pre-Erythrocytic Antigens for Vaccine Development.
Aguiar J.C. et al.: PLoS One. 2015 Aug 20;10(8):e0136109.

- Selection of 27 *P. falciparum* pre-erythrocytic antigens that were then expressed in the wheat germ system
- Tested the protein of sera from humans immunized with *P. falciparum* radiation-attenuated sporozoites (RAS)
- Their results provided evidence to further evaluate these antigens as vaccine candidates

PMID: 26292257, PMCID: PMC4546230

Naturally acquired antibody responses to more than 300 *Plasmodium vivax* proteins in three geographic regions.
Longley R.J. et al.: PLoS Negl Trop Dis. 2017 Sep 11;11(9):e0005888.

- They selected 307 *P. vivax* proteins that had been prepared in the wheat germ system

- They measured antibody responses against those 307 *P. vivax* proteins at the time of *P. vivax* infection and at 2-3 later time-points in three countries
- The data suggest that IgG seropositivity rates including magnitude and longevity are features that are relate to the individual proteins

PMID: 28892517, PMCID: PMC5614652

Identification of highly-protective combinations of *Plasmodium vivax* recombinant proteins for vaccine development.
França C.T. et al.: Elife. 2017 Sep 26;6. pii: e28673.

- They measured total IgG antibodies to 38 *P. vivax* antigens expressed in different systems including wheat germ to investigate the prospective risk of malaria
- Obtained valuable data to establish a clear path forward to testing a multicomponent *P. vivax* vaccine

PMID: 28949293, PMCID: PMC5655538

Antibody profiles to wheat germ cell-free system synthesized *Plasmodium falciparum* proteins correlate with protection from symptomatic malaria in Uganda.
Kanoi B.N. et al.: Vaccine. 2017 Feb 7;35(6):873-881.

- They prepared a library of 1,827 *P. falciparum* proteins derived from 1565 genes representing ~30% of the entire *P. falciparum* genome
- Proteins were expressed in the wheat germ system
- Immunoreactivity to patient sera was determined by the PerkinElmer AlphaScreen™ method (see above)
- Down selected to 53 uncharacterized proteins not previously characterized as vaccine candidates

PMID: 28089547

Immunoscreening of *Plasmodium falciparum* proteins expressed in a wheat germ cell-free system reveals a novel malaria vaccine candidate.
Morita M. et al.: Sci Rep. 2017 Apr 5;7:46086.

- Use of same library of 1,827 *P. falciparum* proteins made in the wheat germ system
- Screened purified IgGs from residents in malaria endemic area to identify antibodies against malaria proteins
- They identified LSA3 as a novel blood-stage vaccine candidate

PMID: 28378857, PMCID: PMC5380959

CFS is actively contributed to projects for identifying vaccine candidates and biomarkers for malaria elimination supported the Global Health Innovative Technology (GHIT) Fund of Japan. You can find more information on those projects on our homepage at:

https://www.cfsciences.com/eg/resources/application-note/454-2019-03-25

Please visit the homepage from GHIT at https://www.ghitfund.org/general/top to learn more about their important work and supported projects.

Follow us on Twitter (@CFSciences) for up-to-date information on new publications, products and the use of our expression system.